Universidade de São Paulo
Escola Superior de Agricultura "Luiz de Queiroz"

Recursos genéticos florestais: Estratégias, gestão e uso sustentado

Weber Antônio Neves do Amaral

Texto sistemático da obra como parte dos requisitos para o Concurso de Livre-Docente junto ao Departamento de Ciências Florestais, ESALQ, USP

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Dedico

*À minha querida **Beatriz Frias Caruso**, quem sempre esteve ao meu lado durante todos estes anos e durante esta jornada juntos. E sem ela, nada disso teria acontecido.*

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RESUMO

Em momentos tão desafiadores que vivemos, refletir, fazer ciência, dissemina-la e debater sobre a importância da biodiversidade se torna ainda mais relevante. A base da vida na escala dos genes, das espécies e dos ecossistemas e suas interações, nos obriga a pensar na geração de valor capturado ou perdido quando não entendemos os papéis e as funções que a biodiversidade possui para o clima, para a segurança alimentar e para nossa saúde. E em especial os recursos genéticos florestais que são a base desta pirâmide que é representada pela biodiversidade. Estudos fundamentais sobre a auto-ecologia de uma espécie, sua fenologia reprodutiva e seus mecanismos associados ao fluxo gênico são fundamentais para uma própria e adequada gestão do potencial que esses recursos genéticos têm para o presente e para o futuro. Apesar da imensa diversidade entre espécies (diversidade inter-específica) que as florestas tropicais, possuem os trabalhos sobre a diversidade dentro de cada espécie (diversidade intra-específica) não recebem tanta atenção quanto deveriam ter. Além disso a falta de informações sobre a distribuição espacial das espécies florestais, os processos de fragmentação e a exploração de habitats naturais tem afetado os padrões dos fluxos genéticos entre e dentro de fragmentos florestais, dificultando ainda mais que estratégias de conservação in situ possam ser adotadas em larga escala no país. Considerando todos os compromissos do Brasil com a restauração dos ecossistema florestais, com a redução das emissões de gases de efeito estufa e considerando importância de uma transição para uma bioeconomia, a adequada gestão dos recursos genéticos florestais pode contribuir de forma significativa com a construção desta transição, desde que ocorra com visão de longo prazo e tornando acessíveis informações, ferramentas e inovações para uso sustentado deste importante pilar da biodiversidade.

Palavras-chave:
recursos genéticos florestais, biodiversidade, diversidade biológica, marcadores moleculares, melhoramento florestal, sustentabilidade, inovação

ABSTRACT

In such challenging times that we live, reflecting, doing science, disseminating it and debating the importance of biodiversity becomes even more relevant. The basis of life on the scale of genes, species and ecosystems and their interactions, forces us to think about the generation of captured or lost value when we do not understand the roles and functions that biodiversity has for climate, food security and for our health. In particular the forest genetic resources that are the base of this pyramid that is represented by biodiversity. Fundamental studies on the auto-ecology of a species, its reproductive phenology and its mechanisms associated with gene flow are essential for proper and adequate management of the potential that these genetic resources have for the present and for the future. Despite the immense diversity among species (inter-specific diversity) that tropical forests have, works on diversity within each species (intra-specific diversity) do not receive as much attention as they should. In addition, the lack of information on the spatial distribution of forest species, fragmentation processes and the exploitation of natural habitats has affected the patterns of genetic flows between and within forest fragments, making it even more difficult for in situ conservation strategies to be adopted on a large scale in the country. Considering all of Brazil's commitments to restoring forest ecosystems, reducing greenhouse gas emissions (GHGs), and considering the importance of a transition to a bioeconomy, the proper management of forest genetic resources can significantly contribute to the construction of this transition, as long as it takes place with a long-term vision and makes accessible information, tools and innovations for the sustainable use of this important pillar of biodiversity.

Keywords:
forest genetic resources, biodiversity, biological diversity, molecular markers, forest breeding, sustainability, innovation.

ÍNDICE

1 INTRODUÇÃO

Este documento sintetiza grande parte das minhas atividades profissionais, como docente e pesquisador dentro da Universidade de São Paulo e em outras Universidades e organizações de pesquisa que tenho atuado nos últimos 36 anos, desde a minha graduação em Engenharia Florestal, na ESALQ em 1985.

Durante estes 36 anos de experiência profissional, eu apresento neste documento, uma linha do tempo, sintetizando grande parte das minhas atividades profissionais, os projetos de pesquisa, as atividades de extensão e as atividades didáticas, que desenvolvi à luz das organizações às quais tive o privilegio de estar ligado. Dentro deste contexto, este documento se estrutura a partir de **uma linha do tempo** (ou uma linha do meu amadurecimento como profissional) desde o início das minhas atividades como:

i. Um aluno de graduação na ESALQ: o contato com o **método científico**;

ii. Minhas atividades profissionais como pesquisador em empresa florestal (Florin Florestamento Integrado, 1986-1989): **recursos genéticos de *Eucalyptus spp*;**

iii. Minha passagem pela Faculdade de Ciências Agronômicas de Botucatu (UNESP, 1989-1997): **a biologia de sementes e reprodutiva das espécies nativas**;

iv. Minha ida aos Estados Unidos para cursar doutorado na Universidade de Harvard (1993-1998): **uso de ferramentas biotecnológicas para entender florescimento de plantas**;

v. Docente da Universidade de São Paulo (1997, ESALQ): **a montagem do Laboratório e a biologia floral**;

vi. Trabalho desenvolvido junto ao IPGRI (International Plant Genetic Resources Institute) em Roma (2000 a 2002), coordenando o programa global em recursos genéticos florestais: **a visão estratégica sobre recursos genéticos florestais;**

vii. Após esse período, eu retornei ao Brasil e a ESALQ desenvolvendo em paralelo às atividades no Departamento de Ciências Florestais, a coordenação do Pólo Nacional de Biocombustíveis (2004-2007), a pedido do Ministério da Agricultura e Abastecimento (MAPA): **a necessidade de uma estratégia nacional para bioenergia.** Em 2013 recebi uma bolsa para professor visitante da Fulbright Foundation para desenvolver pesquisas sobre bioeconomia junto à Universidade de Nebraska em Lincoln, EUA (20011/12);

viii. A partir de julho de 2014, iniciei atividades de colaboração com o professor Aldo Ometto da Universidade de São Paulo da Escola de Engenharia de São Carlos (EESC) dentro de um convênio firmado entre a USP e a Fundação Ellen MacArthur para desenvolvimento de docentes e projetos de pesquisa dentro e fora da USP no tema da economia circular. A relevância deste tema que

constrói e se utiliza das bases teóricas já firmadas a partir dos conceitos da sustentabilidade (dentro do tripé: Ambiental – Social e Econômico). A economia circular se tornou atualmente em um importante marco teórico sobre novos modelos de negócios, em especial sobre como a inovação pode ser utilizada para desenvolvimento de uma **economia regenerativa e circular, trazendo e refletindo sobre a importância dos sistemas biológicos na produção de bens e serviços essenciais para toda a sociedade**, e portanto, reforçando a importância de um visão de longo prazo, sobre a gestão da biodiversidade e em especial dos recursos genéticos florestais.

ix. Atualmente tenho me dedicado a trazer uma nova visão para a pesquisa e o ensino, com intuito desenvolver projetos e disciplinas que possibilitassem a disseminação de informações, metodologias e técnicas associadas a gestão dos recursos genéticos e a biodiversidade de uma forma mais ampla e mais efetiva para diferentes grupos e profissionais. neste período desenvolvi **novos cursos de graduação especialmente voltados para inovação e empreendedorismo** continuando a orientar os meus alunos de pós-graduação e de graduação em suas atividades.

2. ESALQ – Além da experiência de campo: o método científico

Antes mesmo do meu ingresso na ESALQ, eu já estava engajado com problemas ambientais na cidade onde nasci, Araraquara, SP, escrevendo artigos para jornais e participando ativamente em organização não governamental local[1], que naquele momento ainda não eram conhecidas como ONGs e tampouco tinham a relevância que estas organizações possuem hoje. E lendo avidamente as publicações de domingo no Jornal O Estado de São Paulo sobre árvores que eram publicadas na coluna do Engenheiro Agrônomo do Instituto Agronômico de Campinas (IAC), Hermes Moreira.

Já na ESALQ como aluno de graduação, no 1º ano, após uma palestra feita na disciplina de "Introdução da Engenharia Florestal", feita pelo prof. Paulo Y. Kageyama, eu procurei para solicitar um estágio de iniciação científica. E para minha surpresa ele prontamente aceitou este meu pedido e aí comecei a minha carreira como em um jovem pesquisador da área de sementes e melhoramento florestal.

O meu primeiro trabalho de estágio junto ao professor Paulo Kageyama foi auxiliar uma mestranda Inês Sousa Dias com análises associadas à espécie *Mimosa scabrella* (bracatinga).

Após 9 meses de trabalho com a bracatinga, passei a desenvolver um projeto de iniciação científica em parceria com a Companhia Champion celulose e papel sobre dispersão de sementes de *Eucalyptus grandis* em áreas de produção de sementes no Horto Gigante em Mogi Guaçu.

[1] SEMARA – Sociedade de Ecologia e Meio Ambiente da Regiao de Araraquara, fundada pelo prof. Ariovaldo Dellacqua, da UNESP, Campus de Araraquara.

Por mais de 2 anos acompanhei a fenologia reprodutiva deste experimento que era uma Área de Produção de Sementes (APS) muito relevante para o melhoramento florestal desta espécie, sendo que houve a oportunidade de publicação de 3 artigos sobre iniciação de iniciação científica durante este período de projeto de pesquisa.

Encerrando este projeto de pesquisa obtive uma bolsa do Instituto Florestal, SP para desenvolver um trabalho semelhante em várias Estações Experimentais desta organização sobre biologia reprodutiva e dispersão de sementes em áreas de produção de sementes de várias espécies de *Pinus spp.*

Durante todo o período da minha graduação da ESALQ, além da exposição relevante para a minha formação como profissional, com a experiência de campo, para levantamento de dados primários, e em especial para o entendimento dos processos associados à biologia reprodutiva das espécies com as quais eu trabalhei, foi o contato com o método científico. O uso método científico, em especial na elaboração de hipóteses de pesquisa, no desenho de experimentos, com o cuidado na coleta de dados e com a forma de análise desses dados (o desenho experimental a ser utilizado) e como os dados deveriam ser tratados, analisados e interpretados e traduzidos em um artigo de **iniciação científica**, foram o maior legado para a minha como futuro Engenheiro Florestal. Vide artigos publicados durante este período no meu Memorial que dá suporte a este documento.

3. Recursos genéticos de *Eucalyptus spp*

No início dos anos 80 (a partir de 1980), uma importante iniciativa liderada pela Embrapa, CNPF, Curitiba, com a coordenação do Engenheiro Florestal Roberto Alonso, desenvolveu um intenso programa de coleta um de sementes de múltiplas espécies de *Eucalyptus spp*. na Austrália. Esta iniciativa foi movida por demandas especialmente das empresas florestais que tinham interesse na expansão da base genética dos seus povoamentos florestais, os quais até aquele momento se baseavam em um número reduzido de espécies e de base genética reduzida ou mesmo desconhecida. Esta iniciativa da Embrapa se transformou no maior esforço já realizado no Brasil de coleta de material genético nas populações das diversas espécies de **Eucalyptus spp**. na Austrália que conhecemos até hoje.

Em 1986, começo as minhas atividades profissionais na empresa Florin Florestamento Integrado SA localizado na cidade de Jacareí, São Paulo. Fui contratado como pesquisador da área de melhoramento florestal, e dando início ao próprio departamento de pesquisa desta empresa que havia sido recém-criado com a liderança do engenheiro florestal Antônio José Migliorini.

A empresa já fazia parte da rede de empresas associadas ao Instituto de Pesquisas e Estudos Florestais IPEF – ESALQ, Piracicaba e portanto garantindo o acesso a uma rede de experimentos em *Eucalyptus spp*., a qual era coordenada através do seu programa de melhoramento florestal pelo engenheiro florestal Edson Seizo Mori.

A primeira iniciativa que foi desenvolvida após a minha chegada na empresa foi o mapeamento dos experimentos instalados de eucaliptos pela empresa e o entendimento da necessidade ou não de aumento da base genética destas espécies. a partir da assinatura de um

convênio com a Embrapa para que nós pudéssemos instalar novos experimentos a partir destas coletas que foram realizadas pelo engenheiro Roberto Alonso na Austrália.

O levantamento destes experimentos instalados e especial dos povoamentos comerciais implantados pela empresa indicou uma necessidade de busca de novos materiais genéticos que pudessem ser adaptados as novas condições de expansão da empresa no vale do Paraíba em São Paulo no Rio de Janeiro e nas suas adjacências.

Dentro deste contexto foram instaladas foram instalados mais de 20 experimentos de múltiplas espécies e procedências, distribuídos estrategicamente em todas as regiões de influência dos plantios comerciais desta empresa. Além disso, a instalação desta extensa rede de experimentos possibilitou a empresa estar em contato com outras empresas, em especial ter acesso a uma base genética muito mais extensa que apenas os experimentos instalados nas suas propriedades.

Esta rede de experimentos instalados em várias regiões e com diversos materiais genéticos se estruturava (suportada pelo uma profunda base teórica) como uma rede de múltiplas populações, que deveriam funcionar como populações base para melhoramento. Estas populações base (geralmente a partir multi-procedências) eram conduzidas separadamente. E quando necessário, combinadas com outras de populações multi-procedências. Esta estratégia de múltiplas populações base, eram estudadas e quantificadas para a entrada nos programas de melhoramento florestal e para seleção dos melhores materiais genéticos para programas mais avançados de melhoramento, tais como instalação de pomares sementes por mudas e outras estratégias que se iniciavam, particularmente a clonagem de genótipos superiores.

Era cada vez mais evidente para mim que adequada gestão dos recursos genéticos com uma visão de longo prazo seria fundamental para os planos de crescimento e expansão dos plantios florestais, especialmente para áreas onde os sistemas de manejo e de silvicultura não

estivessem tão bem definidos ou para novas áreas, onde não houvesse experimentos disponíveis para balizamento dos plantios comerciais e sobre quais materiais genéticos deveriam ser utilizados.

Naquele momento das Ciências Florestais, em especial da silvicultura de espécies exóticas, era muito rico e tinha no IPEF um órgão importante para balizamento de pesquisas e em especial da integração/interação universidade - empresa. Aliás parte da tese de livre docência do professor Jacques Marcovitch (FEA, USP, e futuro Reitor da USP) e que teve no IPEF, um dos estudos de caso sobre o pioneirismo dos modelos de integração e portanto da inovação trazida para os modelos que se aproximavam as pesquisas realizadas pela Universidade (ESALQ – USP) com a iniciativa privada.

