4FCROPS

Future Crops for
Food, Feed, Fiber and Fuel

Work Package 2

Cropping Possibilities

Task 2.5

Biotechnological Improvement

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INTRODUCTION

Genomics and biotechnology are the modern tools for understanding plants at the various biological and environmental levels, as well as boosting classical plant breeding techniques. The application of new biotech technologies in plant breeding can help provide a more sustainable solution in view of the fact that we are facing a conflict between rising demand and environmental concerns. Advances in plant breeding methods could help boost non-food crops' yield, quality and adaptation to various biotic and abiotic factors (heat, cold, pathogens, water), while reducing the cost of production.

Plants and plant derived materials hold great potential to provide renewable products for the future. In forthcoming decades, the utilisation of crops for energy productions is expected to increase. Biofuels can be produced from a variety of biomass types which are the mainsource (65%) of renewable energy in EU25 (http://ec.europa.eu/research/energy/pdf/bio-mass_en.pdf).

The objective of Task 2.5 was to review the current status for selected non-food crops by the 4FCrops consortium, summarizing aspects of their genetics, genomics and breeding. Nowadays, the available literature forms the basis for current and future efforts to introduce and establish the selected non-food crops, along with strategies to produce new genetic material for biofuel, feed and fiber exploitation.

The current report intends to assess the capacity of biotechnological applications to develop a beneficial pipeline extending from feedstock development to sustainable biomaterials/biofuel production and provide examples of the current state-of-the-art on future non-food crops.

SUGAR-PRODUCING CROPS

Beta Vulgaris L.

Introduction

Sugar beet (*Beta vulgaris* L.), a member of the delicious food group of the *Chenopodiaceae* family, is a plant whose root contains a high concentration of sucrose. It is grown commercially for sugar production. The European Union, the United States, and Russia are the world's three largest sugar beet producers, although only the European Union and Ukraine are significant exporters of sugar from beets. The U.S. harvested 1,004,600 acres of sugar beets in 2008 alone. Beet sugar accounts for 30% of the world's sugar production.

Sugar beet is a hardy biennial plant that can be grown commercially in a wide variety of temperate climates. During its first growing season, it produces a large (1–2 kg) storage root whose dry mass is 15–20% sucrose by weight. If the plant is not harvested at this time, then during its second growing season, nutrients in the root will be used to produce flowers and seeds and the root will decrease in size. In commercial beet production, the root is harvested after the first growing season.

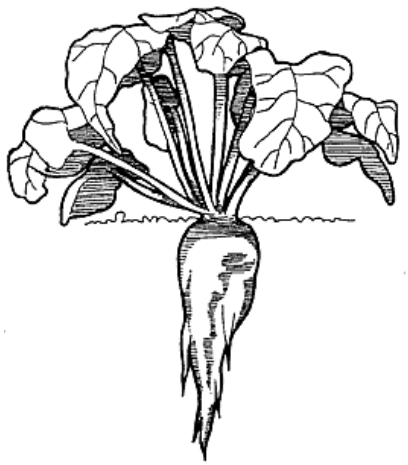

Figure 1: A mature sugar beet plant

Scientific classification	
Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Caryophyllales
Family:	Amaranthaceae
Subfamily:	Chenopodiaceae
Genus:	*Beta*
Species:	*B. vulgaris*

Biotechnology Approaches

Germination studies

There have been studies in order to investigate sugar beet germination. In the recent years, many investigators have shown that crucial role during the germination period play the

plant hormones ABA (Abscisic acid) and ACC (precursor of ethylene) (Hermann et al,, 2007). Molecular approaches used by research groups revealed a set of key regulator genes involved in sugar beet's abiotic stress responses (McGrath et al., 2008). In addition, there have been identified proteins involved specific in germination (de los Reyes, 2003) and in the production of the different parts of the plant such as root, cotyledons and perisperm (Catusse et al., 2008).

Flowering

The sugar beet and its wild progenitor sea beet (*Beta vulgaris ssp. maritima*) are facultative perennials that, under natural growing conditions, exhibit either an annual or a biennial flowering behavior. Many researchers identified that the difference in flowering phenology between annual and biennial sugar beets is determined by a single gene known as the "bolting gene" (Abe et al., 1997).

The effects of the environment on floral initiation in sugar beet have been observed for years but the molecular mechanisms that underlie its control remain to be fully elucidated. As mentioned above, the transition is signified by rapid elongation of the stem (bolting) and tightly linked to the dominant early-bolting (Bolting) gene. In winter-annual ecotypes of *Arabidopsis thaliana*, expression of the MADS-box transcription factor FLOWERING LOCUS C (FLC) during the first growing season creates a facultative vernalization requirement. Reeves et al, (2007) identified FLC homologs in sugar beet by quering expressed sequence tag (EST) databases from *B. vulgaris*.

Molecular markers

Species of the genus *Beta* are grouped into four sections *Beta*, *Corollinae*, *Nanae* and *Procumbentes*. All cultivated beets (sugar, fodder, garden and leaf beet) belong exclusively to the section Beta. With approx. 20 closely and distantly related species and subspecies, the genus provides a suitable system for the comparative study of nuclear genome composition and evolution. Many genus-, section- or species specific repetitive DNA sequences have been analysed from cultivated and wild Beta species (Kubis et al., 1998; Gao et al., 2000), with the most widely used to be the structural modifications of the centromere-specific satellites (Dechyeva et al., 2003; Menzel et al., 2008).

Breeding

Sugar beet is an important agricultural crop, and the results of genome research in this species might be important to the practical implementation in green biotechnology. Currently, a fine-resolution physical map is under construction and a genome-sequencing project is carried out in the framework GABI–Genome Analysis in Biological System Plant (http://www.gabi.de/) aiming to unravel the genome composition of this crop species. Interspecific hybrids and addition lines of *B. vulgaris* are a valuable starting material for plant breeders and an interesting object for fundamental studies on plant genome composition and evolution (Jacobs et al., 2009). Cytoplasmic male sterility (CMS), the maternally inherited

failure to produce functional pollen, has also used in the breeding of sugar beet (Satoh et al. 2004; Yamamoto et al., 2005).

Genetic modification

Transgenic approaches aim to two directions: towards modification of specific traits comprising the increase of pathogen resistance, sugar content and improvement of sugar storage (Graham et al., 1997; Zhang et al., 2000) and towards the tissue specific high protein production in the transgenic plants (Outchkourov et al., 2003; Jaeger et al., 2002).

Disease control is one of the most important goals for biotechnological approaches towards improving sugar beet performance. There are many leaf spot diseases that are detrimental to the plant with the most widespread, destructive but also studied been the *Cercospora* leaf spot (Stahl et al., 2004).

In order to achieve the production of these transgenic lines, *Beta Vulgaris* plants are transformed via the *Argobacterium tumefaciens* transformation technique which is being utilised since 1991 for this plant species (Lindsey et al., 1991; Stahl et al., 2004) and via the biolistic transformation method (Stahl et al., 2004).

Sorghum bicolor L.

Introduction

Sorghum is a single- to multi-culmed C4 plant with perfect flowers; grass species cultivated in diverse and adverse environments from subhumid, hot and dry agro-ecologies, to drought-prone low-to-medium altitudes of the tropics and subtropical regions of the world. This very versatile crop is truly multipurpose, and is used as: (1) grain for food, livestock feed, and industrial products like malt, alcoholic and nonalcoholic beverages, lager beer, stout, and malt drinks; (2) crop residue and silage for livestock feed; (3) chewing cane of the sweet stalk sorghums, (4) household appliances (in fencing and roofing with the dried stalks and as a broom for sweeping with the broomcorn types); and (5) sources of industrial alcohol and household brown sugar with the sweetstalk sorghums. The sorghum plant is composed of two major sections: (1) the vegetative part consisting of the fibrous root system, the culm (stem), and leaves with leaf sheaths wrapping around the node and internode of the culm; and (2) the reproductive portion called inflorescence (panicle) carried on a peduncle (neck, which can be straight or curved (goose neck)) which can be well exerted (short or long neck) or poorly exerted with panicle partially covered by the boot (flag leaf and sheath). The peduncle extends into a central axis of the panicle called rachis, from the nodes of which several branches originate, which bear racemes.

In the preserved germplasm accessions, the unique diversity of sorghum has been classified according to species. There are the cultivated sorghum (Sorghum bicolor L. Moench) and the wild weedy species. Within cultivated sorghums, there are five basic races (race guinea, race candatum, race durra, race bicolor, and race kafir) and ten stable hybrid races.

The taxonomy and evolution of sorghum is well known and documented. The cultivated sorghum, *S. bicolor* (L. Moench) is made up of two crosscompatible subspecies, bicolor and arundinaceum. Bicolor is derived from the domestication of the wild and weedy *Sorghum arundinaceum*.

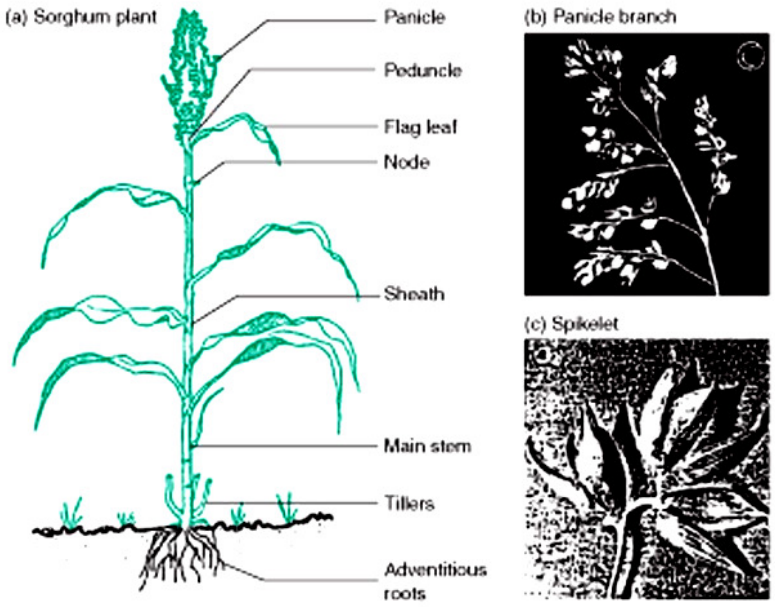

Figure 1: Diagram of the sorghum plant (a) and its components (b and c).