Um dos projetos dentro deste contexto da integração universidade- empresa foi o desenvolvimento de uma pesquisa específica e em parceria com o Laboratório de Celulose e Papel, coordenado pelo professor Luiz Ernesto Georges Barrichelo sobre a integração da floresta com a indústria. Como deveria se dar o desenho de projetos e experimentos da atividade florestal aliados às demandas da indústria. Neste sentido foi conduzida uma pesquisa por mais de 12 meses sobre caracterização tecnológica da madeira, gerando uma publicação, a qual foi publicado na íntegra em evento técnico da ABTCP, em 1987, em co-autoria com o Engenheiro Florestal Fausto R. de Camargo, além do prof. Barrichelo. Apêndice 1. Vide documento na integra nos documentos anexados e que fazem parte do Memorial Circunstanciado.

4. A biologia de sementes e reprodutiva das espécies nativas

A partir do meu desligamento da empresa florestal e a minha transferência como auxiliar de ensino junto à Faculdade de Ciências Agronômicas da Unesp em Botucatu, quando se iniciava o curso de Engenharia Florestal daquela unidade, passei a ter uma atuação com foco em sementes florestais e com as interações que eram realizadas com o tema do melhoramento florestal com o prof. Edson Seizo Mori, que simultaneamente havia sido contratado para a área de melhoramento florestal daquela Universidade.

Durante este período (1989 a 1993), que desenvolvi as minhas atividades de pesquisa, ensino e extensão em Botucatu, tive a oportunidade de ter interações profissionais com o professor João Nakagawa, responsável pela área de sementes agrícolas e com os professores Norberto da Silva e Mauricio Zanotto da área de genética e melhoramento de espécies agrícolas, os quais foram importantes mentores para o meu crescimento profissional além do próprio professor Paulo Y. Kageyama, que naquele momento havia se transformado o meu orientador de mestrado.

O tema da biologia das sementes florestais, e em especial da biologia reprodutiva das espécies florestais, que são totalmente correlacionados aos aspectos associados à fenologia reprodutiva, ou seja, os períodos nos quais e forma cíclica, as flores e suas estruturas reprodutivas se tornavam disponíveis e em conjunto com os sistemas de cruzamento ou sistemas de acasalamento, conhecidos como alogamia e a autogamia (como sendo os 2 principais extremos dos sistemas de cruzamento das plantas) se transformaram nos temas com os quais me dediquei durante o período que estive atuando na Unesp, FCA.

Durante este período concluir a minha dissertação de mestrado estudando a auto-ecologia de uma importante espécie que ocorre em matas ciliares no estado de São Paulo, *Cytharexylum myrianthum* (pau de viola). Uma das espécies indicadoras de estágios de sucessão inicial de matas ciliares, uma espécie que frutifica abundantemente e tem frutos zoocóricos, especialmente por pássaros de tamanho médio. A espécie escolhida para desenvolvimento deste trabalho de pesquisa para a conclusão do das atividades do mestrado porque além de ser um espécie-chave para áreas ciliares, possuem importantes mecanismos de dormência das sementes (físico-mecânico) no solo, formando um banco de sementes que se torna disponível para germinação após a abertura de clareiras e perturbações dos ambientes naturais.

Foram produzidos diversos trabalhos de pesquisa sobre esta espécie, sendo que um deles, publicado na integra em um importante evento internacional sobre biologia de sementes florestais, no México, na Estação Experimental Los Tuxlas, da Universidade Autonoma do Mexico (UNAM), que era naquele período, um dos mais importantes centros de pesquisa de campo sobre a biologia de espécies florestais (Apêndice 2). E vide publicações na integra produzidas e disponíveis no Memorial Circunstanciado, incluindo artigo publicado em revista científica nacional[2] com dados sobre esta mesma espécie.

Além desta espécie, eu me dediquei ao estudo dos mecanismos associados à biologia das sementes e a dispersão destes propágulos em diversas espécies florestais, tendo como ambiente de estudo os fragmentos florestais presentes na fazenda experimental Edgardia, de propriedade da FCA, UNESP, gerando igualmente outras publicações cientificas e orientações de alunos de graduação.

[2] AMARAL, WEBER A. NEVES DO; ANTIQUEIRA LIA MARIS; O. RITTER, HORBACH; MICHELI A..Frutification and germination ecology of Citharexylum myrianthum Cham (Verbenaceae). **Journal of Biotechnology and Biodiversity** . Vol. 4, N.3: pp. 207-215, August, 2013 ISSN: 2179-4804.

5. Uso de ferramentas biotecnológicas para entender florescimento de plantas

Durante o período do meu doutorado nos Estados Unidos tive a oportunidade de participar de aulas, projetos de pesquisa e interagir com docentes e alunos de pós-graduação e graduação da Universidade de Harvard que efetivamente transformou a minha formação como profissional.

Inicialmente desenvolvi um projeto de doutorado com orientação do prof. Otto T. Solbrig, para estudar os efeitos da fragmentação florestal na diversidade genética de espécies arbóreas, usando fragmentos florestais do Estado do Espírito Santo. Infelizmente devido a problemas de campo e perda das populações a serem estudadas, fui obrigado a mudar totalmente o foco e o escopo do meu projeto de doutorado. O que me obrigou a um novo processo de aprendizado e definição de áreas de estudo e que fizessem sentido para mim como profissional e estive alinhada a minha atuação profissional anterior e o que poderia desenvolver futuramente após o encerramento do meu programa de doutorado.

Nesse contexto de busca por um novo projeto e escopo do meu doutorado, mantive contato com o docente recém-contratado pela Universidade de Harvard, o professor David Baum, que já desenvolvia um trabalho muito alinhado com as atividades de pesquisa que eu realizava anteriormente, ou seja, sobre o entendimento dos mecanismos e processos que controlam florescimento em plantas.

Em comum acordo com o meu orientador de doutorado professor Otto T. Solbrig, eu me transferi para trabalhar no laboratório do professor David Baum, para sequenciar genes que controlavam a expressão dos meristemas florais em plantas-modelo (model species).

A espécie escolhida para clonagem do gene foi *Arabidopsis thaliana*. A escolha desta espécie se deu pelo fato da mesma já ter todo o seu sequenciamento genômico realizado, e de já terem sido identificadas potencialmente 3 genes candidatos que pudessem estar associados a conversão dos meristemas vegetativos em reprodutivos especialmente, particularmente os genes Leafy e Ap1, além de outros precursores do processo de reprodução envolvidos com o desenvolvimento e a ontogenia dos meristemas vegetativos. Porém a grande dificuldade era necessidade de haver um treinamento específico que eu pudesse aprender essas novas ferramentas e essas novas técnicas moleculares.

Dentro deste desafio eu obtive uma bolsa de estudos da NSF (National Science Foundation) para realizar um curso de treinamento em biologia molecular no prestigioso Cold Spring Harbour Labs (NJ, USA), que era naquele momento liderado pelo prêmio Nobel, James Watson que foi um dos descobridores do DNA na década de 60.

Após o aprendizado das técnicas de biologia molecular em especialmente da clonagem de genes houve a possibilidade da clonagem do genes homólogos presentes em *Arabidopsis thaliana* em outras espécies. A espécie (e dentro da mesma família: Brasicaceae) candidata escolhida foi *Janopsidium abulense* e *Janopsidium acaule*. Os resultados foram muito promissores, pois houve a identificação do gene *in-situ* e a confirmação do funcionamento e papeis destes genes na conversão dos meristemas vegetativos em florais, e indicando também que particularmente o gene LEAFY poderia ser utilizado (super expressão gênica) ou pelo seu desligamento, acelerando ou evitando o florescimento de plantas a partir do uso da transgenia. Os resultados obtidos foram confirmados a partir de ensaios de hibridização in situ a partir de análises de microscopia eletrônica e de estudos comparativos entre as 2 espécies escolhidas como modelo para testar a viabilidade do gene LEAFY[3] em estudos sobre controle do florescimento em plantas superiores.

[3] SHU, G.; AMARAL, W.A.N., HILEMAN, L. & BAUM, D.. *LEAFY* and the evolution of rosette-flowwering in violet cress (*Jonopsidium acaule*, Brassicaceae). **American Journal of Botany**. EUA, July, 87 (5): 634-641. 2000

6. A montagem do Laboratório e a biologia floral

Após a conclusão não do meu trabalho de doutorado na universidade de Harvard retornei ao Brasil já como docente da Universidade de São Paulo no campus da ESALQ, no departamento de Ciências Florestais.

Com apoio da Fapesp, consegui a atração de um pós-doutor, Dr. Marcelo Dornellas, que me auxiliou para montagem do meu próprio Laboratório no Departamento e no desenvolvimento de linhas de pesquisa associados ao controle dos mecanismos de reprodução em plantas, a partir do uso de técnicas moleculares. Além deste destas iniciativas estive envolvido em projetos de sequenciamento genômico especialmente da bactéria *Xylella fastidiosa* com outros docentes e pesquisadores da ESALQ.

A colaboração com o Dr. Dornelas se tornou muito produtiva, possibilitando a publicação de artigos em periódicos internacionais[4] e diversos outros produtos dos projetos de pesquisa desenvolvidos em parceria com outros pesquisadores, tais como a professora Adriana Martinelli (CENA, USP). Dentro desta parceria o gene LEAFY foi escolhido também como um gene candidato para comparação dos estudos sobre biologia reprodutiva e controles do florescimento (biologia floral) entre as espécies selecionadas.

Além das atividades de pesquisa junto à Esalq, estruturei um novo curso de graduação e cursos de pós-graduação associados aos temas de melhoramento florestal e uso e conservação da biodiversidade respectivamente, e em colaboração com o professor Paulo Kageyama. Em paralelo organizei 3 Simpósios sobre biologia molecular e uso de marcadores

1. [4] DORNELAS, M.C; AMARAL, W.A.N. do; RODRIGUEZ, A.P.M.. *EgLFY* the *Eucalyptus grandis* homolog of the *Arabidopsis* gene *LEAFY* is expressed in reproductive and vegetative tissues. ***Braz. J. Plant Physiol.*** [online]. 2004, vol.16, n.2, pp.105-114. ISSN 1677-9452.

moleculares para as empresas associadas ao IPEF, visando a capacitação dos profissionais brasileiros nestas ferramentas moleculares.

Como as atividades em desenvolvimento nas Estações Experimentais do LCF eram coordenadas pelo professor Mário Ferreira, não houve naquele momento uma demanda específica que fosse exigir a minha atuação como contribuição e suporte das atividades desenvolvidas para gestão dos recursos genéticos existentes naquelas Estações.

7. A visão estratégica sobre recursos genéticos florestais

Em meados de 2000, recebi um honroso convite para assumir a coordenação do programa global de recursos genéticos florestais do Instituto Internacional de Recursos Genéticos de Plantas (IPGRI) localizado em Roma na Itália, e cuja criação ocorrida nos anos 80, se deu pela elaboração de um tratado internacional sobre recursos genéticos de plantas pela FAO (Organizacao Mundial da Alimentacao, ONU), e da preocupação com a erosão dos recursos genéticos e segurança alimentar, ainda tão presentes nos dias atuais.

A importância do programa que passei a coordenar em 2000, pode ser medida pelos projetos e publicações, que foram desenvolvidos e elaboradas durante o período que me dediquei a esta iniciativa. Foram mais de 20 projetos estruturantes sobre conservação e uso dos recursos genéticos florestais em diversas partes do mundo, me proporcionando uma experiência profissional de pesquisa e de entendimento dos problemas globais associados ao os recursos genéticos que foram únicos.

Como coordenador global deste programa, tive a oportunidade também de contribuir com o aporte de recursos financeiros para diversos projetos no Brasil em especialmente para o Departamento de Ciências Florestais, cujo ponto focal junto ao IPGRI era o professor Paulo Y. Kageyama. Neste projeto especificamente, foram produzidos artigos de pesquisa em periódicos internacionais e um livro (hoje um livro fundamental sobre gestão dos recursos genéticos florestais[5]).

A base do entendimento científico para a conservação dos recursos genéticos de plantas, e em especial das espécies florestais passa pelo entendimento dos padrões de

1. [5] VINCETI, B.; AMARAL, W.; B. MEILLEUR. **Challenges in managing forest genetic resources for livehoods:** Examples from Argentina and Brazil. International Plant Genetic Resources Institute-IPGRI, Rome, Italy:, 2004. 271 p.

distribuição espacial da diversidade entre e dentro populações. E a área da genética que aborda essas questões é chamada de genética de populações.

Em ambientes naturais especialmente os ecossistemas florestais, atualmente sofrendo pressões antrópicas com a redução das áreas cobertas por florestas tropicais e a fragmentação consequentemente destes ecossistemas, faz com que estes fluxos de materiais (genéticos: alelos) sejam interrompidos, obrigando aos tomadores de decisão e aos gestores destes recursos, o desenvolvimento de estratégias para mitigação destes problemas em especial para conservação desses recursos genéticos a longo prazo. Dentro deste contexto desafiador são propostas três estratégias para conservação dos recursos genéticos a primeira delas se baseia na **conservação *in-situ***, ou seja, das populações em seus ambientes naturais de ocorrência a segunda estratégia se baseia na coleta do germoplasma ameaçado ou em risco de ameaças e trazê-los para os ambientes fora da sua ocorrência natural, o que chamamos de **conservação *ex situ***. Já a terceira estratégia combina às 2 alternativas e é chamada de **conservação circa-situ.**

Além do papel de contribuir para uma reflexão sobre essas estratégias, eu tive a possibilidade e a honra de interagir com diversas organizações internacionais, Universidades, Centros de Pesquisa e pesquisadores das diversas partes do mundo para auxiliá-los no desenvolvimento de programas nacionais associados à conservação e ao uso dos recursos genéticos florestais, particularmente das espécies não madeireiras[6] e das espécies em risco de extinção[7].

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2. [6] L.T. HONG; W. AMARAL. Research on rattan genetic resources conservation and use: the perspective and strategy of the International Plant Genetic Resources Institute. **Unasylva**. v.52 (205), p.52.2001/2002.

[7] W. CHOUMANE; P. van BREUGEL; T.O.M. BAZUIN; M. BAUM; G.W. AYAD; W. AMARAL. Genetic Diversity of *Pinus Brutia in Syria* as Revealed by DNA Markers. **Forest Genetics.** 2004. Vol. 11, no. 2, pp. 87-101

8. A necessidade de uma estratégia nacional para bioenergia e recursos genéticos florestais

Em 2004, após ter retornado ao Brasil por alguns anos fui convidado pelo Ministério da Agricultura e Abastecimento e em parceria com a ESALQ (gestão do Prof. José Postali Parra e Raul Machado Neto), para liderar o recém-criado Pólo Nacional de Biocombustíveis um *think tank* para pensar e desenhar estratégias sobre biocombustíveis e bioenergia para o Brasil.

Além das atividades de pesquisa e ensino que estava envolvido na ESALQ, ao assumir o Pólo Nacional de Biocombustíveis, pude contribuir para o desenho de uma estratégia nacional para a oferta de matérias-primas com foco em bioenergia e biocombustíveis, a partir de fontes ligno-celulósicos, provenientes de plantações florestais, cana-de-açúcar e de resíduos agrícolas e florestais.

Em um país da mega biodiversidade como o Brasil, a falta de uma estratégia que possibilitasse o levantamento e a identificação de oportunidades para agregação e geração de valor destas matérias-primas ligno-celulósicas não fazia sentido. Além disso, o mundo (visao eurocêntrica e de criação de barreiras técnicas para o Brasil para a exportação de produtos agricolas e florestais), estava buscando vilões que mostrassem que havia uma competição entre a oferta de alimentos e o uso de fontes de matérias-primas voltadas para a bioenergia e ou biocombustíveis[8].

Dentro deste contexto foi desenvolvida uma parceria com a Universidade de Wageningen da Holanda, liderada pelo professor Peter Zubier para estruturação de projetos de

1. [8] AMARAL, W. A. N. & PEDUTO, A. Food Security: The Brazilian Case. 2010. 23p. Series on Trade and Policy Report. Published by the International Institute for Sustainable Development, UK.

pesquisa e consolidação de literatura sobre estes temas e interações, culminando com a elaboração de um livro tinha uma publicação de grande impacto sobre o papel e relações entre biocombustíveis e bioenergia na oferta e produção de alimentos[9].

Além destas atividades realizadas com a Universidade de Wageningen, foram elaborados diversos projetos em parceria com docentes da ESALQ e da USP (Prof. Guilherme Ary Plonski, FEA, Poli) sobre o papel destas matérias-primas na mobilidade, na oferta de energias renováveis e quais as alternativas e possibilidades para a produção sustentável de energia e biocombustíveis[10],

Estas interações e colaborações possibilitaram a elaboração de capítulos de livros sintetizando o estado da arte sobre assunto, palestras e participações em diversos *fora* no país e no exterior, dando suporte a uma **narrativa baseada em dados científicos e comprovados** empiricamente sobre as consequências e impactos da produção de bioenergia no pais, sem emoção ou falta de dados empíricos, que era evidente na ações vindas do exterior, as quais não contribuíam para a imagem do Brasil.

[9] AMARAL, W.A.N. do; MARINHO, J.P.; TARASANTCHI, R. BEBER, A. GIULIANI, E; Environmental sustainability of sugarcane ethanol in Brazil. **In: Sugarcane Ethanol.** Wageningen Academic Publishers, The Netherlands, 2008. Capítulo 5, P. 113-135

[10] AMARAL, W.A.N. do; PLONKI, A. G.; GIULIANI, E. Bionergy Innovation and Sustainable Mobility: Deployment Feedstock Full Potentials. **In: Energy, Transport & the Environment**. Oxford University, London, Springer, 2012. P.77-94

9. Economia regenerativa e circular, trazendo e refletindo sobre a importância dos sistemas biológicos

A parceria realizada com o professor Aldo Ometto da Escola de Engenharia de São Carlos (EESC, USP) possibilitou a expansão das minhas atividades profissionais, incluindo o tema da economia circular como um guarda chuva conceitual que possibilitasse a reunião de diversas áreas do conhecimento e da ciência para o desenvolvimento de projetos, de publicações[11] e para uma reflexão mais profunda sobre os modelos de desenvolvimento econômicos baseados nos processos de extração produção e descarte de materiais e de matérias-primas.

A USP já havia firmado um convênio com a Fundação Ellen MacArthur da Inglaterra para capacitação, transferência de informações e ferramentas associadas às diferentes escolas do saber que contribuíram para a consolidação do conceito da economia circular no ambiente acadêmico e empresarial, inclusive com a visita do Prof. Vahan Agopian (ainda não função anterior a de Reitor), como representante da USP junto ao Conselho da referida Fundação.

O desenvolvimento de uma agenda de pesquisa em economia circular representando a USP e especial representando o eixo dos elementos biológicos no contexto dos sistemas de produção (diagrama da borboleta – butterfly diagram), me possibilitaram a elaboração de diversas publicações, ações de capacitação e desenvolvimento de projetos de pesquisa associados às interfaces entre a economia circular e o tema da sustentabilidade.

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3. [11] ROSSI, EFIGÊNIA; BERTASSINI, ANA CAROLINA; FERREIRA, CAMILA DOS SANTOS; NEVES DO AMARAL, WEBER ANTONIO; OMETTO, ALDO ROBERTO. Circular economy indicators for organizations considering sustainability and business models: Plastic, textile and electro-electronic cases**. Journal of Cleaner Production.** 20-02-20, vol.247, p.119-137. https://doi.org/10.1016/j.jclepro.2019.119137.

O conceito da economia circular ganhou muita visibilidade a partir de uma publicação da Fundação Ellen MacArthur, no Fórum Econômico Mundial, quando foi apresentado um diagrama chamado de diagrama da borboleta onde há fluxos e circulações de produtos em 2 grandes elos ou sistemas: o biológico e o técnico, sendo atualmente a principal figura que simboliza com o conceito da economia circular (Figura 1. Abaixo).

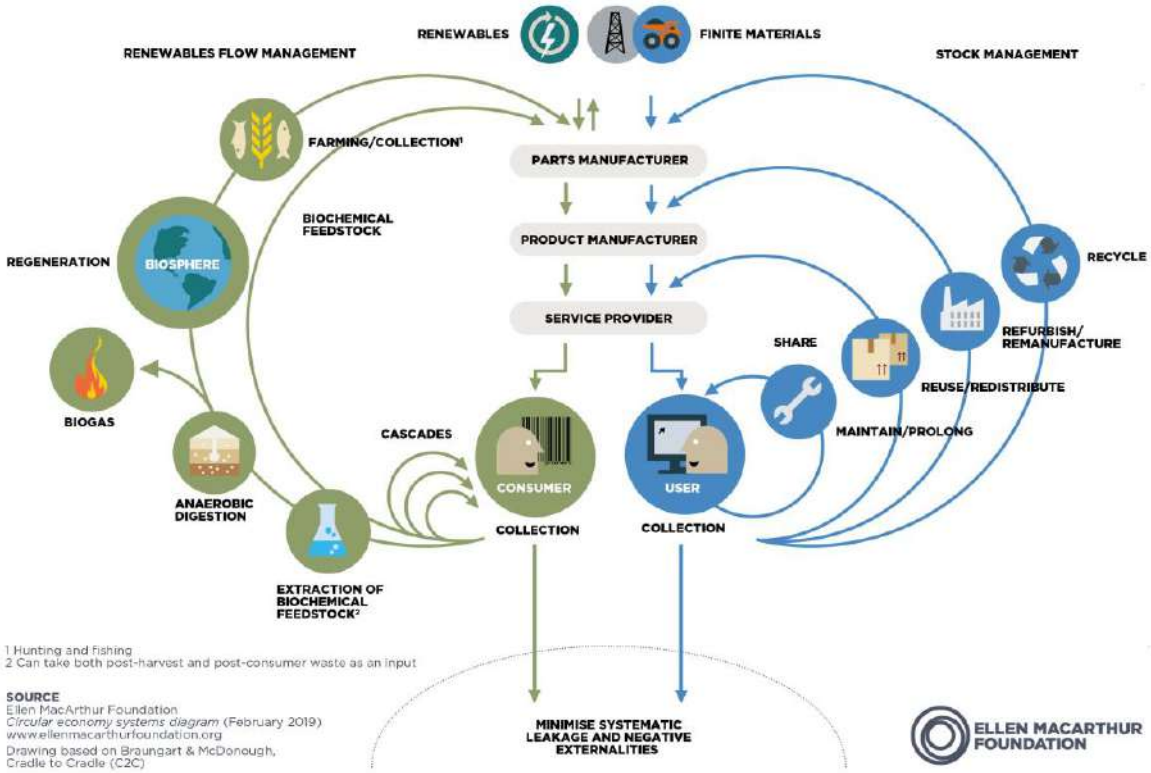


Figura 1. Diagrama da borboleta

Dentro deste conceito de capacitação e elaboração de projetos, há uma grande oportunidade trazida pelos conceitos da economia circular que diz respeito a regeneração dos dos sistemas biológicos, um conceito muito consolidado nas Ciências Florestais.

Atualmente há uma nova linha de reflexão nas interfaces entre sustentabilidade e a Economia Circular, sugerindo que os sistemas de produção particularmente baseados em **agricultura regenerativa** possam substituir os modelos atuais de produção de alimentos de

fibras e de bioenergia. E dentro deste contexto, os sistemas e paisagens mais complexas e portanto mais resilientes, por exemplo aqueles que se baseiam na integração da lavoura pecuária e florestas (sistemas ILPF) os quais vem ganhando grande destaque e escala no Brasil, podem contribuir para a revisão dos atuais modelos de produção e das praticas adotadas atualmente para produção de alimentos, fibras e proteína.

10. Conclusão: uma visão de inovação e empreendedorismo

Durante estes mais de 36 anos de atividade profissional, eu me esforcei e me dediquei para contribuir para Ciências Florestais e de suas múltiplas interfaces com outras áreas do saber, para o desenvolvimento de modelos de desenvolvimento mais sustentáveis e justos, e em especial para formação de futuros talentos do Brasil e do exterior.

Desenvolvi projetos e programas de cooperação nacionais e internacionais, e contribui para a geração de valor dentro do escopo da bioeconomia, a partir da visão que os recursos genéticos florestais (espécies arbóreas) e seu uso sustentado, podem ser as nossas garantias para a conservação da biodiversidade, geração de renda e manutenção dos sistemas de produção e dos serviços ambientais associados.

A formação de talentos, os quais eu acredito ser a grande motivação daqueles que se dedicam ao ensino, pesquisa e extensão, para a formação de novos profissionais, os quais devem transformar o nosso país.

Dentro deste contexto nos últimos anos, eu me capacitei e me dediquei ao desenvolvimento e ao uso de novas ferramentas de ensino didáticas[12] e de engajamento destes talentos para que eles pudessem desenvolver novas competências e ferramentas, com uma visão para inovação e para o empreendedorismo, como duas características fundamentais para a inserção nos mercados de trabalho e especial para transformação da sociedade brasileira.

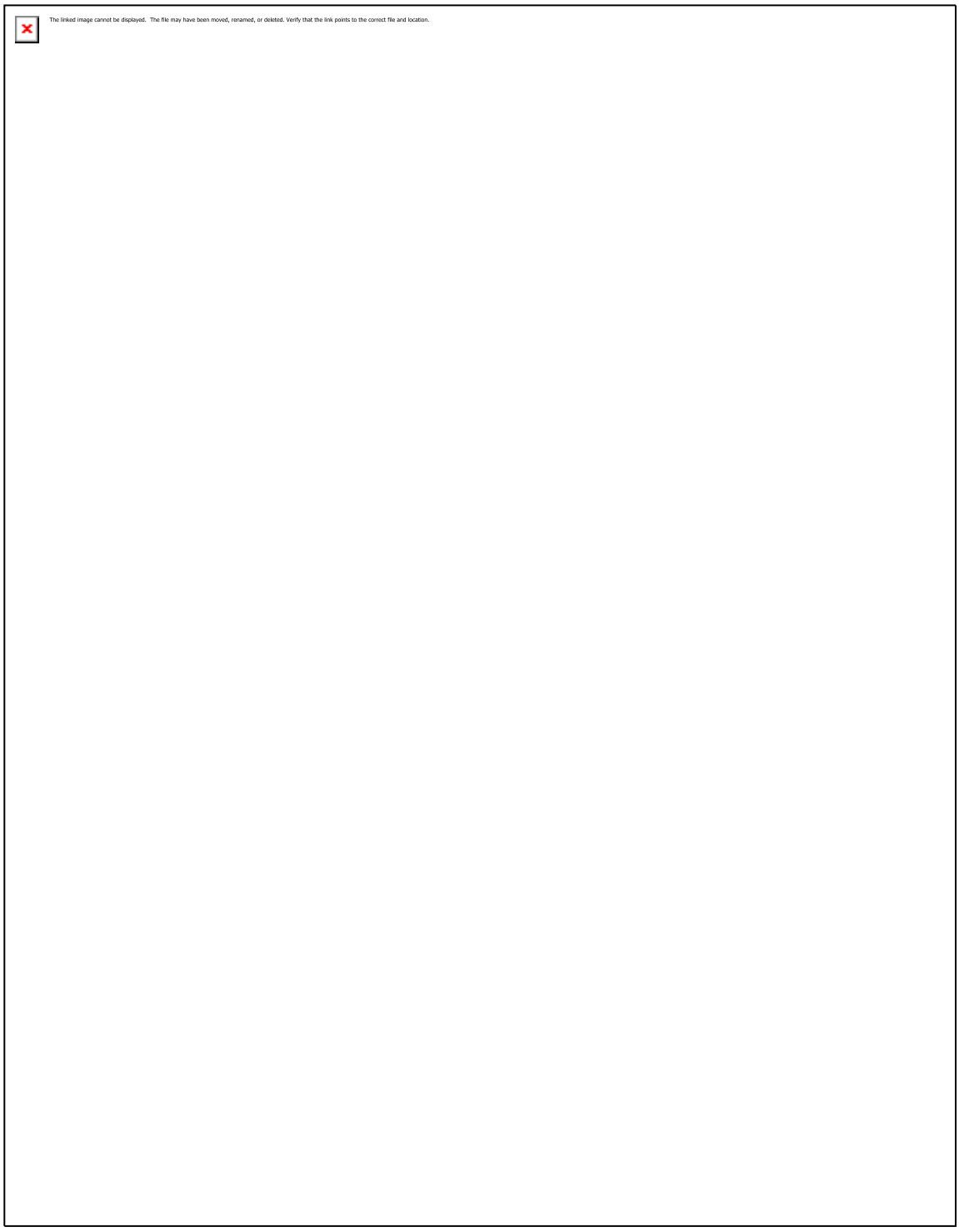
Todos estes anos se constituíram em uma longa e prazerosa jornada profissional, na qual tive a oportunidade de aprender, colaborar, compartilhar com diversos mentores,