Biotechnology approaches

In recent times, more nonconventional breeding approaches have been used to improve sorghum. These include molecular breeding, biotechnological approaches, and farmer participatory plant breeding (PPB). Each of these has their specific uses, which can overlap in some instances in their complementarity with, and enhancing research in conventional breeding. In sorghum, biotechnology tools are now being used in drought-resistance breeding by tagging quantitative trait loci (QTLs) associated with the different types of drought resistance (seedling, pre-flowering, and postflowering stages), for Striga resistance, and genetic mapping for linkage and genomic maps. They are also used for stem borer and midge resistance, grain quality improvement for increased protein, better digestibility, better processing, and incorporation of vitamin A (enhancing yellow endosperm sorghums) and micronutrients (especially Fe, Zn, and Ca); fodder and crop residue quality and digestibility by

incorporation of bmr gene for brown midrib and stay-green trait gene. Newer biotechnological techniques have recently been used in developing alternative foods and industrial applications (as in using sorghum nondigestible protein character in developing biofilms for fruits and vegetable preservation for exports). These several methodologies have been described and recorded, especially for striga resistance and control, herbicide resistance, molecular and linkage mapping, population dynamics of striga, and biodiversity studies in wide crosses.

Genetic screens

Motivated by interest in a range of basic and applied questions, the linkage maps of sorghum have been employed in the "tagging" (mapping) of genes for a large number of traits. The interspecific population has been especially useful for characterization of genes related to domestication, such as seed size, shattering (Paterson et al, 1995), tillering, and rhizomatousness (Paterson et al, 1995). Plant height and flowering time (Ulanch et al, 1996) have been a high priority. Similarly, the importance of hybrid sorghum motivated much research into the genetic control of fertility restoration (Wen et al, 2002). Resistance genes have been tagged for numerous diseases, key insect pests, and also the parasitic weed, striga (Mutengwa et al, 2005). Genes and QTLs have been identified that are related to abiotic stresses including postreproductive stage drought tolerance (stay-green); preharvest sprouting (Carrari et al, 2003), and aluminum tolerance (Magalhaes et al, 2004). Additional morphological characteristics have also been mapped in interspecific and/or intraspecific populations (Feltus et al, 2006). As with most agronomically important crop species, sorghum genomics is an area of active research. In the past 10 years, over 11 genetic linkage maps of sorghum have been published (McIntyre et al., 2001). Sorghum transformation has been reported and repeated by several research groups, both public and private and it is now possible to create transgenic sorghum.

Compared with other cereal grains, *Sorghum bicolor* shows lower protein digestibility. The low digestibility is thought to result from disulfide cross linking in the β- and γ-kafirins (Duodu et al, 2003). In contrast, the single recessive high digestibility/high lysine content (HD) mutation which confers greater grain digestibility exists in sorghum that is thought to result from reduced accumulation of γ- kafirin that allows greater access to the high digestible α-kafarin fraction. In an effort to both clearly define the molecular basis for theHD trait and develop tools to improve the introgression of this difficult-to-screen trait, Winn et al (2009) focused on mapping the QTLs linked to this trait.

DNA markers

With the completion of the genome sequence (RiceGAAS) for rice (*Oryza sativa* L.), the focus of rice genomics research has shifted to the comparison of the rice genome with genomes of other species for gene cloning, breeding, and evolutionary studies. Studies were undertaken in *Sorghum bicolor*, a species which diverged from cultivated rice 40–50 million

years ago (Gaut BS, 2002). Hybridisation-based markers (overgos), in conjunction with fingerprint and BAC end sequence data, were used to build sequence ready BAC contigs for two wild *Oryza* species. When rice overgos were aligned to available *S. bicolor* sequence, 29% of the overgos aligned with three or fewer mismatches; of these, 41% gave positive hybridization signals (Hass-Jacobus et al, 2006). Overgo hybridization patterns supported colinearity of loci in regions of sorghum chromosome 3 and rice chromosome 1 and suggested that a possible genomic inversion occurred in this syntenic region in one of the two genomes after the divergence of *S. bicolor* and *O. sativa*.

Expression profiling

Progress in characterization of the transcriptome has been paralleled by identification of differential gene expression in response to biotic and abiotic factors, including greenbug feeding (Park et al., 2006), dehydration, high salinity and ABA (Buchanan et al., 2005), and methyl jasmonate, salicylic acid, and aminocyclopropane carboxylic acid treatments (Salzman et al., 2005).

Association genetics

Much of the value of the sorghum sequence may be realized through better understanding of the levels and patterns of diversity in extant germ plasm, which can contribute both to functional analysis of specific sorghum genes and to deterministic improvement of sorghum for specific needs and environments. Sorghum is well suited to association mapping methods because of its medium-range patterns of linkage disequilibrium (Hamblin et al, 2005) and its self-pollinating mating system.

Extensive *ex situ* sorghum germplasm collections exist within the U.S. National Plant Germplasm System and ICRISAT. Early characterization of complementary association genetics panels developed by a group of US scientists and by Subprogram 1 of the Generation Challenge Program, is in progress. At present, more than 750 SSR alleles and 1402 SNP alleles discovered in 3.3Mb of sequence (Hamblin et al., 2005; Casa et al., 2005) are freely available from the *Comparative Grass Genomics Center* relational database. Extensive studies of sequence variation in sorghum show that haplotype diversity is low, even when nucleotide diversity is high: for regions of average length 671 bp surveyed in 17 accessions, the median number of haplotypes was three and the mode was two (Hamblin et al, 2005). Common sequence variation can therefore be captured in a small sample of accessions.

Genetic mapping

Linkage mapping in sorghum takes advantage of its straightforward diploid genetics, amenability to inbreeding, high levels of DNA polymorphism between *Sorghum* species, and manageable levels of DNA polymorphism within *S. bicolor*. High-density reference maps of one intraspecific *S. bicolor* (Kleinet al., 2000; Menz et al., 2002) and one interspecific *S. bicolor* x *S. propinquum* (Bowers et al., 2003) cross provide about 2600 sequence-tagged-sites

(based on low-copy probes that have been sequenced), 2454 AFLPs, and 1375 sequence-scanned based on sequences of genetically anchored BAC clones) loci. These two maps share one common parent (*S. bicolor* "BTx623") and are essentially collinear (Feltus et al, 2006). Cytological characterization of the individual sorghum chromosomes has provided a generally adopted numbering system (Kim et al, 2005). More than 800 markers mapped in sorghum are derived from other taxa (hence serve as comparative anchors) and additional sorghum markers have been mapped directly in other taxa, or can be plotted based on sequence similarity. Anchoring of the sorghum maps to those of rice, maize (Bowers et al., 2003) sugarcane (Ming et al, 1998), millet, switchgrass (Missaoui et al, 2005), bermuda grass, and others provides for the cross utilization of results to simultaneously advance knowledge of many important crops.

Physical mapping

Sorghum was the first angiosperm for which a BAC library was published (Woo et al, 1994). Estimates of the physical size of the sorghum genome range from 700 Mbp based on Cot analysis (Peterson et al, 2002) to 772Mbp based on flow cytometry. This makes the sorghum genome about 60% larger than that of rice, but only about 1/4 the size of the genomes of maize or human. Peterson et al, (2002) using DNA renaturation kinetic analysis showed the comparative composition of the sorghum genome.

Genome sequence

The shotgun sequencing of a leading US sorghum inbred, BTx623, is now complete, with □10.5million reads (□8X coverage) deposited in the NCBI Trace Archive. Early analysis confirms that the sorghum genome sequence will be a suitable substrate for a complete and high-quality annotation. Alignments of the preliminary assembly to sorghum methyl-filtered sequence; sorghum, maize, and sugarcane transcript assemblies; and the Arabidopsis and rice proteomes confirms the base-level accuracy of the assembly and correct local structure of protein-coding loci. Additional resources from reduced-representation sequencing will contribute to the identification of expressed portions of the genome sequence. The sorghum gene space is presently represented by approximately 204 000 expressed sequence tags, many of which have been clustered into □22000 unigenes representing more than 20 diverse libraries from several genotypes (Pratt et al, 2005). About 500 000 methyl-filtered (MF) reads that provide an estimated 1X coverage of the MF-estimated gene space (Bedell et al, 2005) have been assembled into contigs (SAMIs, http://magi.plantgenomics.iastate.edu).

LIGNOCELLULOSIC FEEDSTOCKS (WOODY & HERBACEOUS)

Eucalyptus spp.

Introduction

Native to the Australian continent and its northern neighbours, Eucalyptus is the most widely planted hardwood tree in the world and constitutes one of the world's main sources of biomass. Its versatility and rapid annual growth are still being explored in plantations already estimated at 19 million ha, spread over 37 countries and accounting for 16% of forest plantation areas worldwide (Carbonnier, 2004). India is the largest planter (8 million ha), followed by Brazil (3 million ha) (Junghans et al., 2003), while in Australia and particularly Tasmania, there has also been a move towards growing Eucalyptus in plantations in addition to the 41 million ha of natural Eucalyptus forest (Junghans et al., 2003). Amongst 700 Eucalyptus species, while *E. grandis* is the most widely cultivated species in subtropical and warm temperate regions, *E. camaldulensis* is the most common species in arid and semi-arid lands and *E. globulus* is the main species in temperate climates free of severe frosts. Elite clones are mainly used in Brazil by the cellulose and paper industry because of wood quality and high volume yield. Mature trees also provide strong and durable timber which is the major use of Eucalyptus, along with fuel supply, in countries such as India. The name *eucalyptus* comes from the Greek: ευκάλυπτος, *eukályptos*, meaning "well covered", or "beautiful bark" according to linguist Ahmed Seddik.

Eucalyptus has attracted attention from global development researchers and environmentalists. It is a fast-growing source of wood, its oil can be used for cleaning and functions as a natural insecticide, and it is sometimes used to drain swamps and thereby reduce the risk of malaria. Outside their natural ranges, eucalypts are lauded for their beneficial economic impact on poor populations and derided for being invasive water-suckers, leading to controversy over their total impact.

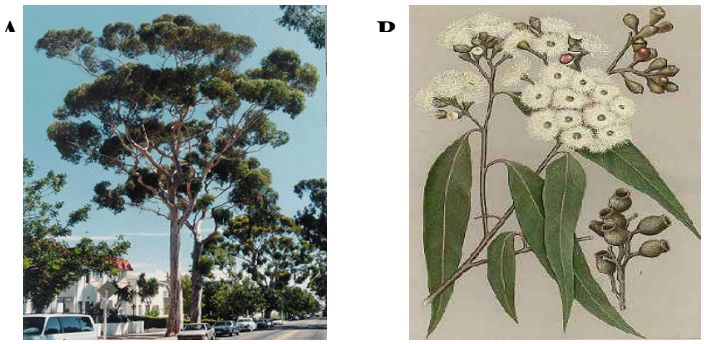

Figure 1. A) A mature *Eucalyptus* tree; B) Flowers and seeds of *Eucalyptus maculata* Hook

Biotechnology approaches
Genome research

Eucalypts are diploid plants with a haploid chromosome number of 11. The size of the genome has been estimated for several eucalypt species and their hybrids to range from 370 to 700 million Mbps.