[12] Uso da metodologia CANVAS em processos de ensino e aprendizado em Engenharia Florestal e Gestão Ambiental, Weber Antônio Neves do Amaral e Francides Gomes da Silva Junior wana@usp.br Anais do 2o Congresso de Graduação da Universidade de São Paulo 05 e 06 de julho de 2016 - Campus USP “Luiz de Queiroz” - Piracicaba/SP

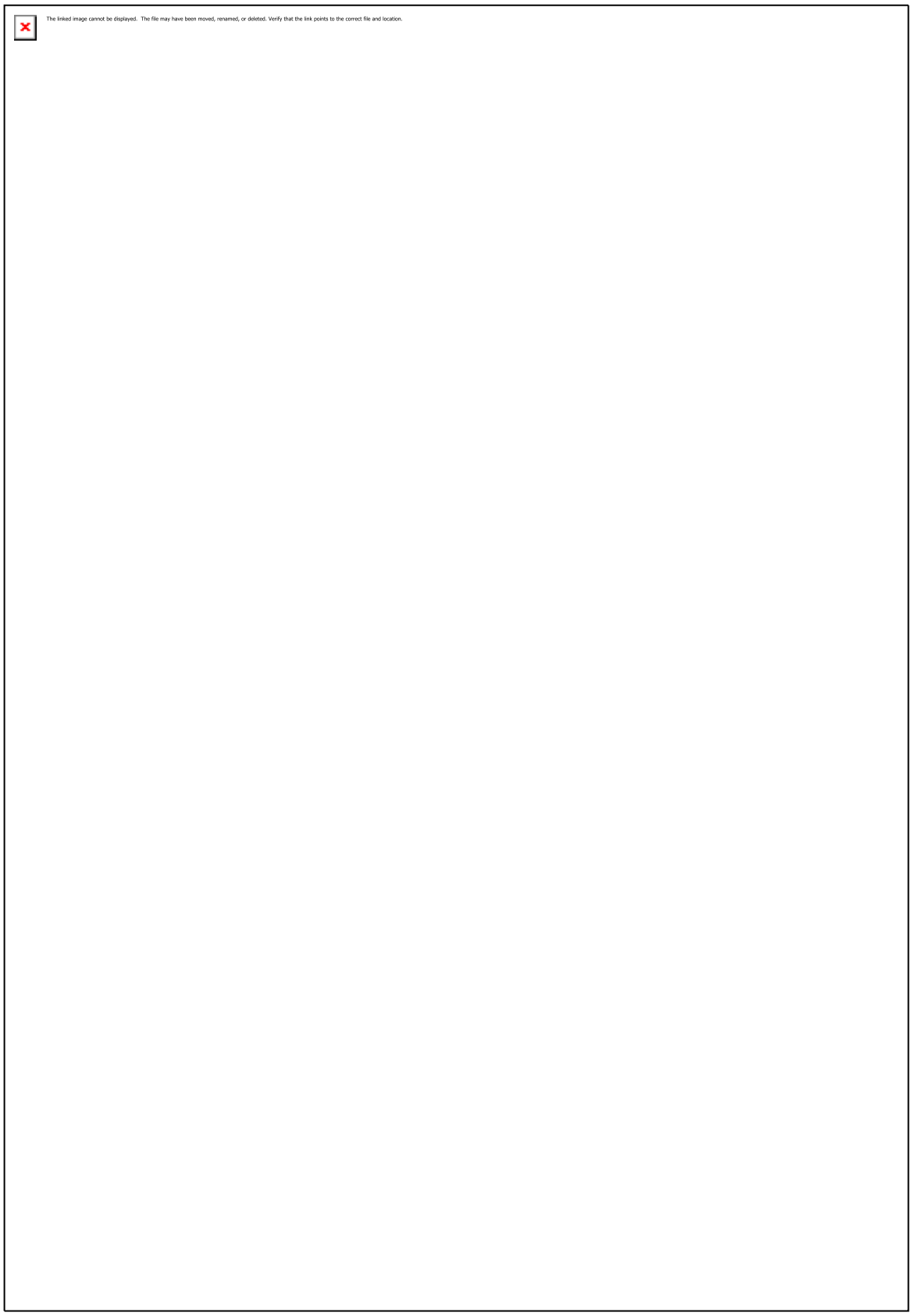
colaboradores, amigos e colegas, os quais contribuíram significativamente para todas as atividades que eu descrevi e comentei neste meu documento. Sem a participação e a colaboração com estas pessoas eu não teria tido a competência e as condições para este meu desenvolvimento profissional, que descrevi acima. Um agradecimento a todos aqueles mencionados neste documento e aqueles que não mencionei, mas que fazem parte importante e relevante desta minha carreira profissional. Meu muito obrigado a todos vocês.

APÊNDICE

1. *Artigo apresentado na íntegra no 20º. Congresso ABCP, SP, 1987.*



2. *Artigo apresentado na íntegra no Taller sobre Frugivoria e Dispersao de Sementes, México, 1993.*



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Frutification and germination ecology of *Citharexylum myrianthum* Cham (Verbenaceae)

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ABSTRACT

(Fructification and germination ecology of Citharexylum myrianthum Cham [Verbenaceae]). Citharexylum myrianthum Cham is a tree species that is abundant in riparian forests, widely used in the restoration of degraded areas, especially for areas with flood tendency. This study aimed to provide information on the mode of dispersal and germination of C. myrianthum. The process of fruition and seedling has been analyzed in a field trial in Botucatu/SP. The evaluations included aspects of the fruit maturation, fruit and pyrenes dispersal and predation and ecophysiology of germination. The fruits are dispersed mainly by birds and predation not affecting germination. This study allowed concluding that the species has good germination potential, especially under the light - characterizing himself as pioneered - and that its fruits have good viability in the soil and form seed bank for more than 12 months. These are important traits that make C. myrianthum useful in restoration of riparian forests.
Keywords: Zoocoric dispersion, Germination, Fruits predation.

Frutificação e ecologia da germinação de *Citharexylum myrianthum* Cham (Verbenaceae)

RESUMO

(Frutificação e ecologia da germinação de *Citharexylum myrianthum* Cham [Verbenaceae]). *Citharexylum myrianthum* Cham (tarumã) é uma espécie arbórea que ocorre abundantemente na mata ciliar e é utilizada em programas de recuperação de áreas degradadas, especialmente em áreas com solos encharcados. Este trabalho teve por objetivos fornecer informações sobre o modo de dispersão e germinação do tarumã. Foram realizadas análises da maturação dos frutos produzidos, de viabilidade dos pirênios, de dispersão e predação e de ecofisiologia da germinação. Os frutos são dispersos, principalmente, por aves generalistas e a germinação é pouco afetada pela predação pré-dispersão. A espécie apresentou um bom potencial germinativo em regime de luz, caracterizando-se como pioneira e, seus frutos mostraram boa viabilidade no solo, formando banco de sementes por mais de 12 meses. As características avaliadas demonstraram o grande potencial da espécie para ser utilizada na restauração de matas ciliares, a partir de sementes e mudas.
Palavras chave: Dispersão zoocórica, Germinação, Predação de frutos.

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INTRODUÇÃO

A substituição da vegetação nativa devido à expansão da fronteira agropecuária e das malhas urbanas, assim como do desmatamento indiscriminado, acarreta na fragmentação dos ecossistemas florestais, aumentando o grau de isolamento entre os fragmentos (Primack e Rodrigues, 2001). A fragmentação de habitats é uma forte ameaça `a diversidade biológica e a causa primária de extinção de espécies (Wilcox e Murphy, 1985). Dessa forma, os fragmentos florestais restantes sofrem grande pressão antrópica, agravando este problema e levando ao aparecimento de extensas áreas degradadas vizinhas às florestas secundárias.

Citharexylum myrianthum Cham (família Verbenaceae), é uma espécie nativa conhecida popularmente como tarumã ou pau de viola, de ocorrência ampla no nordeste, sudeste e sul do Brasil, em formações de Cerrado, Caatinga e Mata Atlântica (Salimena et al., 2013). Atinge 15-25 metros de altura e floresce durante os meses de novembro e dezembro, apresentando frutos maduros a partir de janeiro (Lorenzi, 2002). É descrita na literatura como uma espécie pioneira, apresentando ótima regeneração natural em vários estágios da sucessão secundária e produzindo anualmente uma grande quantidade de flores e frutos (Reitz et al., 1979; Lorenzi, 1992; Carvalho, 2004). Por este motivo, é muito utilizada em programas de recomposição de matas ciliares ou para arborização urbana (Torres et al., 1993), além de finalidades paisagísticas em praças e parques.

Os frutos do tarumã apresentam síndrome zoocórica, sendo dispersos principalmente por aves (frutos com coloração atrativa, localização exposta e conspícua) que são adaptações para a dispersão ornitocórica (Snow, 1971; Van Der Pijl, 1982), e mamíferos, notadamente dispersa pelo macaco-bugio (*Alouatta fusca*) (Carvalho, 2004). Segundo Andrade (2005) a polinização é feita por diversas espécies de esfingídeos, beija flores e até mesmo borboletas, sendo os esfingídeos de hábito crepuscular e noturno e os beija flores e borboletas de hábito diurno. A dispersão dos frutos e sementes

Para produção de sementes e mudas, os frutos são colhidos diretamente da árvore, quando começam a ser procurados por aves. A extração é feita por maceração para retirada da polpa dos caroços, sendo as sementes dispostas em ambiente ventilado para secagem. Após o beneficiamento do fruto, a unidade prática de manipulação é a metade do pirênio (semipirênio), e não a semente propriamente dita, que se encontra firmemente aderida ao semipirênio em contato com o ar. As sementes são distribuídas para germinação logo que colhidas em canteiros semi-sombreados. A taxa de germinação geralmente é superior a 80% e não há necessidade de quebra de dormência (Zanon et al., 1997; IBF, 2013). Após o transplante das mudas (quando atingem 4-6 cm), estas precisam receber sol direto, devendo ser plantadas em áreas abertas (Bueno e Leonhardt, 2011). O desenvolvimento das plantas no campo é rápido, podendo atingir quatro metros em dois anos de idade (Zanon et al., 1997; IBF, 2013).

Estudos considerando aspectos silviculturais de espécies nativas com grande importância econômica e ambiental são fundamentais para subsidiar planos de manejo, recuperação ambiental e conservação. Considerando este aspecto, este estudo teve por objetivo fornecer informações sobre a maturação fisiológica de frutos, dispersão de sementes e germinação de tarumã, as quais podem ser utilizadas para identificação do potencial desta espécie a partir da semeadura direta ou plantio a partir de mudas em projetos de restauração de matas ciliares.

MATERIAL E MÉTODOS

A área de estudo está localizada na Fazenda Experimental Edgárdia (22° 47’ 30” S e 48° 26’ 15” W), localizada na bacia do rio Capivara, no município de Botucatu, Estado de São Paulo. A Fazenda possui uma área de aproximadamente 500 hectares e pertence à Universidade Estadual Paulista. A altitude média da região é de 577 m e o clima é classificado, segundo Koeppen como tipo Cfa ou temperado úmido, com verão quente (Martins, 1993). Os solos encontrados no local de estudo são predominantemente associações de solos aluviais, glei húmico eutrófico e latossolo vermelho-amarelo (Carvalho et al., 1991).

Foram observados neste estudo cinco indivíduos adultos de tarumã com DAP médio de 30 cm, altura média de 21,3 m e área média da copa de 34,6 metros quadrados. Para análise da maturação dos frutos, foram realizadas oito coletas em intervalos de uma semana, iniciadas 35 dias após o pico de floração. As características analisadas foram: teor de umidade do fruto e pirênio, peso da matéria seca, cor, comprimento, diâmetro, germinação e composição química. Os frutos coletados foram agrupados visualmente quanto à cor em quatro grupos: verdes, amarelos,

amarelo/avermelhados e vermelhos. As dimensões dos frutos (comprimento e diâmetro) foram avaliadas por classe de cor, empregando-se um paquímetro com exatidão de 0,1mm. A porcentagem de umidade dos frutos e pirênios foi determinada em base úmida em estufa, a 105° C, com quatro repetições de 20 frutos ou pirênios cada, no delineamento inteiramente casualizado (Brasil, 1992). Para a determinação do peso seco das estruturas, utilizaram-se os mesmos frutos e pirênios submetidos aos testes de umidade. Os testes de germinação foram conduzidos em câmaras germinadoras do tipo germ-box, sobre papel toalha, com oito repetições de 25 pirênios despolpados, no delineamento inteiramente casualizado, com avaliação aos 30 dias.

Na composição química dos frutos avaliou-se a composição centesimal de proteínas, amido, ácidos graxos, fibras e cinzas, conforme a metodologia de Teles (1981) para o amido e a metodologia da Association of Official Analytical Chemists (AOAC, 1975) para os outros componentes.

Na análise de viabilidade dos pirênios ao longo do tempo, foi empregado um ensaio de campo com 20 sacos de tela de sombrite 50% em que se colocaram 50 pirênios. Os 20 sacos ou repetições foram aleatoriamente distribuídos em clareiras próximas às plantas mães e, após um ano, cinco foram abertos para análise da germinação, predação e degradação dos pirênios e, três sacos para a determinação do teor de umidade (Pagano, 1985; Sork, 1987). O teste de germinação foi conduzido em laboratório à temperatura constante de 25°C em presença de luz. Para o estudo do grau de injúria dos frutos e pirênios coletados próximos à planta-mãe foram instaladas quatro bandejas de $1m^2$, localizadas a 2m da base do tronco das árvores. As variáveis analisadas foram: pirênios inteiros e quebrados, frutos inteiros e danificados.

Na dispersão da espécie por ornitocoria foram realizadas observações a olho nu e com auxílio de binóculos. A metodologia foi adaptada de Silva (1988), considerando como unidade padrão de observação a hora-planta. As observações ocorreram no período de 05h30min as 09h30min e de 17h:00min as 19h:00min, totalizando 40 horas de observações. Foram registradas as espécies que se alimentavam dos frutos, e a frequência de visitação. Para a identificação das aves, foram utilizadas redes de nylon de 3m de altura alocadas ao redor de uma das árvores para a captura. Fezes de aves foram coletadas na projeção da copa e em poleiros identificados a fim de verificar a presença de frutos e pirênios, o grau de degradação, germinação e umidade destes.

O efeito da luz na germinação foi estudado em um ensaio no laboratório, em câmara germinadora, com 25 pirênios em papel e oito repetições de quatro tratamentos, combinando presença e ausência de luz com temperatura constante a 25° C e variável a 20-30° C (Brasil, 1992).

RESULTADOS E DISCUSSÃO

Maturação dos frutos

Na maturação dos frutos foi observado um aumento em diâmetro em relação ao comprimento (Tabela 1). Os frutos aumentaram em média 60% de diâmetro, enquanto que o comprimento sofreu um acréscimo de 22%. Foi observado a partir do estádio intermediário que houve um aumento no comprimento dos frutos, apesar de a germinação ser baixa. O aumento no volume dos frutos ocorreu devido ao crescimento do mesocarpo, que passa a acumular água no fruto maduro. Nos frutos verdes, o peso da matéria seca dos pirênios representa 44% do peso da matéria seca do fruto, já no fruto maduro, esse valor corresponde a 37%, indicando que, apesar de possuir maior porcentagem de água, os frutos maduros ganham proporcionalmente mais matéria seca que os pirênios, considerando os estádios de maturação. Além disso, os frutos tendem a ganhar água, e o pirênio a perdê-la durante a maturação. Com a formação da semente, a umidade vai diminuindo em função do aumento de matéria seca até o ponto em que o teor de água da semente oscila de acordo com a umidade do ambiente (Carvalho e Nakagawa, 2000).

Quanto à porcentagem de germinação houve diferenças entre os estádios de maturação. Os frutos verdes e intermediários apresentaram uma menor porcentagem de germinação, enquanto que os frutos maduros e os frutos nos estádios intermediário-maduro apresentaram maior germinação, podendo ser utilizados eficientemente na produção de mudas desta espécie.

No tarumã, observa-se que o comprimento, diâmetro, umidade e matéria seca dos frutos e pirênios são bons indicativos da maturação fisiológica da espécie. Além disso, a classificação dos frutos de acordo com a coloração também foi considerada eficiente para aferir o grau de maturidade e percentual de germinação dos frutos e pirênios. Assim como observado para cedro

(*Cedrela fissilis*) (Corvello et al., 1999), que alcança a maturidade fisiológica com teor de água entre 50 e 60% e os frutos apresentam coloração marrom-esverdeada a marrom-clara. O tamanho dos frutos e a coloração das sementes também foram bons indicadores do ponto de maturação fisiológica para a quaresmeira (*Tibouchinagranulosa*) (Lopes et al., 2005). Para o jacarandá-da-bahia (*Dalbergia nigra*), o grau de umidade e o peso da matéria seca foram os índices que melhor caracterizaram a maturação fisiológica e época de colheita das sementes (Martins e Silva, 1997), assim como para *Podocarpus lambertii (Ragagnin et al., 1994)*.

Tabela 1 – Percentual germinação, comprimento e diâmetro em milímetros, percentual de umidade e peso em matéria seca dos frutos e pirênios de tarumã, nos diferentes estádios fisiológicos (Verde, intermediário, Intermediário/maduro e Maduro), São Paulo.

Estádio	Germinação (%)	Comprimento (mm)**	Diâmetro (mm)	Umidade (%)		Matéria seca (g/10unid)	
				Fruto	Pirênio	Fruto	Pirênio
Verde	30 b*	9,7 c	6,1 d	62,4 c	17,4 a	0,90 b	0,41 b
Intermediário	33 b	12,1 a	7,6 c	60,4 d	15,6 ab	1,10 b	0,43 b
Intermed/maduro	46 ab	11,0 b	8,2 b	64,0 b	14,7 b	1,39 ab	0,55 ab
Maduro	68 a	11,9 a	9,7 a	71,6 a	14,2 c	1,76 a	0,66 a
Média	**44,2**	**11,2**	**7,9**	**63,9**	**15,3**	**1,29**	**0,51**

*As médias seguidas pela mesma letra não diferem entre si pelo teste de Tukey a 5% de probabilidade de erro.** Valores de comprimento e diâmetro em milímetros, porcentagem de umidade e matéria seca em gramas por 10 unidades.

A análise química de frutos em diferentes estádios de maturação revela que estes são amiláceos e possuem elevada porcentagem de fibras, que se acumulam provavelmente no pericarpo, ou seja, na camada externa do pirênio (Figura 1).

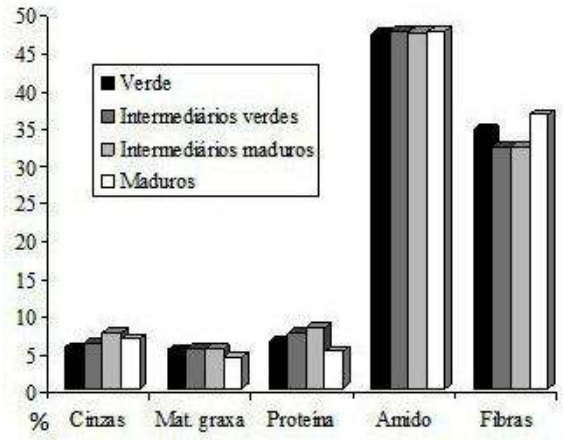

Figura1: Composição de cinzas, matéria graxa, proteína, amido e fibras dos frutos de tarumã em diferentes estádios de maturação na amostra desidratada. São Paulo.

Os frutos maduros apresentam uma menor porcentagem de matéria graxa e proteína que os verdes, sendo que o teor de amido praticamente não varia durante a maturação. Nos frutos intermediário-maduros há um aumento acentuado no teor de proteína e cinzas, com manutenção dos teores de matéria graxa. A razão do aumento do teor de proteína na metade do processo de maturação pode estar relacionada com o aumento da síntese protéica, sendo que, após atingir um determinado valor, as proteínas começam a se degradar.

Como os frutos de tarumã apresentam pequena mudança na sua composição química durante a maturação, pode-se esperar que os compostos analisados não foram os fatores mais importantes que condicionam a presença ou não de predadores dos frutos. Os resultados da análise química indicam, por outro lado que, os frutos maduros, pobres em proteínas e matéria graxa, são consumidos por um grande número de animais, o que aumenta a probabilidade de sucesso da dispersão, já que há uma maior chance dos frutos caírem em local adequado ao estabelecimento.

Perda da viabilidade dos pirênios no tempo

A média de frutos inteiros encontrados foi de quatro frutos por amostra e 64 pirênios. Destes, 28,7% e 50,4% dos pirênios não apresentavam sinais de degradação. Este fato pode ter ocorrido

pela deterioração dos frutos e pirênios, da perda por animais e pela malha da tela.

A germinação média dos pirênios armazenados no solo foi de 19,5%, e a umidade média presente nos frutos armazenados foi de 16,9%. A porcentagem de germinação encontrada após um ano é alta, sendo que as sementes permanecem viáveis por um período superior a doze meses, levando à formação de um banco de sementes (Hall e Swaine, 1980; De Foresta e Prevost, 1986; Garwood, 1989; Levin, 1990, Zanon et al., 1997). Sabe-se que as espécies da vegetação em crescimento representam pequena amostra do conteúdo genético da comunidade e que o banco de sementes não germinadas que permanecem no solo pode conter grande número de genótipos diferentes da população adulta. O conhecimento básico do banco de sementes permite que se realizem previsões sobre o potencial florístico existente no processo de sucessão que se segue. Este tipo de estudo é fundamental para o entendimento do estabelecimento e evolução de um ecossistema florestal e consequentemente para o gerenciamento e implantação de planos de manejo e de recuperação florestal (Nobrega et al., 2009).

O banco de sementes de tarumã pode ter um papel importante na manutenção da base genética das populações em situações em que houve a eliminação das árvores existentes anteriormente. A formação de um banco de sementes do solo auxilia, ainda, a atenuar os efeitos causados pelos anos de menor produção de frutos, funcionando como um estabilizador do número de indivíduos que podem colonizar uma determinada área, aumentado a probabilidade de estabelecimento e colonização por novos indivíduos.

A permanência das sementes viáveis no solo, levando a formação de um banco de sementes é uma característica típica de espécies pioneiras, como descritas por Carvalho (2004). Por ser uma espécie que ocorre preferencialmente em áreas úmidas e brejos (Lorenzi, 1992), após as sementes de tarumã terem sido dispersas da planta mãe, pode ocorrer dispersão secundária pela água, inclusive depois de um ano da sua produção (Amaral, 1993). Além disso, as sementes de tarumã podem ser armazenadas por um período de 360 dias em câmara seca (Zanon et al*.,* 1997).

Dispersão de frutos e pirênios

A porcentagem de frutos inteiros nas bandejas foi de 24%, um valor relativamente baixo pela quantidade de frutos que caem da árvore mãe pela gravidade, entretanto a polpa encontra-se muito susceptível aos danos provocados pela precipitação entre uma coleta e outra. Já a porcentagem de pirênios quebrados foi de 29,1%, o que pode estar relacionado com a predação por aves.

A metodologia usada para a análise da predação, baseada no grau de injúria, mostrou-se pouco precisa, pois, como o intervalo entre as coletas foi de uma semana e a polpa dos frutos maduros pode ter sido danificada pela chuva. Já na predação pré-dispersão, se observou a presença da larva de *Anastrepha* sp. (mosca das frutas). A porcentagem de frutos maduros mantidos em observação, que possuíam a larva de *Anastrepha* sp., foi de 75%, enquanto que para os frutos intermediários-maduros foi de 31%. Este resultado indica que a infestação ocorre com maior intensidade na fase final da maturação.

Observações sobre a avifauna

Em observações sobre a avifauna foram identificadas 17 espécies de aves, de onze famílias, visitando as árvores ao longo do dia, sendo que, de todas as espécies visitantes, apenas seis foram observadas alimentando-se dos frutos, com a frequência de visitação destas alcançando 52% do total observado (Tabela 2). As famílias com maior número de espécies visitantes foram Tyrannidae (4) e Columbidade (3). Machado e Rosa (2005) também identificaram Tyrannidae como a família com maior número de espécies visitantes, tendo observado cinco espécies, sendo três diferentes das aqui listadas.

Além disso, embora neste estudo não se tenha observado a ingestão dos frutos por *Tyrannus melancholicus, Tangara sayaca* e *Pitangus sulphuratus*, no estudo de Machado e Rosa (2005) são identificadas estas espécies como consumidoras que engolem o fruto inteiro.

Tabela 2 – Espécies de aves, famílias, hábito e frequência de visitação observada nas árvores de tarumã, São Paulo

Espécie	Família	Hábito	Frequência (%)
Patagioenas picazuro Temminck*	Columbidade	g,f	26
Patagioenas cayennensis Bonnaterre*	Columbidade	g,f	15
Geotrygon violacea Temminck*	Columbidade	g.f	11
Crotophaga ani Linnaeus	Cuculidae	i,c	0,2
Guira guira Gmelin	Cuculidae	i,c	0,2
Tangara sayaca Linnaeus	Fringilidae	f,b	7,5
Synallaxis spixi Sclater	Furnariidae	i,c	2,7
Gnorimopsar chopi Vieillot	Icteridae	O	4,2
Turdus amaurochalinus Cabanis*	Muscicapidae	o.f	7,7
Forpus xanthopterygius Spix*	Psittacidae	g,f	2,9
Sporophila lineola Linnaeus	Thraupidae	G	0,2
Turdus rufiventris Vieillot*	Turdidae	o,f	1,5
Colonia colonus Vieillot	Tyrannidae	I	0,2
Myiodynastes maculatus Statius Muller	Tyrannidae	I	9
Tyrannus melancholicus Vieillot	Tyrannidae	I	8,5
Pitangus sulphuratus Linnaeus	Tyrannidae	O	1,8
Cyclarhis gujanensis Gmelin	Vireonidae	i,f	1,4

* Espécies de aves que se alimentam dos frutos; i: insetívoros; f: frugíveros; g: granívoros; o: onívoros; c: carnívoros; b: brotos.

A presença de pássaros insetívoros na fase final da maturação dos frutos pode estar relacionada ao grande número de insetos encontrados na planta. As larvas de *Anastrepha* sp. infestam os frutos maduros e, servem de alimento para muitas aves insetívoras. A larva se alimenta da polpa do fruto maduro, porém não causa nenhum dano aos pirênios, já que o endocarpo é muito resistente à penetração.

Dentre as espécies de aves observadas alimentando-se dos frutos, a que pode provocar maiores danos aos pirênios seria a *Forpus xanthopterygius,* que, devido ao bico duro, é capaz de quebrar o endocarpo dos pirênios. Por outro lado *Columba picazuro, C. cayannensis* e *Geotrygon violacea* alimentam-se dos frutos e os ingerem, podendo provocar danos físicos e reduzindo a germinação. Entretanto, estas espécies de aves, talvez sejam as mais eficientes na dispersão de sementes, mesmo com os danos provocados, pois, voam longas distâncias e transportam os pirênios para novas áreas.

Quando se avaliam de forma conjunta os resultados das análises químicas dos frutos, estes são pobres em nutrientes, confirmando-se a hipótese de dispersão dos frutos por aves generalistas. A forma não agrupada com que os frutos são dispersos, já que não mais de dois pirênios foram encontrados nas fezes, confirma as hipóteses levantadas por Shupp et al. (1989), quanto aos mecanismos de dispersão de sementes de espécies pioneiras, que contribuem para uma menor taxa de predação e consequentemente confere maior sucesso no estabelecimento de plântulas.

Ecofisiologia da germinação

Os testes de germinação em diferentes temperaturas revelaram uma maior porcentagem de plântulas germinadas quando se utilizaram frutos frescos à 25ºC em presença de luz (45%) enquanto a menor porcentagem foi de 9,7% em frutos secos à 25ºC com luz. Os tratamentos de fruto seco e fresco entre 20-30 ºC com luz tiveram valores aproximados de 14,9 e 18,3% respectivamente, sem diferenciação de acordo com o Teste de Tukey à 5% de probabilidade (dados não apresentados). Estes resultados diferem aos de Zanon et al. (1997) que testou a germinação de tarumã utilizando frutos frescos em temperaturas de 20, 25 e 30 graus Celsius associados a quatro tipos de substrato: papel-toalha, papel mata-borrão, areia e vermiculita. Com exceção do papel toalha onde as melhores taxas de germinação se deram a 30 ^{o} C, todos os outros substratos obtiveram germinação significativa a 25^{o} C.

Esta diferença de temperatura ideal para germinação nos dois estudos poderia ser atribuída ao tipo de substrato utilizado, porém Alves et al. (2006) testaram a germinação de tarumã em

substratos micorrízicos e vermiculita além de um substrato controle composto de casca de arroz e areia. O tempo médio de germinação não diferiu de forma significativa, sendo de 40 dias para substrato controle e micorrizado e 41 dias para vermiculita. Neste estudo foi utilizada uma casa de vegetação sem monitoramento da temperatura e nem o grau de exposição à luz.