The nuclear genome of eucalypts has been represented by maps generated through linkage analysis of DNA based polymorphic markers (Shepherd & Jones, 2005). These markers include microsatellites or simple sequence repeats (SSRs), RAPDs, AFLPs and RFLPs ((Thamarus et al., 2002). Some of these markers are dominant, such as RAPDs and AFLPs. Microsatellites and RFLPs on the other hand are codominant markers and are generally more informative as they are multiallelic. Although dominant markers are relatively cheap they have limited transferability, whereas codominant markers have a much broader transferability and are potentially more informative in crosses with up to four alleles segregating such as outcross F2 and pseudo-testcrosses (Bundock et al., 2000).

Organelle genome structure and inheritance has also been investigated in eucalypts. Studies have found the chloroplast to be maternally inherited in eucalypts (McKinnon et al., 2001b). By comparing the *E. globulus* chloroplast genome sequence with that of other sequenced species including *Nicotiana tobacum* and *Oenothera elata*, very high homology was identified in the coding regions between these species and high divergence in the intragenic regions, which were also a source of microsatellites. Eucalypt mitochondria have been less well characterized. It has been reported that the mitochondria are maternally inherited in *E. globules*. Rengel et al. (2009) have reported the sequencing, assembly and annotation of approximately 10,000 ESTs derived from a normalized full-length secondary xylem cDNA library as well as subtractive libraries. The EST assembly generated a set of 3,857 wood-related unigenes including 2,461 contigs (Cg) and 1,396 singletons (Sg) that were named 'EUCAWOOD' creating a valuable resource for functional genomics studies of wood formation and molecular breeding in this economically important genus. About 65% of the EUCAWOOD sequences produced matches with poplar, grapevine, *Arabidopsis* and rice protein sequence databases. The number of sequences has significantly increased (Costa da Cruz et al., 2008; Novaes et al., 2008; Qiu et al., 2008) but is still low in comparison to other forest tree species such as poplar or pine. The major part of this new data set is composed of short sequences whose number is expected to increase dramatically in the future thanks to the development of the high throughput '454' technology (Novaes et al., 2008).

Species	Modified trait	Gene(s) altered	References
E. camaldulensis	Cellulose	cbd, cel1	Shani et al., 2003
E. camaldulensis	Lignin	C4H, CAD, Ntlim1	Chen et al., 2001; Kawaoka et al., 2003; Valerio et al., 2003
E. camaldulensis	Stress resistance	DREB1A	Hibino et al., 2002; Kondo et al., 2002 ; 2003
E. camaldulensis	Salt stress tolerance	cod A	Yamada-Watanabe et al., 2003
E. camaldulensis	Insect/herbicide resistance	cry3A, bar	Harcourt et al., 2000
E. grandis	Cellulose	cbd, cel1	Shani et al., 2003
E. grandis · E. urophylla	Lignin	CAD	Tournier et al., 2003
E. urophylla	Bacterial wilt resistance	cecropin D	Shao et al., 2002

Figure 2: Research involving genetic modification in Eucalyptus species (Poke et al., 2005)

Quantitative Trait Locus Mapping

The construction of a comprehensive microsatellite-based linkage map for commercial species of *Eucalyptus* is now well advanced (Brondani and Grattapaglia, 2002). In the context of Genolyptus project (Grattapaglia, 2004) a target was established to develop and map 1,000 microsatellite markers. Sources for this large number of microsatellites are now becoming available, not only from the enriched library approach, but also from a shotgun genomic library, a large set of ESTs, and from BAC end sequences. The availability of transportable, multiallelic, PCR-based co-dominant microsatellite loci provides a fundamental tool to carry out linkage and quantitative trait locus (QTL) analysis in eucalypts and allows researchers to move from phenotypes to target genomic regions controlling traits of interest.

Transcript abundance, measured for 2,608 genes in the differentiating xylem of a 91 (*E. grandis* X *E. globulus*) X *E. grandis* backcross progeny was correlated with diameter variation, revealing coordinated down-regulation of genes encoding enzymes of the lignin biosynthesis and associated methylation pathways in fast growing individuals (Kirst et al., 2004). Quantitative trait locus (QTL) analysis of transcript levels of lignin-related genes showed that their mRNA abundance is regulated by two genetic loci, demonstrating coordinated genetic control over lignin biosynthesis.

Association mapping

Genetic mapping became accessible to several forest tree species in the beginning of the 90's based on the combination of the speedy and inexpensive generation of dominant RAPD and AFLP markers and the pseudo-testcross strategy in two-generation pedigrees or the use of the haploid genetics of conifers. Concomitant to this development, linkage maps of co-dominant markers led to the construction of integrated RFLP maps for a few species (Devey et al., 1994) and the possibility of comparative mapping (Komulainen et al., 2003; Krutovsky et al., 2004). However it soon became clear that true advancements in QTL validation across pedigrees and eventually marker assisted selection in forest trees, would strongly depend on the

availability of higher throughput, higher polymorphism typing systems such as microsatellites, organized in dense genetic maps (Brondani et al., 1998; Grattapaglia, 2000). In the last few years a number of studies reported genetic maps for forest trees built with combinations of several hundred RAPD and AFLP markers together with some tens of EST, genes and microsatellites (Brondani et al., 2002;Achere et al., 2004). Linkage maps with around one hundred microsatellites were reported for *Pinus taeda* (Zhou et al., 2003) and *Populus* (Yin et al., 2004). However to allow a more precise comparison of QTL position and validation of putative QTL across pedigrees larger sets of microsatellites are clearly necessary.

Using marker-based methods, significant narrow-sense heritability of foliar defense chemicals in a natural population of Eucalyptus melliodora was found (Andrew et al., 2005).

With the rapid advancement of genome projects generating a large amount of sequence information and single nucleotide polymorphism (SNP) (one-letter variations in the DNA sequence that contribute to differences among individuals) data, plant genomics has experienced a growing interest in an alternative approach for the identification of genes underlying quantitative traits. Some laboratories have started association mapping work for wood traits, both in Pines (Brown et al., 2001) and in *Eucalyptus* (Thumma et al., 2005), by sampling trees in the wild or from breeding programs that display contrasting phenotypes for wood quality traits. Külheim et al., (2009) discovered 8,631 SNPs across the species *Eucalyptus globulus*, *E. nitens*, *E. camaldulensis* and *E. loxophleba.*

Another marker-based technique is the one that uses microsatellites for the detection of differences and mapping of Eucalyptus species genetic backgrounds. One hundred thirty seven autosomal microsatellite markers have been published to date for species of *Eucalyptus* and 70 from *E. grandis* and *E. urophylla* (Brondani et al., 2002). Recently, a set of 35 chloroplast DNA microsatellites were developed based on the full cp-DNA sequence of *E. globules*. Bordani et al. (2006) reported the construction of a consensus genetic linkage map covering all 11 linkage groups of *Eucalyptus* including a total 234 mapped loci making it, according to their knowledge, the most complete genetic map of *Eucalyptus* and of a forest tree to date based exclusively on interspecific transferable microsatellites.

EST programmes

Similar to the efforts in poplar, sequencing projects often form part of large genomics programs undertaken by national or international consortia. The biggest on-going joint public and private programs were founded in Brazil, demonstrating the economic importance of Eucalyptus at the state level in this country. The Genolyptus project (http://genolyptus.ucb.br/genolyptus-english.jsp), which includes thirteen companies, seven universities and Embrapa (Brazilian Enterprise for Agricultural Research) is targeting wood quality and disease resistance. Divided into several topics (Grattapaglia, 2004), the research program aims to translate genomics knowledge into improved tree breeding technologies. In

2004, the sequencing project reached about 150,000 ESTs for *E. grandis* (50%), *E. globulus* (16%), *E.urophylla* (10%) and *E. pellita* (10%), the remaining 14%for six other species.

The second Brazilian consortium, FOREST (http://est.cbmeg. unicamp.br/pgl/research/forests.html), is made up of twenty laboratories from San Paulo and four companies. The isolation of 124,000 sequences (12 cDNA libraries) was obtained mainly from *E. grandis*, representing different organs under different growth conditions and related to environmental stress tolerance (Furtado et al., 2004). In addition, OJI Paper has 60,000 ESTs.

Another independent French public genome sequencing program (26,000 ESTs from *E. gunnii*) was also named FOR*EST* (http://www.genoscope.cns.fr). These Eucalyptus sequences were isolated in the framework of the Toulouse University - CNRS research programm, focusingontheregulationof lignin biosynthesis (http://www.smcv.ups-tlse.fr/root/equipes/regulation/equipe en.php) and frost tolerance (http://www.smcv. ups-tlse.fr/root/equipes/stressfroid/ equipe en.php). The sequences are being released on GenBank. In Australia, the CSIRO forestry research programs (http://www.ffp.csiro.au/ tigr/molecular/) led to the isolation of EST collections from *E. grandis* and *E. grandis* X *E. nitens* hybrids. A current research project on wood quality is based on a 5000 cDNA library from Eucalyptus xylem. Moreover, the same research groups are also interested in flowering and cold tolerance (Fullard and Moran, 2003). At the University of Melbourne, 93 sequences of cambial genes from *E. globulus* have been posted in GenBank (accession no AW191301–AW191393) and, from them, 43 could be annotated (Bossinger and Leitch, 2000).

Tissue Culture of Eucalyptus

Conventionally, eucalyptus is propagated through seeds. However, due to segregation of genes, the seed-raised population is highly heterogeneous. Most Eucalyptus can be propagated vegetatively using traditional stem cutting techniques. However, in vitro micropropagation and rooting, followed by transfer to soil, is becoming increasingly popular because of the prospects of rapid genetic gain. These micropropagated plants are not necessarily used in the establishment of plantations, but are useful source material for the development of clonal hedges and hydroponic systems as sources of cuttings. Micropropagation by microcuttings is commonly carried out on *E. camaldulensis*, *E. globulus*, *E. grandis*, *E. nitens*, *E. tereticornis*, *E. urophylla* and *E. gunnil*. In addition to these conventional multiplication procedures, the production of somatic embryos, which renders micropropagation highly cost effective, would be a powerful tool for the establishment of plantations of elite Eucalyptus genotypes (Bandyopadhyay et al., 1999; Bandyopadhyay and Hamill, 2000). Dhawan and Saxena (2004) have been successful in multiplying three species of eucalyptus i.e., *E. tereticornis, E. camaldulensis* and *E. citriodora*.

Hybridization

Molecular genetics can play a major role in understanding hybrid inviability and identifying compatible individuals and in implementing advanced generation hybridization. For example, DNA markers showed that high levels of segregation distortion occur in both a selfed *E. gunnii* x *globulus* F2 (Vaillancourt et al., 1995) and an *E. grandis* x (*E. grandis* x *globulus*) backcross (Myburg et al., 2000).

There is also a clear role for marker-assisted selection (MAS) in advanced generation hybridization strategies (Griffin et al., 2000). Marker assisted backcrossing has great potential to speed the introgression of favourable genes into the recurrent species (Myburg et al., 2000). It is being proposed to aid with the introgression of salt tolerant genes into *E. grandis* and *E. globulus* through hybridization with salt-tolerant selections of *E. camaldulensis* in Australia (Dale et al., 2000).

Populus spp.

Introduction

Populus is a genus of 25–35 species of deciduous flowering plants in the family Salicaceae, native to most of the Northern Hemisphere. English names variously applied to different species include poplar, aspen, and cottonwood. The genus has a large genetic diversity, and can grow from anywhere between 15–50 m tall, with trunks of up to 2.5 m diameter. In addition to their value for wood products, members of the genus Populus (poplars) provide a range of ecological services, including carbon sequestration, bioremediation, nutrient cycling, biofiltration and diverse habitats. They are also widely used model organisms for tree molecular biology and biotechnology. The sequencing of the poplar genome to an approximately 6X depth adds to a long list of important attributes for research. These include facile transformation, vegetative propagation, rapid growth, modest genome size and extensive expressed sequence tags.

Biotechnology approaches

Genome sequencing

The publication of the draft sequence of *Populus* (Tuskan et al., 2006) based on the genotype Nisqually-1 creates a new resource for bioenergy applications in that it contains all the genes to build a tree. Breaking the code requires more work than simply obtaining the DNA sequence itself – the process of decoding the genome sequence is called annotation (Stein, 2001). The challenge in poplar is to identify the various alleles that control trait variation within the species or interspecific pedigree of interest, since each gene may have many alleles within each species.

Genes that condition plant responses to the hormones auxin and cytokinin, known to have dramatic effects on tree growth, development and architecture, have also been annotated

(Kalluri et al., 2007; Ramirez-Carvajal et al., 2008). There is also the annotation of gene families that belong to mitogen-activated kinase family (Nicole et al., 2006), to the heat shock protein family (Waters et al, 2008) and to the LIM protein family (Arnaud et al., 2007).

QTLs

Most bioenergy-related traits are quantitative, meaning that they are typically either oligogenic (few to several loci regulate variation in the trait) or polygenic (many loci are involved) with a measurable effect of the environment on trait expression. Quantitative trait loci (QTL) associated with variation in these traits can be identified using QTL mapping. When QTL are identified in a well-designed study, it reinforces that the trait is heritable, and simultaneously identifies a genomic region that contains the gene(s) involved.

QTL for stem height, stem circumference, stem volume, number of sylleptic branches and total aboveground biomass were recently identified (Rae et al., 2008) and these data complement earlier studies that identified QTL for aboveground, belowground, leaf, stem and coarse root biomass, as well as the proportion of biomass allocated aboveground to leaves and to stems, and allocated belowground, and belowground specifically to coarse roots, and into fine roots (Wullschleger et al., 2005). In a long-lived species such as poplar, it is also essential to understand how biomass production changes with maturity (or in the case of SRC, the individual stools and the entire stand) with understanding being required at the genetic and physiological/morphological level. A number of studies have reported QTL in the population used for this study at a single time point, for single stem plants usually during early phases of growth. More recently, a QTL mapping identified regions of genetic control for biomass yield in poplar (Rae et al., 2009).

EST programmes

Poplars deploy an array of combined defense strategies against herbivores that can be grouped as chemical and physical defenses, direct and indirect defenses, constitutive and induced defenses, as well as local and systemic defenses. Several recent studies have been conducted on the molecular mechanisms underlying inducible defenses against herbivores in poplar (Major and Constabel, 2006; Miranda et al., 2007). Another study was the gene expression analysis in autumn leaves of poplar, in which Bhalerao et al (2003) preparing cDNA libraries and obtaining ESTs, identified genes that are involved only in the leaf senescence.

The poplar EST database from Umea Plant Science Centre (Umea, Sweden) was used by Ferreira et al. (2006) in order to contribute to the knowledge of the molecular mechanisms underlying Euphrates poplar tolerance/resistance to high temperatures. In September 2004, opened to the public a Populus expressed sequence tag (EST) database (POPULUS DB) which was created from 19 cDNA libraries each originating from different Populus tree tissues (Sterky et al., 2004). The database consists of 102,019 ESTs, assembled into a unigene set of 11,885 clusters and 12,759 non-clustered singletons corresponding altogether to 24,644 unique

sequences or transcripts (POPULUSDB). Moreau et al (2004) used this dataset for in silico transcript profiling of a particular process in the woody tissues of the Populus stem: the programmed death of xylem fibers.

As part of the poplar genome sequencing project and the development of genomic resources for poplar, Ralph et al (2008), generated a full-length (FL)-cDNA collection using RNA from xylem, phloem and cambium, and green shoot tips and leaves from the *P. trichocarpa* Nisqually-1 genotype, as well as insect-attacked leaves of the *P. trichocarpa* X *P. deltoides* hybrid.

ForestTreeDB is intended as a resource that centralizes large-scale expressed sequence tag (EST) sequencing results from several tree species (http://foresttree.org/ftdb) (Pavy et al, 2006). It currently encompasses 344878 quality sequences from 68 libraries from diverse organs of conifer and hybrid poplar trees, and aims to be continuously enriched. In an earlier study, around 14,000 expressed sequence tags (ESTs) representing genes involved in abiotic stress responses from several normalized and subtracted cDNA libraries produced from control, stress- exposed ,and desert-grown *P.euphratica* trees were sequenced (Brosche et al., 2005). A microarray with a unigene set of 6,340 ESTs enriched in stress-related genes was constructed (Brosche et al., 2005) and used in the characterization of the transcriptional responses to gradual soil water depletion (Bogeat-Triboulot et al., 2007).

Microarrays

Transcript profiling using microarrays has made possible to test the potential involvement of thousands of genes in a biological process, thus providing valuable information for the selection of target genes. This technology has already generated important data on gene expression profiles during the transdifferentiation of mesophyll cells into xylem cells in *Zinnia elegans* and identified candidate genes involved in xylem formation in hybrid aspen (*Populus tremula* x *tremuloides*) (Hertzberg et al., 2001). Schrader et al. (2004) have reported a high-resolution transcript profile across the cambial zone of aspen (*Populus tremula*) for more than 13,000 genes. Ninety-five publicly available DNA microarray datasets were obtained from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/) by Ogata et al., (2009). They performed a co-expression network analysis using the datasets and extracted “co-expression modules,” comprising co-expressed genes, which are tightly interconnected to each other. Information on the experiments contributing to gene-to-gene connections in co-expression modules was associated with the modules. Their database is available at http://webs2.kazusa.or.jp/kagiana/cop/.

Transformation system

A very widely used transformation method to produce transgenic poplar plants is that of the *Agrobacterium tumefaciens* transformation method (Arisi et al., 1997; Noctor et al., 1998). New advances have been achived in plastid transformation. In most angiosperm plant

species, plastid genes are maternally inherited, and therefore, transgenes in these plastids are not disseminated by pollen. This makes plastid transformation a valuable tool for the creation and cultivation of genetically modified plants that are biologically contained, thus posing lower environmental risks (Daniell, 2007). Okumura et al. (2006) reported an efficient chloroplast transformation method by bombarding poplar leaves with 0.6-mm gold particles. However, the most recent transformation technique that has been reported is the one of Liu et al (2009) in which they succeeded the transformation of whole poplar plants by exposing them hydroponically to 3, 3', 4, 4'-tetrachlorobiphenyl (CB77).

Characteristics of interest

Over the past few years, there have been several reports regarding the study of poplar's lignin content and its metabolic pathways (Poke et al., 2006; Leple et al., 2007).

Many characteristics of growth have also been studied in poplar. Transgenic manipulation of poplar growth was accomplished by enhancing biosynthesis of the phytohormone gibberellin through overexpression of the gene encoding GA 20-oxidase (Eriksson et al., 2000) and more recently, genes were identified that play crucial role in the perception of light and the regulation of the circadian clock (Loivamki et al, 2007).

Populus species (cottonwoods, poplars, and aspens, hereafter referred to collectively as poplar) are often ecological foundation species and include the most widely distributed trees in the Northern Hemisphere. The phenolic metabolites produced by poplar are thought to be important determinants of community structure and ecosystem dynamics (Whitham et al., 2006). Poplar leaves typically accumulate several classes of phenolic metabolites, including the salicylate-derived phenolic glycosides (PGs), flavonoids such as flavonol glycosides, anthocyanins, and proanthocyanidins (PAs; or condensed tannins), and numerous small phenolic acids and their esters (Lindroth and Hwang, 1996). In the literature, there are many reports available on the study of flavonoid and PA biosynthetic pathways genes (Mellway et al., 2009; Wilkins et al., 2009).

Cynara cardanculus L.

Introduction

Cynara cardunculus L. is a diploid (2n=34) out crossing perennial species native to the Mediterranean basin. It belongs to the Asteraceae family and includes the globe artichoke (var. *scolymus* L.), the cultivated cardoon (var. *altilis* DC), and the wild cardoon (var. *sylvestris* (Lamk) Fiori). Molecular, cytogenetic and isozyme evidence suggests that *Cynara cardunculus* var. s*ylvestris* is the wild ancestor of the globe artichoke and cultivated cardoon (Rottenberg A and Zohary, 1996; Raccuia et al., 2004). While the former is vegetatively propagated, the latter is propagated by seeds. The existence of this complex primary genepool, containingthe wild prog enitor and the two crops showing different reproductive strategies, is unique among crop

species, making the study of genepools and germplasm differentiation particularly interesting for the utilization of Cynara genetic resources (Pagnota and Noorani, 2010). The crop remains of regional importance in Spain, Italy, Greece and the south of France, where it is used in traditional dishes. In spite of its old origin and its good flavour the cardoon has never become a widespread crop. For instance in Spain, that is one of the countries that most cultivated cardoon, has only 1000 ha (96% irrigated lands) for this crop. Named varieties are difficult to find outside the Mediterranean region. Wild and cultivated forms of *C. cardunculus* are allogamous and perennial.