Com relação às características das plântulas estabelecidas a partir de frutos e pirênios, não houve significância estatística nas características avaliadas. O comprimento médio do sistema radicular foi de 26,3 cm, o comprimento médio da parte aérea foi de 9,1 cm e o número de folhas foi de 5 pares. O peso total de matéria seca encontrada foi de 8,7 g e 12,2 g, para frutos e pirênios respectivamente, após 114 dias de cultivo. As plântulas possuem um sistema radicular desenvolvido, possivelmente pela necessidade de fixação em locais encharcados, sendo que mesmo os frutos que não sofreram remoção da polpa, por aves ou pela água, germinaram, indicando que não há presença de substâncias inibidoras da germinação.

Estudos abordando a ecologia de espécies florestais nativas são importantes, pois fornecem subsídios para programas de conservação e recuperação de ecossistemas florestais. Os dados aqui apresentados contribuem para a tomada de decisões que levem a uma melhor produção de mudas e manejo da espécie e sua utilização em programas de restauração de matas ciliares. Assim, observa-se que os frutos desta espécie tem um maior potencial germinativo quando alcançam o estádio intermediário/maduro e maduro e um menor teor de úmida do pirênio. A espécie é caracterizada como pioneira e os frutos permanecem viáveis no banco de sementes do solo por um longo período e são dispersos, principalmente, por aves generalistas.

CONCLUSÕES

Os frutos de tarumã tendem a ganhar umidade durante a maturação e os pirênios a perder, sendo que a predação que sofrem quando maduros, por larvas de *Anastrepha* sp., não afeta a germinação. Esta espécie é geralmente consumida e dispersa por aves generalistas. Quando no solo, os pirênios podem permanecer viáveis por um período superior a doze meses, contribuindo para a formação do banco de sementes do solo. Estas características reforçam o potencial desta espécie para uso em programas e projetos de restauração de matas ciliares a partir da semeadura direta e por mudas.

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Anexo 4.

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LEAFY AND THE EVOLUTION OF ROSETTE FLOWERING IN VIOLET CRESS (*JONOPSIDIUM ACAULE*, BRASSICACEAE)[1]

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Arabidopsis and most other Brassicaceae produce an elongated inflorescence of mainly ebracteate flowers. However, the early-flowering species violet cress (*Jonopsidium acaule*) and a handful of other species produce flowers singly in the axils of rosette leaves. In *Arabidopsis* the gene *LEAFY* (*LFY*) is implicated in both the determination of flower meristem identity and in the suppression of leaves (bracts) that would otherwise subtend the flowers. In this study we examined the role of *LFY* homologs in the evolution of rosette flowering in violet cress. We cloned two *LFY* homologs, *vcLFY1* and *vcLFY2*, from violet cress. Their exon sequences show ~90% nucleotide similarity with *Arabidopsis LFY* and 99% similarity to each other. We used in situ hybridization to study *vcLFY* expression in violet cress. The patterns were very similar to *LFY* in *Arabidopsis* except for stronger expression in the shoot apical meristem outside of the region of flower meristem initiation. It is possible that the relatively diffuse expression of *vcLFY* contributes to the lack of bract suppression in violet cress. Additionally, the earliest flowers produced by violet cress express *vcLFY*, suggesting that accelerated flowering in violet cress could also result from changes in the regulation of *vcLFY*.

Key words: *Arabidopsis*; Brassicaceae; evolution of development; flowering; gene expression; inflorescence; in situ hybridization; *LEAFY*.

The placement of flowers on a plant has profound ecological significance. It is, therefore, desirable that we understand how flower disposition is regulated in a given species and how it becomes modified in the course of evolution. The approach taken in this paper is to compare a well-studied model system, *Arabidopsis* [*Arabidopsis thaliana* (L.)Heynh.], with another member of Brassicaceae, violet cress [*Jonopsidium acaule* (Desf.)Rchb.]. Whereas *Arabidopsis* flowers are borne on elongated, leafless, inflorescences, violet cress flowers emerge from the axils of rosette leaves (Figs. 1–2). Here we explore the possible role of a floral meristem identity gene, *LEAFY* (*LFY*), in the evolution of rosette flowering.

The vegetative phase of development in *Arabidopsis*, violet cress, and almost all other Brassicaceae involves the formation of a rosette: a series of spirally arranged leaves with relatively short internodes. The meristems in the axils of these rosette leaves have the potential to produce new shoots called paraclades. The transition to flowering in most Brassicaceae (but not violet cress) results in the shoot apical meristem switching from producing leaves (with quiescent paraclades) to producing flowers without subtending leaves (Hempel and Feldman, 1994). Soon after the onset of flower production, the internodes of the primary axis elongate, carrying upward the flowers and the youngest leaves ("cauline leaves"). Flowering generally also results in the derepression of the distalmost paraclades, such that they elongate and start producing lateral flowers (Hempel and Feldman, 1994). The elongated portion of the plant, including the upper paraclades and the flowering axis, is usually termed an "inflorescence" (see Weberling [1989] for refined terminology).

Violet cress is one of a few rosette flowering species of Brassicaceae (Fig. 1). In such plants reproduction begins early and flowers are produced in the axils of leaves, which are indistinguishable from the leaves that precede flowering. We will use the term bract for any leaf subtending a flower and the term bracteate (vs. ebracteate) to describe flowers that are associated with a bract. One interpretation of the violet cress pattern is that solitary flowers are produced from the rosette rather than on an inflorescence. However, the fact that we use the appellation "rosette flowering" should not be taken as a rejection of two other equally valid interpretations: (1) bracteate flowers are produced on a compressed inflorescence; or (2) each flower is a reduced paraclade with a single, terminal flower. Regardless of which of these models is favored, we need to explain three distinct features of rosette flowering: precocious reproduction, the lack of bract suppression, and reduced internode elongation. In this paper, we focus on the possible genetic basis of the first two of these phenomena.

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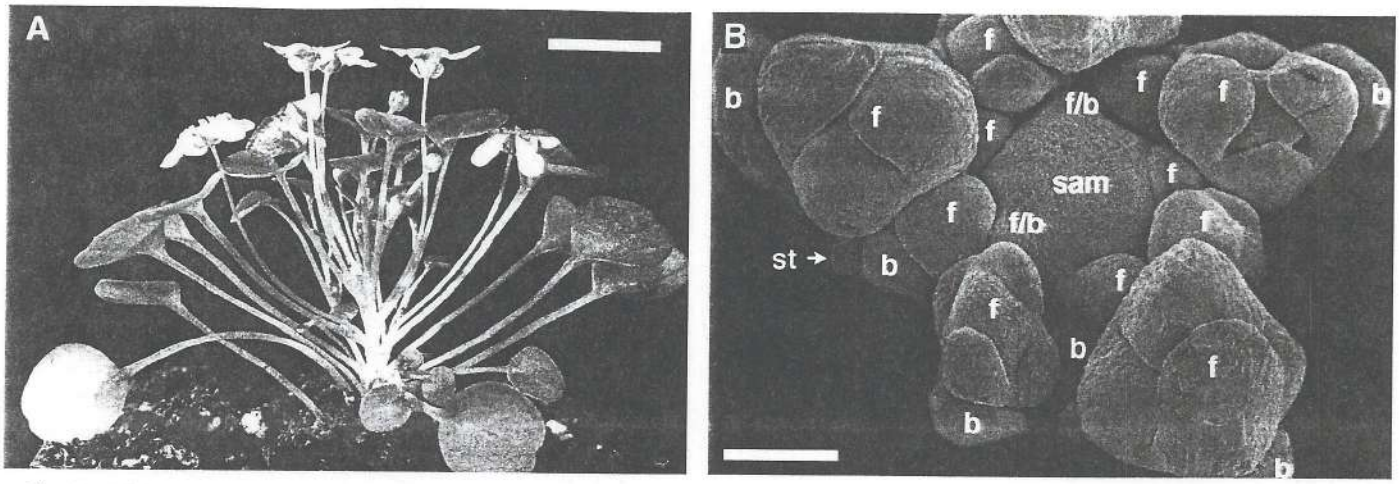


Fig. 1. Violet cress morphology. (A) Photograph of a flowering violet cress plant. Scale bar = 1 cm. (B) Scanning electron micrograph of the shoot apical meristem of a flowering plant. Scale bar = 100 μm.
Figure Abbreviations: b, bract primordium; b/f, combined bract/flower primordium; f, flower primordium; pc, paraclade; sam, shoot apical meristem; st, stipule.

The strategy we have taken to elucidate the genetic basis of rosette flowering in violet cress is to identify candidate genes based on the extensive knowledge of the genetic regulation of floral meristem identity in *Arabidopsis* (e.g., Weigel, 1995; Yanofsky, 1995; Simon, Igeño, and Coupland, 1996; Bradley et al., 1996, 1997; Lee et al., 1997; Ruiz-García et al., 1997; Blázquez et al., 1997, 1998; Parcy et al., 1998; Busch, Bomblies, and Weigel, 1999). There are several genes that might play a role in the origin of rosette flowering (e.g., *TERMINAL FLOWER 1*; *APETALA 1*). However, one obvious candidate gene is *LFY* because *Arabidopsis* plants overexpressing *LFY* show precocious flowering (Weigel and Nilsson, 1995; Blázquez et al., 1997) and the formation of flowers in the axils of rosette leaves (Weigel and Nilsson, 1995; Wagner, Sablowski, and Meyerowitz, 1999). This shows that changes in *LFY* activity or expression could contribute to the evolution of rosette flowering.

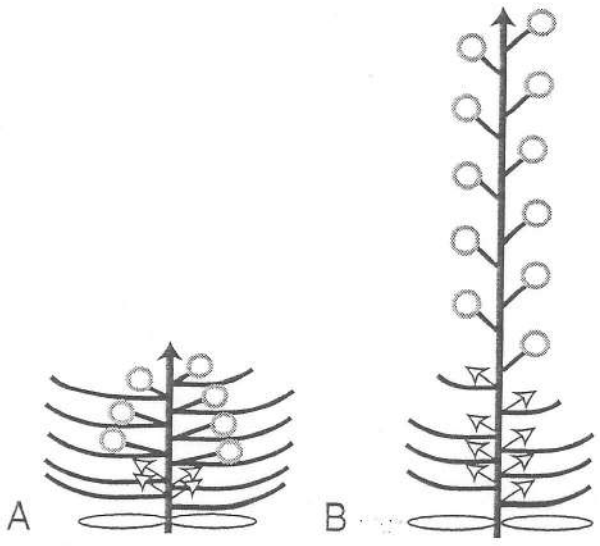
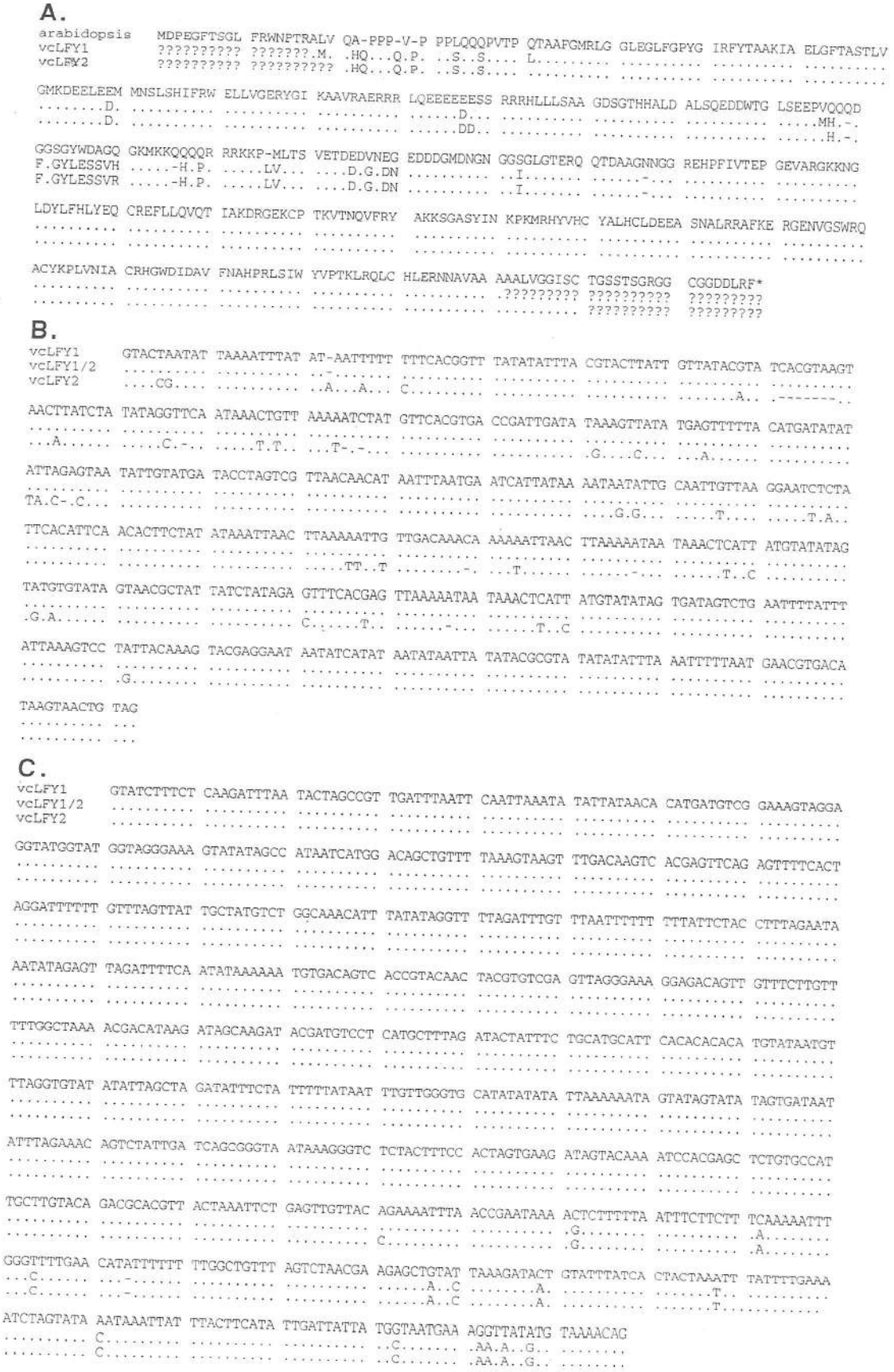


Fig. 2. Schematic diagrams of plant architecture. (A) Violet cress. (B) *Arabidopsis*. Ovals = cotyledons, curved lines = leaves, circles = flowers, open arrows = paraclades, and closed arrows = primary shoot apical meristems.