Figure 1. *Cynara cardunculus* L.

Biotechnology approaches

Genome mapping

The genome research of *Cynara cardunculus* L, unlike other species belonging to Asteraceae (Compositae) family (i.e. sunflower, lettuce and chicory), is far behind. The species is highly heterozygous and suffers marked inbreeding depression when forced to self-fertilize.

Artichoke breeding programs have traditionally aimed at improving earliness, head yield and quality, resistance to disease (such as Verticillium) and nematodes (Arce et al., 2004; Miguel et all., 2004; Esteva et al., 2004). Breeding programmes have been based on intraclonal selection (Pècaut 1983; Mauromicale and Copani 1989) or hybridization among varietal groups followed by selection (Baznisky and Zohary, 1994). Therefore, efforts have been made in cynara genome research, especially development of genomic resources and tools for basic and applied genetics, genomics, and breeding research. These resources and tools include different types of DNA markers such as randomly amplified polymorphic DNA (RAPD) (Lanteri et al., 2001; Sonnante et al., 2002), amplified fragment length polymorphism (AFLP) (Lanteri et al., 2004b, Portis et al., 2005a; 2005b) and simple sequence repeat (SSR) or microsatellites (Acquadro et al., 2005a; 2005b, 2009).

Recently, large-scale sequencing work *in Cynara cardunuculus* has been generating about 33M of pair-end 75bp sequences corresponding to 2,500 Mbp (2.3X genome coverage of artichoke) (Sonnante et al., 2011) and a collection of 36,321 ESTs, generated from the 'Green Globe' variety of *C. cardunculus* var. *scolymus,* as part of the output of the Compositae Genome Project ("http://compgenomics.ucdavis.edu") (Scagglione et al., 2009). The

cataloguing of these sequences in public databases is providing useful information to develop markers with high resolving power, such as SNPs thus initiating an era of *in silico* cynara marker discovery.

Molecular markers

In order to understand the function of specific genes and their role in metabolic pathways, as also to identify the key steps in their coregulation mechanisms, several approaches have been exploited. The identification of the genetic basis of metabolite variation in *A. thaliana* has been pioneered by Keurentjes et al. (2006), by applying quantitative trait loci (QTL) analyses on a large metabolomics data set. However, the *C. cardunculus* genome is still poorly mapped. In order to move to a crossing strategy for breeding, a greater knowledge of globe artichoke genome will be essential.

There has been reported the isolation and characterization of a gene involved in involved in both chlorogenic acid and lignin biosynthesis (Moglia et al., 2009) and an enzyme involved in the phenylpropanoid pathway (De Paolis et al., 2008). Moreover, gene sequences encoding hydroxycinnamoyltransferase (HCT and HQT), involved in the synthesis of chlorogenic acid, have been recently identified, characterized and incorporated within the developing globe artichoke linkage maps (Comino et al., 2009).

Comperative proteomics

Although molecular markers have been developed and applied to produce a genetic map the *Cynara cardunculus* genome remains poorly researched. The genome is fixed in time, but the proteome is very plastic, depending on tissue type, developmental stage and age, and is also strongly modulated by the environment. The differential response of related proteomes to the same set of biotic and abiotic factors allows the genetic mapping of expressed genes (Thiellement et al., 2002). A proteomic analysis compares gene products involved across the full range of physiological processes, and illustrates the dynamic nature of cell/tissue processes (Rajjou et al., 2006). The first leaf proteome analysis for globe artichoke has been performed (Acquadro et al., 2009) and mass spectrometry- (MS-) was used for the identification of selected protein spots. Comparison of global protein expression profiles of green and etiolated fleshy stalks of cultivated cardoon indicated that, during etiolation, the differentially expressed proteins were involved mainly in starch metabolism and stress response (Guarino et al., 2010).

Tissue culture

Generally, globe artichoke is propagated vegetatively by offshoots, stumps or dried shoots harvested from commercial fields at the end of the production cycle; however, the potential for the spread of pest (nematodes, fungi and viruses) using the current propagation technique is very high leading to significant economic losses. It has been demonstrated that plants obtained with the meristem tip culture technique shown improved field performance with

respect to both qualitative and quantitative traits, and this can compensate for the higher cost of the planting material (Saccardo et al., 2007).

The availability of an efficient protocol for the in vitro production of haploid plants and subsequent diploidisation would greatly speed the development of the homozygous material needed for F1 hybrid breeding (Lanteri and Portis, 2008). The first reported attempts to culture anthers from five Italian cultivars resulted only in the production of callus (Motzo and Deidda, 1993). Although microspores can now be reproducibly cultured, development beyond the second division has not yet been attained, presumably because of non-optimal culture conditions (Stamigna et al., 2004). Haploid production via gynogenesis has been also been unsuccessful although *in situ* gynogenesis using fertilisation with irradiated pollen has been reported by the INRA station (Lanteri and Portis, 2008). However, this method is at present not sufficiently reproducible for general use (Stamigna et al., 2004).

FIBER CROPS

Cannabis sativa L.

Introduction

Cannabis sativa is an annual plant in the Cannabaceae family. It's one of the oldest known domesticated plants and today is cultivated throughout the world for psychoactive cannabinoids, durable fiber, and nutritious seed. Different parts of the plant have different uses, and different varieties are cultivated in different ways and harvested at different times, depending on the purpose for which it is grown. Cannabis can be separated into psychoactive and nonpsychoactive cultivars according to the ratio of D9-tetrahydrocannabinol (THC,) the primary psychoactive agent, and cannabidiol (CBD) (Hillig and Mahlberg, 2004).

Figure 1*:* *Cannabis sativa L.* Plant parts and seeds.

Hemp is a dioecious, herbaceous annual plant with a four to six month growing season. Dioecy, by definition, means that pistillate (female) and staminate (male) flowers are presented on separate plants. Marijuana can be propagated in two ways: by seed or by cloning. Seeds are a result of sexual reproduction between a pistillate and staminate plant and produce new individuals with recombinant genotypes. Isolated female plants will produce prolific floral buds with a high THC content. Cloning is a form of asexual reproduction that allows for preservation of the genotype due tolack of meiotic recombination. This form of propagation is desirable to the grower because it perpetuates the unique characteristics of the parent plant. It also generates a population of nearly identical, all-pistillate, fast-growing and evenly maturing Cannabis plants. To propagate marijuana by cloning, a cutting is removed from the parent plant and induced to form a new root system. Root systems typically develop in three to six weeks and the clones are then ready to be transplanted into larger containers. Plant development can be accelerated by supplying excess nutrients, carbon dioxide and light. With a sudden shift from twenty-four hour daylight to a twelve hour light regime to mimic autumn conditions, marijuana plants can be forced to flower before they are eight weeks of age.

Biotechnology approaches

Inheritance of traits

The class of secondary products unique to the dioecious species *Cannabis sativa L.* (hemp) is the terpenophenolic substances known as cannabinoids, which accumulate mainly in the glandular trichomes of the plant (Hammond and Mahleberg, 1977). Over 60 cannabinoids are known (de Zeeuw et al., 1972a), the most abundant being cannabidiol (CBD) and D-9-tetrahydrocannabinol (THC). Small and Beckstead (1973) were the first to systematically survey a wide number of Cannabis accessions for variability in cannabinoid composition. De Meijer et al (1992), in a survey of large Cannabis collection, also found that plants belonging to the same population often show distinct CBD/THC ratios.

DNA markers

Today, the concept of Cannabis as a monotypic genus is widely accepted; taxonomical, morphological and biometrical studies confirm the continuity of its gene pool despite the extremely high variation found within and between populations. In the last few years, the existence of just a single species within the genus has been confirmed by molecular marker studies that show a limited segregation of the different groups within the genus Cannabis and an extremely high degree of polymorphism, estimated to be of the same magnitude within and between populations (Faeti et al., 1996; Forapani et al., 2001). Within the dioecious populations, the presence of a high number of male- specific markers, presumably associated with the Y chromosome, was found by RAPD and amplified fragment length polymorphism (AFLPs) analysis (Mandolino et al., 2004; Flachowsky et al., 2001). Alghanim and Almirall

(2003) developed 11 microsatellite markers that found to be useful for DNA typing and for assessing genetic relatedness in *Cannabis*.

Improvement of bio-components of interest

Hemp is thought to be likely the first plant cultivated by mankind for its textile use (Lu and Clarke, 1995). The methods today for modifcation of hemp fibers, in order to make them finer, cleaner, softer and more suitable for processing on machines of higher efficiency than traditional hemp machines, are chemical, chemomechanical and mechanical methods. One recently advanced method is the modification of hemp fibers with sodium hydroxide solutions under different conditions, in order to partially extract lignin, pectins and hemicelluloses, and separate the fiber bundles (Kostic et al, 2008). The quality of modified hemp fibers can be characterised by determining their chemical composition, fineness, mechanical and sorption properties.

Distinguishing Hemp from Marijuana

Currently available methods of analysis for THC in Cannabis leaf material are high-performance liquid chromatography (HPLC) with UV detection (Rustichelli et al., 1998), gas chromatography with flame-ionization detection (GC–FID) (de Meijer et al., 1992), and a screening method based on HPLC. These methods are used for purposes like the detection of higher than the maximum allowed concentration of the psychoactive cannabinoid, Δ9-tetrahydrocannabinol, in industrial fiber hemp. Additionally, Hewavitharana et al. (2005) developed a new method based on mass spectrometry (MS) by which the total THC concentration can be determined accurately leaving outside closely or co-eluting compounds from the sample.

Hibiscus cannabinus L.

Introduction

Kenaf (*Hibiscus cannabinus* L., *Malvaceae*) is a warm season annual fiber crop closely related to cotton (*Gossypium hirsutum* L., Malvaceae) and okra (*Abelmoschus esculentus* L., *Malvaceae*) that can be successfully produced in a large portion of the United States, particularly in the southern states. As the commercial use of kenaf continues to diversify from its historical role as a cordage crop (rope, twine, and sackcloth) to its various new applications including paper products, building materials, absorbents, and livestock feed, choices within the decision matrix will continue to increase and involve issues ranging from basic agricultural production methods to marketing of kenaf products. These management decisions will require an understanding of the many different facets of kenaf production as a fiber, feed, and seed crop.

Figure 2. Leaves and plants of kenaf cultivars 'Everglades 41' (top and left) and 'Tainung #2' (bottom and right).

Biotechnology approaches
Transformation techniques

A very efficient method for transformation of kenaf plants using the *Agrobacterium tumefaciens* has been developed (Banks et al., 1993). More recently, the influence of *Agrobacterium* strain, temperature, host tissue wounding, acetosyringone, v*irG/virE* genes and host cell division on T-DNA expression in the kenaf shoot apex were investigated (Srivatanakul et al., 2001). Besides the conventional method of *Agrobacterium* transformation using callus or cells in tissue culture, efforts have been made for *in planta* transformation of kenaf (Kojima et al., 2004). Reports on protocols for isolation, electrofusion and culture of kenaf protoplasts as an initial step in plant improvement strategies have been made made (Reichert and Liu, 1996). More recently, Liang et al. (2002) developed a protoplast isolation protocol for kenaf leaf tissue from potted plants in order to study replication of *Hibiscus* chlorotic ringspot virus (HCRSV). Another approach used for the transient transformation of kenaf leaves is the biolistic method (Sanford et al., 1993; Liang et al., 2002).

Components of interest

Lignin and cellulose are the high abundant biopolymers in plant cell wall. In the recent years, the demand of vegetable fibers has increased steadily, due to their good mechanical and biodegrability proprieties and due to the increase of biocomposite material production. Kenaf (*Hibiscus cannabinus* L.) is an important herbaceous plant cultivated mainly as source of vegetable fibers. In particular, lignin fibers are mainly present in the stem cortical external part (bark); the internal part (core) furnishes light and absorbent wood due to its high content of cellulose. Research groups have been focused on the study of major genes involved in the lignin and cellulose biosynthesis pathways (Ruotolo et al., 2007)**.** In addition to the studies

made for kenaf cell wal, the chemical composition of the essential oil of kenaf (*Hibiscus cannabinus*) has also been examined (Kobaisy et al., 2001).

Linum usitatissimum L.

Introduction

Flax (*Linum usitatissimum*), also known as linseed, is a member of the genus *Linum* in the family *Linaceae*. It is native to the region extending from the eastern Mediterranean to India and was probably first domesticated in the Fertile Crescent. Flax was extensively cultivated in ancient Ethiopia and ancient Egypt. In a prehistoric cave in the Republic of Georgia dyed flax fibers have been found that date to 34,000BC. *L. usitassimum* is an economically important crop that is grown either for its fiber (fiber flax) or for its oil (oilseed flax). Both flax and linseed are specialised developments of a single species, which originates from the Mediterranean and Southwest Asian regions as mentioned above.

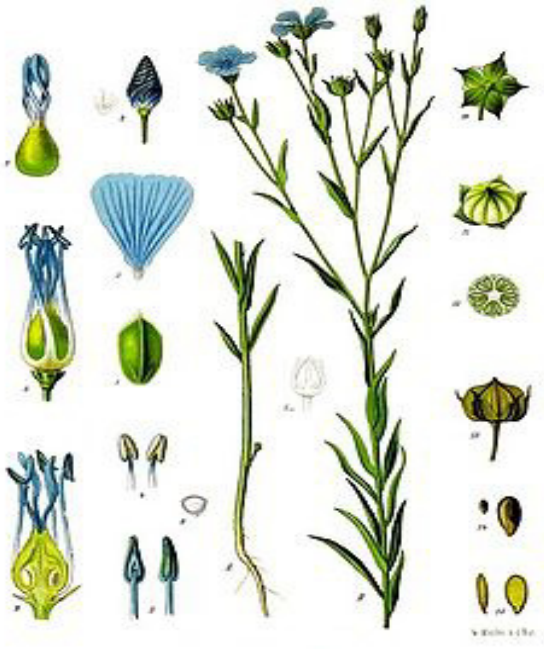

Figure 1. Organs of the flax plant

The terms flax and linseed are often used interchangeably, in North America 'flaxseed' describes flax of a human edible form, and 'linseed' describes flax used for industrial oils. For a useful description of the composition of flaxseed, see http://www.flaxcouncil.ca/FlaxPrimer_Chptr1.pdf.

Biotechnology approaches

Tissue and organ culture

The first report of the species capacity to initiate buds from decapitated hypocotyl sections was made by Link and Eggera (1946). Interestingly, an *in vitro* system was employed to study a pathogen as early as 1957 when the production of aerial-mycelium and uredospores by *Melampsora lini* (Pers) H. Lev. on flax leaves in tissue culture was reported by Turel and Ledingham (1957). Work on improving and optimizing culture medium for this species was reported by Ibrahim (1971) and this work was followed up by a study on biochemical differentiation and phenolic compounds from flax tissue cultures by Liau and Ibrahim (1973). Tissue culture, protoplasts and morphogenes have widened the scope of applications in this species (Gamborg and Shyluk, 1976). This species can be induced to regenerate shoots and roots readily, and though some considerable genotypic variation has been observed, this species can be classed as largely amenable to *in vitro* regeneration.

Flax embryos were one of the first embryos cultivated *in vitro*. Later, the experiments of Erdelska (Erdelska et al., 1973) and Pretova (1986) described flax embryo development *in vitro* from the globular stage onwards.

Flax was shown to be responsive in explant culture systems to a wide range of growth regulators including thidiazuraon (Bretagne et al., 1994; Jain and Rashid, 2001).

A further elucidation of the genetic basis of in vitro regeneration responses was reported by Bonell and Lassaga (2002). The plasticity of the response of this species has also enabledstudies on the derivation of salt-tolerant lines from in vitro cultures (McHughen and Swartz, 1984) on differential stress tolerance from somaclonal variants (O'connor et al., 1991) and on inducing albino mutations as a tool for genetic analysis and cell biology (Bretagne-Sagnard et al., 1996). An assessment of induction of somaclonal variation was also performed in 1992. A supplementary study on hydroxyl radical formation during in vitro morphogenesis of flax and the effects of free radical chemistry modification on morphogenic and embryogenic response has also been reported (Obert et al., 2004b).

Somatic embryos of flax were first derived from immature zygotic embryos (Pretova and Williams, 1986). Somatic embryogenesis has been further investigated in flax, with a fascinating application of somatic embryogenesis described by Ling and Binding (1992) when this mode of regeneration was described as a means of regeneration from protoplasts. An initial report by Cunha and Ferreira (1996) was followed by a more detailed study by the same authors (Cunha and Fernandes-Ferreira, 1999). In addition, the free sterol content variation during the process of somatic embryogenesis was reported (Cunha and Ferreira, 1997) and this work was extended to the determination of the esterified acids content (Cunha and Ferreira, 2003). Somatic embryos of flax were induced by an indirect method from callus by Tejavathi et al. (2000), but growth progression of the shoot apex was not achieved. Another report was made

by Dedicova et al. (2000) on the regeneration of shoots and embryo-like structures (ELS) from hypocotyl segments. Progress on embryogenesis in flax has been reviewed (Pretova and Obert, 2003) but knowledge regarding factors that affect and control ELS formation in this plant species is still insufficient and often contradictory.

Protoplasts can be isolated from most tissues of flax or linseed. Protoplasts have been widely used in a number of biochemical and physiological studies.

Genome mapping

In the past few years, several types of molecular markers including random amplified polymorphics DNA (RAPDs), restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs) and EST-SSRs have been employed to analyze flax genetic diversity (Adugna et al., 2006; Cloutier, 2009). Sixty three QTLs for eighteen important agronomic traits have been recently identified (Vromans, 2006), providing new targets for manipulation using biotechnology.

Transformation techniques

The first reports of the successful gene transfer by *Agrobacterium* into flax were relatively quickly after the original breakthrough in tobacco as early as 1986. The regeneration of transformed flax shoots via a callus stage has been described (Basiran et al., 1987). Regeneration of flax plants transformed by *Agrobacterium* rhizogenes was reported in 1988. However, the first successful verified transformation of flax using *Agrobacterium tumefaciens* and the consequent production of glyphosate-tolerant plants was described by Jordan and McHughen (1988) and successful uptake of a resistance gene to the herbicide sulphonylurea was reported by McSheffrey et al. (1992) and later an improved transformation procedure was published by Dong and McHughen (1993). However, Mlynarova et al. (1994) published a high efficiency method, used for a number of other studies. Rakousky et al. (1999) developed a transformation system based on the antibiotic selection agent hygromycin.

Though all methods to this date had been based on Agrobacterium-mediated transformation, Ling and Binding (1997) reported regeneration of transgenic plants through direct transformation of protoplasts with PEG and *A. tumefaciens* mediated transformation of plastocytes. Wijayanto and McHughen (1999) reported transformation of flax using particle bombardment and such an approach was adopted for transient expression studies of various seed-specific promoters. More applied transformation targets were reported by Ayliffe et al. (2002) investigating up-regulated genes at rust infection sites.

Oilseed crops

Brassica carinata A. Braun

Introduction

Ethiopian or Abyssinian mustard (*Brassica carinata A. Braun*) is an amphidiploid with one genome from *Brassica nigra* L. Koch and the other from *Brassica oleracea* L. (genome BBCC, 2*n*=34).

With the additional agronomic advantage of its better tolerance to semi-arid conditions (Malik, 1990), the species has recently gained the interest of researchers in Canada and Spain (Raney et al., 1995; Velasco et al., 1995a). As appealing as it may be, from an agronomic standpoint, the stigma of low-quality oil from the seed of B. carinata is a legacy of its long-standing mediocrity. The reason for this is its high erucic acid content, which is reported to be in the range of 35±44% (Becker et al., 1999). This is above the level acceptable, from a nutritional stand- point, in canola-quality rapeseed, which has now replaced all the traditional high-erucic acid types in Europe and Canada (Downey, 1990).

Brassica carinata is an adequate oil-bearing crop that is well-adapted to marginal regions (i.e., Andalusia (Spain), which is one of the poorest regions of the EU). Non food cultures in set-aside lands can significantly decrease the enormous amount of subsidies spent for agricultural overproduction in Europe, which leads to an increase in farmer incomes as well as the creation of new employment (Dorado et al., 2004). This crop is drought-resistant and grown in arid regions such as Andalusia.

Figure 1: A mature *Brassica carinata* plant

Biotechnology approaches

Inheritance of traits

Ethiopian mustard possesses a number of agronomic advantages over other oilseed crops. The restricted amount of genetic variability available in natural *B. carinata* for traits like inbuilt resistance to drought, diseases and pests has constrained the breeding programmes aimed at improvement of the crop. Erucic acid is a trait which in high content is undesirable for use of *B. carinata* as a vegetable oil. Although efforts have been made to improve its quality, much has to be done to use natural variations that might exist within the species for fatty acid contents (Alemayehu and Becker, 2001).