In *Arabidopsis*, *lfy* mutants fail to produce normal flowers (Schultz and Haughn, 1991; Huala and Sussex, 1992; Weigel et al., 1992). In other species where mutants in *LFY* homologs are available, flower formation is similarly disrupted (Coen et al., 1990; Hofer et al., 1997; Souer et al., 1998). Likewise, plants overexpressing *LFY* genes produce ectopic flowers (Weigel and Nilsson, 1995; Souer et al., 1998). Thus, despite species-to-species variation in their expression (see Coen et al., 1990; Weigel et al., 1992; Anthony, James, and Jordan, 1993; Kelly, Bonnlander, and Meeks-Wagner, 1995; Weigel and Nilsson, 1995; Hofer et al., 1997; Pouteau et al., 1997; Kyozuka et al., 1998; Souer et al., 1998), *LFY* homologs appear to play a conserved role in the regulation of flower meristem formation (Weigel and Nilsson, 1995). Additionally, *LFY* has been implicated in the suppression of bracts in *Arabidopsis* (Coen and Nugent, 1994).

Models of the function of *LFY* (e.g., Schultz and Haughn, 1991; Weigel et al., 1992; Weigel and Nilson, 1995; Parcy et al., 1998) suggest that its transcription is activated by exogenous factors (e.g., daylength, vernalization) and endogenous factors (a developmental clock) via hormones such as gibberellic acid (Blázquez et al., 1998) and several flowering-time genes (as reviewed in Weigel, 1995; Levy and Dean, 1998). LFY protein then binds to the promoters of target genes (especially, *APETALA 1*, *APETALA 3*, and *AGAMOUS*) and, in the presence of necessary coregulators, causes shoot meristems to differentiate into flowers (Lee et al., 1997; Parcy et al., 1998; Busch, Bomblies, and Weigel, 1999; Wagner, Sablowski, and Meyerowitz, 1999).

Violet cress is one of five species in the genus *Jonopsidium* Reichenb. (Heywood, 1992; Morales, 1993). These species are annual herbs growing in North Africa, Spain, Portugal, and Italy. Within *Jonopsidium*, three species produce an inflorescence in which most (but not all) flowers are ebracteate, whereas two species (*J. acaule*


A.
arabidopsis MDPEGFTSGL FRWNPTRALV QA-PPP-V-P PPLQQQPVTP QTAAFGMRLG GLEGLFGPYG IRFYTAAKIA ELGFTASTLV
vcLFY1 ?????????? ???????.M. .HQ...Q.P. ..S..S.... L.....
vcLFY2 ?????????? ?????????? .HQ...Q.P. ..S..S....
GMKDEELEEM MNSLSHIFRW ELLVGERYGI KAAVRAERRR LQEEEEEESS RRRHLLLSAA GDSGTHHALD ALSQEDDWTG LSEEPVQQQD
.....D. .....DD.. .....MH.-.
.....D. .....H.-.
GGSGYWDAGQ GKMKKQQQQR RRKKP-MLTS VETDEDVNEG EDDDGMDNGN GGSGLGTERQ QTDAAGNNGG REHPPIVTEP GEVARGKKNG
F.GYLESSVH .....-H.P. .....LV... ....D.G.DN ..I..... .....-...
F.GYLESSVR .....-H.P. .....LV... ....D.G.DN ..I..... .....-...
LDYLFHLYEQ CREFLLQVQT IAKDRGEKCP TKVTNQVFRY AKKSGASYIN KPKMRHYVHC YALHCLDEEA SNALRRAFKE RGENVGSWRQ
ACYKPLVNIA CRHGWDIDAV FNAHPRLSIW YVPTKLRQLC HLERNNAVAA AAALVGGISC TGSSTSGRGG CGGDDLRF*
.?????????? ?????????? ?????????
?????????? ?????????
B.
vcLFY1 GTACTAATAT TAAAATTTAT AT-AATTTTT TTTCACGGTT TATATATTTA CGTACTTATT GTTATACGTA TCACGTAAGT
vcLFY1/2 ..-.....
vcLFY2 ....CG.... ..A...A... C.....
.....A.. .-------..
AACTTATCTA TATAGGTTCA ATAAACTGTT AAAAATCTAT GTTCACGTGA CCGATTGATA TAAAGTTATA TGAGTTTTTA CATGATATAT
...A..... .....C.-.. .....T.T.. ...T-.-... .....G....C... ...A.....
ATTAGAGTAA TATTGTATGA TACCTAGTCG TTAACAACAT AATTTAATGA ATCATTATAA AATAATATTG CAATTGTTAA GGAATCTCTA
TA.C-.C... ....G.G... .....T.... .....T.A..
TTCACATTCA ACACTTCTAT ATAAATTAAC TTAAAAATTG TTGACAAACA AAAAATTAAC TTAAAAATAA TAAACTCATT ATGTATATAG
.....TT..T .....-. ...T..... .....-... .....T..C
TATGTGTATA GTAACGCTAT TATCTATAGA GTTTCACGAG TTAAAAATAA TAAACTCATT ATGTATATAG TGATAGTCTG AATTTTATTT
.G.A..... C.....T.. .....-... .....T..C
ATTAAAGTCC TATTACAAAG TACGAGGAAT AATATCATAT AATATAATTA TATACGCGTA TATATATTTA AATTTTTAAT GAACGTGACA
.G.....
TAAGTAACTG TAG
C.
vcLFY1 GTATCTTTCT CAAGATTTAA TACTAGCCGT TGATTTAATT CAATTAAATA TATTATAACA CATGATGTCG GAAAGTAGGA
vcLFY1/2
vcLFY2
GGTATGGTAT GGTAGGGAAA GTATATAGCC ATAATCATGG ACAGCTGTTT TAAAGTAAGT TTGACAAGTC ACGAGTTCAG AGTTTTCACT
AGGATTTTTT GTTTAGTTAT TGCTATGTCT GGCAAACATT TATATAGGTT TTAGATTTGT TTAATTTTTT TTTATTCTAC CTTTAGAATA
AATATAGAGT TAGATTTTCA ATATAAAAAA TGTGACAGTC ACCGTACAAC TACGTGTCGA GTTAGGGAAA GGAGACAGTT GTTTCTTGTT
TTTGGCTAAA ACGACATAAG ATAGCAAGAT ACGATGTCCT CATGCTTTAG ATACTATTTC TGCATGCATT CACACACACA TGTATAATGT
TTAGGTGTAT ATATTAGCTA GATATTTCTA TTTTTATAAT TTGTTGGGTG CATATATATA TTAAAAAATA GTATAGTATA TAGTGATAAT
ATTTAGAAAC AGTCTATTGA TCAGCGGGTA ATAAAGGGTC TCTACTTTCC ACTAGTGAAG ATAGTACAAA ATCCACGAGC TCTGTGCCAT
TGCTTGTACA GACGCACGTT ACTAAATTCT GAGTTGTTAC AGAAAATTTA ACCGAATAAA ACTCTTTTTA ATTTCTTCTT TCAAAAATTT
.G..... .A.....
C..... .G..... .A.....
GGGTTTTGAA CATATTTTTT TTGGCTGTTT AGTCTAACGA AGAGCTGTAT TAAAGATACT GTATTTATCA CTACTAAATT TATTTTGAAA
...C..... ...-..... .....A..C .....A. .....T..
...C..... ...-..... .....A..C .....A. .....T.....
ATCTAGTATA AATAAATTAT TTACTTCATA TTGATTATTA TGGTAATGAA AGGTTATATG TAAAACAG
C..... ..C..... .AA.A..G.. .....
C..... ..C..... .AA.A..G.. .....

and *J. albiflorum* Durieu) are fully bracteate (Heywood, 1992). Violet cress (*J. acaule*) is thought to originate from Portugal but is cultivated as a garden ornamental (Heywood, 1992). Molecular phylogenetic analyses have confirmed that the genus *Jonopsidium* is closely related to an inflorescence-bearing, Mediterranean genus, *Cochlearia* (Zunk et al., 1996). This suggests that violet cress represents a lineage that evolved rosette flowering from an inflorescence-bearing ancestor.

MATERIALS AND METHODS

Plant materials—Seeds of violet cress were obtained from the Colección de Germplasma de Crucíferas, Instituto Nacional de Investigaciones Agrarias, Madrid, Spain (accession number 496–1782–83, from Utrecht Botanical Garden). A voucher specimen of our cultivated lines (Baum 373) is deposited in the Gray Herbarium (Harvard University). Seeds for *Arabidopsis*, ecotype *landsberg erecta*, were obtained from the *Arabidopsis* Biological Resource Center (Ohio State University). Seeds were surface-sterilized and planted on agar plates in minimum Murashige and Skoog medium (Sigma, St. Louis, Missouri, USA). The plates were treated at 4°C for 1 wk and then were transferred to a growth chamber with 16 h of light and 8 h of dark, under a light density of 140–153 $\mu mol \cdot m^{-2} \cdot s^{-1}$, at 21°C during the daylight cycle and 17°C during the dark cycle. One-week-old seedlings were transplanted into pots.

Exogenous application of gibberellic acid has been shown to influence *LFY* expression (Blázquez et al., 1998). To investigate whether there is a similar effect in violet cress, some plants were surface sprayed twice a week with 100 μmol/L gibberellic acid (GA_3, Sigma, St. Louis, Missouri, USA) in 10 mmol/L Tris-HCl (pH 8.0).

Scanning electron microscopy (SEM)—Shoot apical meristems of flowering violet cress plants were fixed in FAA (formalin-acetic acid-alcohol) and stored in 70% ethanol at 4°C. Shoot tips were dehydrated in a series of ethanols, critical-point dried, and sputter-coated with gold-palladium. SEM was conducted using an Amray AMR model 100 microscope. Images were scanned and backgrounds were blackened using Adobe Photoshop 5.0 (Adobe Systems, Inc., Mountain View, California, USA).

Amplification, cloning, and sequencing—A ~3 kilobasepair (kbp) fragment of *LFY* was amplified (eLONGase kit; Gibco BRL, Gaithersburg, Maryland, USA) from violet cress genomic DNA using primers 001F (ATGGATCCTGAAGGTTTCACG) and 1198R (ACAGCTAATACCGCCAACTAA) designed based on published sequences of *LFY* homologs. The single product (~3 kbp) was purified and cloned using the pGEM-T Easy Vector (Promega, Madison, Wisconsin, USA). Five clones were purified and sequenced using internal primers designed from published *LFY* sequences and by primer walking. Cleaned cycle-sequencing reactions (BigDye; PE Applied Biosystems, Foster City, California, USA) were visualized on an ABI 377 automated DNA sequencer (PE Applied Biosystems, Foster City, California, USA). Sequences were assembled and edited using Sequencher 3.0 (Gene Codes Inc., Ann Arbor, Michigan, USA).

In situ hybridization—Plants harvested at various developmental stages were fixed in 4% paraformaldehyde for 24 h. At the time of fixation the stage of development was determined by counting the number of visible (>2 mm) leaves (excluding cotyledons). Fixed plants were dissected and embedded in paraffin (Paraplast+; Oxford Labware, St. Louis, Missouri, USA). A microtome was used to cut sections at 6 μm. These were mounted on Poly-D-lysine coated slides and then dewaxed in xylene (three washes of 10 min at room temperature).

We used antisense and sense riboprobes derived from an *Arabidopsis LFY* cDNA clone (Weigel et al., 1992) and from violet cress *LFY* (*vcLFY*) exons. The latter probe was generated by amplifying exons I and III of *vcLFY* and subcloning the PCR products into the TA PCR-cloning vector (Invitrogen, Carlsbad, California, USA). For all the probes, digoxigenin-labeled sense and antisense probes were synthesized from the clones by in vitro transcription using SP6 and T7 RNA polymerases (Boehringer-Mannheim, Indianapolis, Indiana, USA). The violet cress probe used for in situ hybridization was an equimolar mix of the exon I and exon III probes.

Probe hydrolysis, prehybridization, and hybridization were modified from Shu et al. (in press). Proteinase K treatment used 20 μg/mL for 20 min at 37°C, and RNase A treatment used 5 μg/mL for 20 min at 37°C. Each millilitre of prehybridization/hybridization solution contained: 125 μL 10X in situ hybridization buffer (3.0 mol/L NaCl, 0.1 mol/L Tris pH 6.8, 0.1 mol/L Na phosphate pH 6.8, 50 mmol/L EDTA), 500 μL deionized formamide, 250 μL 50% dextran sulfate, 25 μL 20 mg/mL tRNA, 60 μL 5 mg/mL poly A, and 40 μL ddH_2O. Hybridization was run at 52°C overnight. A range of post-hybridization washing stringencies were used, the highest being 0.25 × SSC at 42°C for 30 min. Immunolocalization and colorimetric detection used color substrates NBT and X-phosphate (Boehringer-Mannheim, Indianapolis, Indiana, USA). Sections were observed using an Olympus BX60 compound microscope under dark-field. Image resizing and background masking was carried out using Adobe Photoshop 5.0 (Adobe Systems Inc., Mountain View, California, USA).

RESULTS

Shoot morphological development—Once flowering commenced in violet cress (usually at about node 9), all subsequent nodes produced both a flower and a subtending bract (Fig. 1A). These paired structures appeared as a single bulge on the flanks of the shoot apical meristem. Soon afterwards, however, paired primordia could be distinguished: an apical flower primordium and a more basal bract primordium (Fig. 1B). As they matured, leaves and bracts developed a pair of linear stipules (Fig. 1B). Violet cress flowers were observed to pass through a similar series of developmental stages to those defined for *Arabidopsis* by Smyth, Bowman, and Meyerowitz (1990).

Isolation of LEAFY homologs—Alignment of the five clone sequences with *Arabidopsis LFY* (Weigel et al., 1992) showed that they were *LFY* homologs, here named *vcLFY*. Intron/exon boundaries were inferred based on intron positions in *LFY* (Wiegel et al., 1992). The inferred exons of *vcLFY* and *LFY* showed 90% nucleotide identity and 91.5–92.3% amino acid identity (Fig. 3). In addition, there were seven inferred single amino acid insertion/deletion events separating *vcLFY* and *LFY*. Most of the divergence in inferred protein sequence was localized in the proline-rich domain of exon I and the acidic domain of exon II (Weigel et al., 1992; Fig. 3). There was perfect

←

Fig. 3. (A) Alignment of the inferred amino acid sequences of *LFY* and *vcLFY*. (B–C) Alignment of introns I (B) and II (C) for *vcLFY1*, *vcLFY2*, and the putative hybrid sequence *vcLFY1/2*. *Arabidopsis LFY* was too divergent to include in intron alignments. Note that *vcLFY1/2* resembles *vcLFY1* for intron I but *vcLFY2* for intron II.

conservation of amino acid sequence in the second half of exon II and in exon III, suggesting that these regions have been under the strongest selective constraint. The *vcLFY* and *LFY* intron sequences were so divergent that alignment was impossible.