A way to develop low erucic acid genotypes is through induced mutagenesis using ethyl methane sulphonate as the mutagen. It's been successful the developing diverse *B. carinata* mutant progenies with reduced erucic acid content, high oleic acid and high oil content which is a significant advance towards the development of Ethiopian mustard lines with canola characteristics (Sheikh et al., 2009).

The change of the properties of *Brassica carinata* can be achieved also through an efficient system of mutagenesis using ultraviolet (UV) light irradiation of isolated microspores (Barro et al., 2002).

Breeding achievements

Microspore culture in combination with induced mutations can speed up breeding programmes, since homozygous doubled haploid (DH) lines exhibiting modifed agronomic traits can be rapidly obtained (Maluszynski et al., 1995). Although microspore embryogenesis has been reported for *B. carinata*, information on differences in embryogenic response between genotypes is scanty. Regeneration has also been reported for cotyledons (Narasimhulu and Chopra, 1988) and hypocotyls. The frequency of plant regeneration from cultured cells and tissues of *B. carinata* has been relatively low in the past. But shoots of *B. carinata* can be regenerated at high frequency (100%) via selection of explants and manipulation of culture medium.

Among the economically important Brassica crops, plants have been produced from protoplasts of *B. oleracaa* (Kao et al., 1990), *B. nigra* (Gupta et al., 1991), *B.juncea* (Kirti and Chopra, 1990), and *B. napus* (Thomzik and Haln 1990). In *B. carinata*, plants have been produced from hypocotyl protoplasts at a moderate frequency (Choung et al., 1987) and from cotyledon protoplasts of *B. carinata* using agarose embedding techniques (Jaiswal et al., 1990). Another approach involves high frequency regeneration from hypocotyl protoplasts of *B. carinata* without agarose embedding for three divergent genotypes (Narasimhulu et al., 1992).

Plant transformation

Plant transformation systems have been developed for all the major Brassica species and also for *B. carinata* opening the way for genetic engineering to obtain transgenic plants with modified agronomic traits. Mutation techniques have been widely used to improve yield, disease and pest resistance in crops.

Although transgenic *B. carinata* plants have been produced at a relatively low frequency (1.5%) through *Agrobacterium*-mediated gene transformation (Narasimhulu et al., 1992), an efficient and reliable genetic transformation methodology for this species has been developed, using cotyledonary petiole and hypocotyl explants from *in vitro* grown seedlings.

Improvement of bio-components of interest

Genetic engineering of plants offers many opportunities for the agrochemical, food processing, and pharmaceutical industries to develop new products and manufacturing processes. There have been engineered *B. carinata* plants to express a gene for the protein hirudin, which is a potent thrombin inhibitor. *B. carinata* is chosen for these studies because of its low frequency of outcrossing, amenability to tissue culture techniques high frequency of multiple shoot regeneration per explant, and its transformation frequency using *Agrobacterium*-mediated gene transfer. When using *B. carinata* for genetic transformation, the co-suppression approach is attractive, because the high transformation rate in this species affords the opportunity to generate the high numbers of transgenic lines necessary to observe a co-suppression event.

Molecular markers

The genus *Brassica* contains a number of species of outstanding agronomical importance and, as a consequence, has received considerable attention in the context of crop improvement by both conventional plant breeding and biotechnological programmes. These approaches often require reliable chromosome identification and karyotypical analysis in order, for example, to assay chromosome variation following in vitro culture and regeneration, to characterise substitution or addition lines, or simply to integrate genetic and physical maps.

The number of 18S-5.8S-25S rDNA loci in *Brassica* diploids (*Brassica nigra, Brassica oleracea* and *Brassica campestris*) and allotetraploids (*Brassica carinata, Brassica juncea* and *Brassica napus*) was first described by the Maluszynska and Heslop-Harrison in 1993. Further investigations described more precisely the genomic distribution of rDNA sites on prometaphase and metaphase chromosomes, and later determined their transcriptional activity.

Brassica napus L.

Introduction

Brassica napus, also known as rape, oilseed rape, rapa, rapeseed and (in the case of one particular group of cultivars) canola, is a bright yellow flowering member of the family Brassicaceae (mustard or cabbage family). The name derives from the Latin for turnip, *rāpum* or *rāpa*, and is first recorded in English at the end of the 14th century. Older writers usually distinguished the turnip and rape by the adjectives *round* and *long* (*-rooted*) respectively.

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Subclass:	Dilleniidae
Order:	Brassicales
Family:	Brassicaceae
Genus:	*Brassica*
Variety:	***B. napus***

Figure 1. *Brassica napus* (rapeseed)

Oilseed rape (rapeseed; *Brassica napus* L., genome AACC, 2*n* = 38) arises from spontaneous hybridization between turnip (*Brassica rapa*) (AA, 2*n* = 20) and cabbage (*Brassica oleracea*) (CC, 2*n* = 18). It is the most important oilseed crop in Europe and the second one over the world after soybean (*Glycine max*).

Biotechnology approaches

Regeneration and transformation techniques

Organogenesis is an indispensable tool for plant regeneration using tissue culture techniques and for plant transformation. Regeneration of plants via organogenesis has been accomplished from various tissues such as cotyledons (Ono et al., 1994), hypocotyls (Yang et

al., 1991), peduncle segments (Eapen and George, 1997), leaves, thin cell layers of epidermal and subepidermal cells (Klimaszewska and Keller, 1985), roots (Xu et al., 1982), and protoplasts (Hu et al., 1999). However, hypocotyl segments remain the most desirable explants for tissue culture and have been used for most Brassica species because of their ability to regenerate.

Somatic embryogenesis, which has been the subject of increasing research in the genus, has become one of the most desired pathways in the regeneration of plants via tissue culture because it bypasses the necessity of time-consuming and costly manipulation of individual explants, which is a problem with organogenesis. Microspores or anthers have been somatic embryogenesis explants of choice in most Brassica species. Somatic embryos have been obtained from hypocotyls, protoplast-derived colonies, and immature cotyledons (Turgut et al., 1998) in *B. napus.*

Protoplast fusion allows the creation of hybrid and cybrid combinations of species that are sexually incompatible, thus facilitating the transfer of genes from a related, but sexually incompatible species, to another without genetic transformation. This technology has allowed not only intrageneric hybridizations, but the production of intergeneric hybrids and cybrids as well. Various desirable traits from the parents have been transferred to the hybrids and cybrids using this technology. One success of protoplast fusion has been the production of disease-resistant hybrids. Somatic hybrids that are resistant to bacterial soft rot have been produced by the fusion of *B. rapa and B. oleracea* protoplasts (Ren et al., 2000). Interspecific hybrids have been produced by fusing mesophyll protoplasts of *B. juncea* and *B. spinescens* (Kirti et al., 1991b). Protoplast fusion between *B. oleracea* and *Moricandia nitens*, a C3–C4 photosynthesis intermediate wild species, resulted in the production of intergeneric hybrids that expressed a gas-exchange character that was intermediate between the two parents.

Molecular markers

Several markers have been used to assess the genetic fidelity of *in vitro*-grown plants such as isozymes, RFLPs and RAPDs. However, these markers are suboptimal for genetic identification, giving way to improved PCR fingerprinting technology. In *B. oleracea* var. botrytis, Leroy et al. (2000) have used inter-simple sequence repeat (ISSR) markers to analyze the genetic stability of somatic embryos derived from hypocotyls. They did not find any polymorphism between different regenerants. However, in cauliflower callus, out of the 224 calluses analyzed, six exhibited original patterns, and in one of these PCR patterns differed at four polymorphic loci. The most frequent primer used for detection of polymorphisms was (CAA) (Leroy et al., 2001).

A better knowledge of genetic determinism of oil content will be relevant for the breeders to control the genetic advance of the crop. By using different segregating rapeseed populations, recent studies reported the identification of numerous QTL (7 to 14 regions per

study) involved in the control of oil content, which is consistent with the polygenic determinism of the trait (Renard et al., 2006). Each of the QTL accounted for less than 10% of the total oil content variance (Renard et al, 2006, Bancroft et al, 2006). Some of these QTL coincided with loci controlling erucic acid content, suggesting that it is a major determinant for oil content in oilseed rape. Additive effects were shown to be the main factors controlling oil content (Renard et al, 2006, Bancroft et al, 2006), with individual additive effect of the different alleles ranging from 0.2 to 1.2%. In addition, strong environmental effects underlie variations in oil content (Turner et al, 2003). Yield-related traits (such as biomass, harvest index, plant architecture, adaptation, resistance to biotic and abiotic constraints) may also indirectly affect yield by affecting the yield-component traits or by other, unknown mechanisms. Increasing evidence suggests that ‘‘fine-mapped’’ quantitative trait loci (QTL) or genes identified as affecting crop yield involve diverse pathways, such as seed number (Burstin et al. 2007; Xie et al. 2008; Xing et al. 2008; Xue et al. 2008), seed weight (Shomura et al. 2008; Wang et al. 2008; Xing et al. 2008; Xue et al. 2008), flowering time (Xie et al. 2008; Xue et al. 2008), plant height (Xie et al. 2008; Xue et al. 2008), branching (Xing et al. 2008), biomass yield (Burstin et al. 2007), resistance and tolerance to biotic and abiotic stresses Warrington et al. 2008), and root architecture (Hochholdinger et al. 2008).

Genetic transformation

Transformation systems have been developed in almost all the economically important species of Brassica such as *B. juncea* (Barfield and Pua, 1991), *B. napus* (Moloney et al., 1989), *B. rapa* (Radke et al., 1992), *B. oleracea* (De Block et al., 1989), *B. nigra* (Gupta et al., 1993), and *B. carinata*. Oil quality improvement has been an important target for Brassica transformation (Liu et al., 2001). Brassica oil is in great global demand and technology is available to custom-tune fatty acid profiles in seeds. Other target traits that were investigated for improvement through genetic transformation were insect resistance (Halfhill et al., 2001), salt tolerance (Pan et al, 2009) and male sterility (Jagannath et al., 2002).

LIST OF GENES OF IMPORTANCE RECENTLY INTRODUCED IN BRASSICA CROPS AND THEIR FUNCTIONS

Species	Gene introduced	Function	Reference
Brassica napus L.	crsI-1	Sulfonylurea resistance	Blackshaw et al., 1994
Brassica napus L.	Bxn	Bromoxynil resistance	Zhong et al., 1997
Brassica napus L.	d12-desaturase	Production of high g-linolenic acid	Liu et al., 2001
Brassica napus L.	Garm FatA1	Increase in enzyme activity towards acyl-acyl carrier protein (ACP)	Facciotti et al., 1999
Brassica napus L.	phbA.phbB.phBc or bktB.phbB.phbC	Production of poly(b-hydroxybutyrate)(PHB)	Houmiel et al., 1999
Brassica napus L.	CrtB	Increase in carotenoid production	Shewmaker et al., 1999
Brassica napus L.	Truncated synthetic Bt Cry1A (c)	Resistance to diamondback moth and cabbage looper	Stewart et al., 1996; Halfhill et al., 2001
B. rapa ssp.	pekinensis Synthetic Bt Cry1c	Resistance to diamondback moth	Cho et al., 2001
B. rapa (syn. B. campestris) ssp. Parachinensis	Synthetic Bt Cry1Ab, Cry1Ac	Resistance to diamondback moth	Xiang et al., 2000
Brassica carinata	OBHIRT (oleosin–hirudin) fusion protein	Production of hirudin	Chaudhary et al., 1998
Rutabaga (B. napobrassica)	Bt Cry1A (c)	Resistance to cabbage caterpillar(Pieris rapas)	Li et al., 1995
Broccoli (B. oleracea L.var. italica)	Bt Cry1A (c)	Resistance to diamondback moth	Metz et al., 1995b; Cao et al., 1999
Brassica oleracea var. capitata (cabbage)	Bt Cry1Ab3	Resistance to diamondback moth larvae	Jin et al., 2000
Brassica juncea	Bacterial CodA	Enhanced salt and cold tolerance	Prasad et al., 2000
Brassica juncea	Barnase	Male sterility	Jagannath et al., 2001

PERENNIAL GRASSES

Miscanthus sp.

Introduction

The genus *Miscanthus* belongs to the tribe *Andropogoneae* in the family *Poaceae* and was first described by Andersson (1855). Is a perennial C4-grass and has its origins in the tropics and subtropics, but different species are found throughout a wide climatic range in East Asia. It is an environmentally benign plant that can be grown at a low level of fertiliser input, especially nitrogen (Lewandowski et al., 2000). *Miscanthus* biomass can be used as solid biofuel e.g. for co-combustion with coal (Wagenaar and Vandenheuvel, 1997), a source of industrial fibre for paper pulp (Cappelletto et al., 2000), insulation material, hard boards and plant potting mixtures and used whole for roof thatching (Kjeldsen et al., 1999).

Figure 1. Different *Miscanthus sp*. ecotypes and reproductive organs

Compared with other C-4 genera, miscanthus is more tolerant to the cool climate of north-west Europe (Beale & Long, 1995). Once established, miscanthus is harvested annually and in Denmark needs a rotation of minimum 10–12 yr in order to depreciate establishment costs (Parsby, 1996). The European investigations during the first decade were almost exclusively conducted with one genotype, the sterile, triploid hybrid *M. x giganteus* (Hodkinson & Renvoize, 2001). In northern Europe *M.* x *giganteus* was difficult to establish and had a rather poor combustion quality because it did not senesce, which delayed leaching of minerals from the crop during winter (Jørgensen, 1997; Venendaal *et al*, 1997). Therefore, the genetic base of miscanthus has been broadened in Europe by collecting and screening existing genotypes and by developing the breeding methods (Deuter & Abraham, 1998).

Biotechnology approaches

Genetic mapping

First molecular studies in *Miscanthus* used isozymes (Von Wuhlish et al. 1994) and AFLPs. The AFLP approach is widely used to analyze the genetic diversity of European species of *Miscanthus.* The technique is an adequate and powerful tool to evaluate genetic

diversification, to analyse the success of hybridizations and to find wrong classifications (Greef et al, 1997).

An "offspring cross" mapping strategy in combination with the random amplified polymorphic DNA (RAPD) assay has been used by Atienza et al (2002) in order to construct the first genetic map of the species *Miscanthus sinensis* ($2n = 2x = 38$). In addition, 17–18S rRNA has been found useful for elucidating the ancient evolutionary history of angiosperm families or flowering plants (Chaw et al. 1997) owing to its conserved nature; and the internal transcribed spacer region (ITS) has been widely used for phylogenetic reconstruction at specific or generic levels (Baldwin 1992). Another way that was studied in order to collect and detect genetic variation in Miscanthus species is through single pollen grain polymerase chain reaction (PCR).

M. sinensis is the donor of two of the three genomes of *M x giganteus*. The Mendelian segregation of five microsatellite loci tested by Hernández et al (2001) illustrates the usefulness of these markers for *Miscanthus* mapping, and the high levels of variability and reproducibility associated with microsatellite markers will allow them to be used as anchor markers between genetic maps of *Miscanthus* and maize.

Breeding achievements

Although vegetative propagation systems like rhizome division or *in vitro* axillary shoot propagation are available, the development of an efficient embryogenic cell culture system in *Miscanthus* is desirable. Embryogenic culture systems have already been established in the triploid hybrid *Miscanthus* x *ogiformis* Honda Giganteus' (Lewandowski and Kahnt, 1993; Holme and Petersen, 1996). With the purpose of improving the callus culture system of *Miscanthus* x *ogiformis* Honda Giganteus',nproline was included in MS and N6 callus induction and suspension culture media. Effects of proline were investigated on embryogenic callus formation, growth of suspension cultures and plant regeneration (Bæksted Holme et al, 1997). Petersen (1997) demonstrated that plant regeneration from *Miscanthus* x *ogiformis* Honda 'Giganteus' could be improved considerably by adding low concentrations of BA during callus induction, compared to previous investigations (Holme and Petersen, 1996).

Panicum virgatum L.

Introduction

Switchgrass (*Panicum virgatum* L.) is a perennial C4 grass propagated by seed that can be established at low cost and risk and requires very low inputs while giving high biomass yields geven on marginal soils. Since the early 1990s the crop has been developed as a model herbaceous energy crop for ethanol and electricity production in the USA and in Canada and it is also being considered as a paper pulp production feedstock. In Europe switchgrass has been introduced as a potential energy crop only recently. Several studies on growth and yield showed

encouraging results, and it is now clear that switchgrass can be cultivated both in North and South Europe. Additionally, economic analysis under different scenarios found switchgrass to be more profitable than other conventional crops e.g. maize or alfalfa. The crop has the potential to play a role in supporting policies to increase the use of durable products, reduce CO2 emissions, utilise marginal and set aside lands and provide new economic activities for rural communities.

It is a self-incompatible and largely cross-pollinated species with and genome constitution varying from diploid to decaploid. Natural populations are broadly classified into two main ecotypes, lowland and upland, based on morphology and natural habitat. Lowland types are usually tetraploid with genetic composition of (2n = 4x = 36) with a DNA content of approximately 3 pg. Most of the upland types are either hexaploid (2n = 6x = 54) or octaploid (2n = 8x = 72) with octaploid DNA content of 5.9 to 6.2 pg 2C–1 (Lu et al., 1998). In a species such as switchgrass there exists a great deal of phenotypic variation derived from latitudinal adaptation across its natural range and local adaptation to soil, temperature, and moisture conditions (Casler et al., 2007). It is still largely undomesticated thus large gains might be realized through fixation of beneficial alleles in breeding populations.

Biotechnology approaches

Hybridization

It has been shown that heterosis exists in switchgrass for agronomic traits including biomass yield (Vogel et al. 2007). Switchgrass is a cross-pollinated species, and cross pollination is enforced by a gametophytic self-compatibility system that is similar to the S-Z incompatibility system found in other Poaceae (Martinez-Reyna and Vogel, 2002). No allelopathy has been reported in switchgrass (Carroll and Somerville, 2009). The existence of gametophytic self-incompatibility in switchgrass may make it possible to develop switchgrass hybrid cultivars using the method illustrated in Figure1. Improvements in tissue culture for clonally propagating plants, such as the node propagation method should make hybrids based on self-incompatibility commercially feasible (Martinez-Reyna and Vogel, 2008).

Genetic transformation

Transformation of switchgrass cells has been obtained by PEG-mediated DNA uptake of protoplasts (Mazarei et al., 2008) and particle bombardment of immature inflorescence-derived embryogenic callus (Richards et al., 2001). Herbicide resistance and visual markers have been used to identify transformants. Switchgrass was reported being genetically transformed via *Agrobacterium* only the past few years. Recently, another transient transformation system the Fast Agro-mediated Seedling Transformation (FAST) has been developed providing a rapid, efficient and economical assay of gene function in intact plants with minimal manual handling and without dedicated device (Li et al., 2009). Actually, transformation projects with the aim to down-regulate genes in the lignin pathway are currently

underway in several grasses including switchgrass (Noble Foundation Press Release;URL:http://www.noble.org/Press_Release/ForageImprovement/BiomassGrant/index.html; USDA., 2006). Recently, it has been reported the successful engineering of switchgrass for the synthesis of polyhydroxybutyrate (PHB) which is high molecular weight polyester (Somleva et al., 2008), demonstrating that this high-yielding biomass crop is amenable to the complex metabolic engineering strategies necessary to produce high-value biomaterials with lignocellulose-derived biofuels.

Genome mapping

ESTs and genomic microsatellites are being developed for switchgrass (Tobias et al., 2008) and should be a good source of molecular markers. 61,585 high-quality ESTs have been generated and seventy-three percent of the assembled consensus sequences could be aligned with the sorghum, indicating a high degree of similarity (Tobias et al., 2008). Chloroplast polymorphisms and random amplified polymorphic DNA (RAPD) markers have been used to evaluate diversity among cultivars and natural populations of switchgrass (Hultquist et al., 1996; Missaoui et al. 2006). Microsatellite markers developed from conserved grass (CG), tall fescue (TF) and switchgrass ESTs were assessed on parents and a subset of this mapping population (Saha et al. 2007). The genetic variability within and among 31 switchgrass populations obtained from Germplasm Resources Information Network (GRIN) has been assessed by EST-SSR markers.

Tissue culture technology

Regeneration from cells or tissues cultured *in vitro* is a fundamental requirement for most applications of plant biotechnology. Tissue culture protocols for both direct and callus-mediated shoot regeneration and somatic embryogenesis are available for switchgrass. Switchgrass plants can be regenerated from mature caryopses, young leaf segments and in vitro-developed inflorescences through somatic embryogenesis and organogenesis (Alexandrova et al., 1996). However, the most effective approach seems to be the nodal culture that is possible to produce approximately 500 plantlets from one parent plant in 12 wk. Advanced regeneration techniques have been recently developed for switchgrass at the University of Tennessee, including production of flowers from tissue (node) culture (McLaughlin and Kszos, 2005).

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