Three of the five sequenced clones fell into a class, here named *vcLFY1*. These three *vcLFY1* clones differed from their shared consensus sequence by 0, 1, and 2 sequenced bases. These discrepancies are tentatively ascribed to nucleotide misincorporation during the polymerase chain reaction (PCR) rather than allelic variation. One clone, here labeled *vcLFY2*, differed from *vcLFY1* by 57 substitutions and 15 insertion/deletions. The differences between *vcLFY1* and *vcLFY2* were concentrated in the introns (Fig. 3B–C) with only two differences in the deduced amino acid sequence (Fig. 3A). The fifth clone was identical to *vcLFY1* for most of the sequence, but the 3′ end of intron II was identical to *vcLFY2* (Fig. 3C). This sequence is, thus, most likely a recombinant between the other classes generated during PCR (Bradley and Hillis, 1997). Consequently, while at least two distinct *LFY*-like sequences were detected in violet cress, there is no clear evidence for more than two *vcLFY* genes. The probe used for in situ hybridization was composed of exon I from *vcLFY1* and exon III from *vcLFY2*. Given the high sequence similarity in these exons the combined probe would, most likely, detect either transcript.

Gene expression—Both the *LFY* and *vcLFY* antisense probes gave a localized, reddish signal in some sections (Fig. 4). In contrast, adjacent sections hybridized to sense probes never showed any such signal (e.g., Fig. 4A-B insets). Therefore, we here interpret an accumulation of staining as representing an accumulation of *LFY/vcLFY* transcript.

Expression was low or undetectable in young violet cress seedlings before the nine-leaf stage (data not shown). The first flowers produced were associated with weak but distinct *vcLFY* expression in the shoot apical meristem and the presumptive flower and bract primordia (Fig. 4A). Compared to *Arabidopsis* seedlings producing their first flower primordia (12-leaf stage; Fig. 4B), violet cress plants showed much more diffuse expression with almost as much transcript localized in the shoot apical meristem as in the flower primordium. The basic pattern of *vcLFY* expression across the primary shoot apex remained unchanged in older violet cress plants (Fig. 4C-D).

The changes in expression with flower stage were similar for violet cress as reported for *Arabidopsis* (Weigel et al., 1992). Flowers at stages 1–2 showed expression evenly across the apex of the primordium (Fig. 4E–F). In stage 3, both violet cress (Fig. 4G) and *Arabidopsis* flowers (Fig. 4H) showed transcript accumulation in the outer (calyx) whorl. In both species, older flowers showed maximal expression in developing stamens and ovaries (e.g., Fig. 4I–J).

The spatial distribution of *vcLFY* in GA-treated seedlings is qualitatively similar to that seen without hormone application. However, GA-treated seedlings appeared to have enhanced *vcLFY* expression, especially in leaves and the apical dome (compare Fig. 4C vs. K). While this effect cannot be quantified without use of a constitutively expressed control probe, it suggests that *vcLFY* expression is sensitive to GA levels in much the same way as *LFY* in *Arabidopsis* (Blázquez et al., 1998).

DISCUSSION

Molecular evolution in the LFY gene family—Two *vcLFY* sequence classes were detected in the violet cress genome. Without additional work, it cannot be determined conclusively whether *vcLFY1* and *vcLFY2* represent distinct loci or two alleles at a single locus. Nonetheless, the relatively high divergence in the introns suggests that these genes have not been subject to recombinational homogenization and that they, therefore, might represent duplicated *LFY* paralogs. If so, the great similarity of *vcLFY1* and *vcLFY2* exons to each other as compared to *Arabidopsis* (Fig. 3) suggests either that the gene duplication event occurred much after the divergence of *Arabidopsis* and violet cress or that there has been effective concerted evolution.

In considering the possibility that *vcLFY1* and *VCLFY2* represent distinct loci, it is necessary to evaluate whether violet cress is diploid or tetraploid. The chromosome number of violet cress is $2n = 24$ (Heywood, 1992). Given the frequent occurrence of $2n = 12$ in *Cochlearia* and *Iberis*, the putative close relatives of *Jonopsidium*, it is likely that violet cress has a tetraploid ancestry. Since the range of chromosome numbers within *Jonopsidium* is $2n = 22–36$ (Luque and Lifante, 1991; Heywood, 1992) it is most plausible that tetraploidization occurred at the base of the genus. Nonetheless, although this scenario is plausible, without examining other *Jonopsidium* species for *LFY* copy number, one cannot rule out there having been a specific gene duplication event on the lineage leading to *J. acaule*.

The implications of the possible duplication of *LFY* for the evolution of rosette flowering remain uncertain. Blazquéz et al., (1997) showed that increasing *LFY* copy number in *Arabidopsis* can increase *LFY* expression and induce precocious flowering. However, even if violet cress has two *LFY* loci, this would not necessarily imply increased expression because evolutionary dosage compensation is likely. The lack of stop codons and the high sequence similarity in the *vcLFY* exons relative to the introns suggests that both genes are functional. However, without paralog-specific probes for in situ hybridization we do not know whether both genes are expressed in the same pattern. Therefore, further work is needed to determine the number of *vcLFY* paralogs and how, if at all, their expression differs.

Comparison of LFY and vcLFY expression—*LFY* expression in wild-type *Arabidopsis* was characterized by Weigel et al. (1992) using in situ hybridization. Use of a reporter construct suggested additional *LFY* expression in the vegetative phase (Blázquez et al., 1997). Similarly, immunolocalization of LFY protein showed that whereas RNA levels decline in the center of flowers at stage 3 (Fig. 4H; Weigel et al., 1992), protein levels persist evenly across the primordium (Parcy et al., 1998). Apart from these minor discrepancies, all three methods provided similar information and suggested that *LFY* is upregulated when *Arabidopsis* plants begin to flower and that the

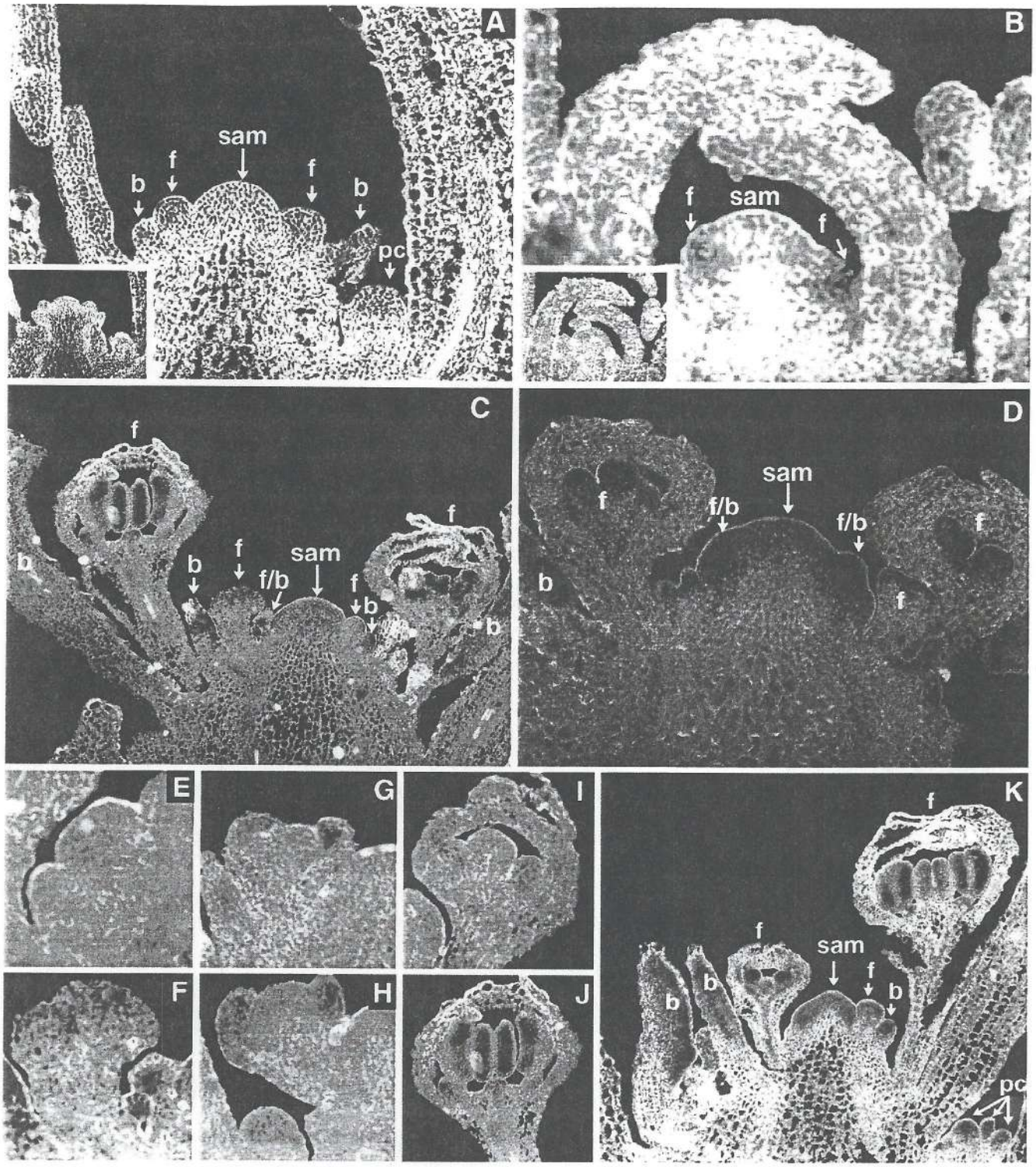


Fig. 4. In situ hybridization results. (A–D) Longitudinal sections of violet cress (A, C, D) or *Arabidopsis* (B) shoot apices probed with antisense or sense probes. (A) Nine-leaf violet cress with *vcLFY* antisense and sense (inset) probes. Two flower primordia with subtending bract primordia are visible. (B) Twelve-leaf *Arabidopsis* probed with antisense or sense (inset) *LFY* probes. Two presumptive flower primordia visible. (C) Twelve-leaf violet cress probed with antisense *vcLFY*. (D) Mature (>20 leaves) violet cress probed with antisense *LFY* probe. (E–J) Longitudinal sections of violet cress (E–G, I–J) or *Arabidopsis* (H) flower primordia probed with *vcLFY* (E–G, I–J) or *LFY* (H) probes. Stages of flower development follow Smyth, Bowman, and Meyerowitz (1990). (E) Stage 1. (F) Stage 2. (G–H) Stage 3. (I) Stage 6. (J) Stage 7–8. (K) Longitudinal section of the shoot apex of a 12-leaf, GA-treated violet cress plant probed with antisense *vcLFY* probe.

gene product accumulates in developing flower primordia. Therefore, it is reasonable to suppose that in situ hybridization in violet cress can provide useful information on the pattern of *vcLFY* expression during violet cress development.

In general, the pattern of *vcLFY* expression that we observed in violet cress is similar to that found in *Arabidopsis*. In both species, *vcLFY* signal is weak in young vegetative tissue and is much stronger in flower primordia. Young *Arabidopsis* leaves frequently express *LFY* (e.g., Blázquez et al., 1997) in much the same way as we have observed for *vcLFY* in violet cress. Flower primordia of *Arabidopsis* and violet cress show similar stage-specific patterns of *LFY/vcLFY* expression. Finally, in both species, gene expression appears to increase after the application of exogenous GA (Blázquez et al., 1997) and GA accelerates flowering (Blázquez et al., 1997; Shu et al., unpublished data). Therefore, our data suggest overall conservation in the regulation of *vcLFY* and *LFY*.

Relative to *Arabidopsis*, violet cress shows stronger *vcLFY* expression in leaves/bracts and in the shoot apical meristem (Fig. 4A–D). The presence of *LFY* homolog expression in bracts has been observed in other, distantly related species such as *Antirrhinum* (Coen et al., 1990) and, given the lack of bracts in *Arabidopsis*, this can hardly be treated as a meaningful difference. However, the presence of *vcLFY* transcript in the shoot apical meristem in areas that are not destined to make floral primordia is noteworthy because, in *Arabidopsis*, the spatial regulation of *LFY* expression seems to be important for normal bract suppression. Expression of *LFY* under the control of the more or less constitutive *35S* promoter results in plants failing to suppress bracts (Weigel and Nilsson, 1995). In contrast, overexpression of *LFY* by the introduction of additional copies under the control of the endogenous *LFY* promoter results in early flowering, but not the production of bracts (Blázquez et al., 1997). These observations suggest that it is changes in the spatial or temporal pattern of expression rather than expression level per se that causes bract formation in *35S::LFY Arabidopsis*. It is, therefore, a possibility that an expanded zone of *vcLFY* expression is the proximate cause of bract formation in violet cress.

In long-day growth conditions the number of true leaves below the first flower in violet cress is 8.1 ± 1.2 (Amaral, 1998). This contrasts with 18.7 ± 3.4 leaves for the inflorescence flowering *Jonopsidium abulense*, under the same growth conditions (Amaral, 1998). Furthermore, unlike *J. abulense* and *Arabidopsis*, the position of the first flower produced by violet cress is only slightly affected by daylength. Violet cress grown in short days produced 13.8% additional leaves before flowering (9.2 ± 1.3 leaves; Amaral, 1998), whereas short-day grown *J. abulense* produced 32.1% more leaves than long-day grown plants (24.7 ± 3.0 leaves; Amaral, 1998). Thus, the life-history strategy of violet cress seems to entail rapid commencement of reproduction regardless of environmental cues.

In principle, the evolution of precocious flowering could involve genetic changes either upstream or downstream of *LFY*. That is to say, it could reflect an early upregulation of *LFY* or it could result from flowers being formed in the absence of *LFY* activity. The in situ hybridization data show that the earliest flower primordia produced by violet cress express *vcLFY*. This suggests that precocious flowering in violet cress could arise from developmentally accelerated initiation of *LFY* expression, or a more rapid increase in transcript level to the threshold needed for flowering. One possible cause of precocious *LFY* expression is gene duplication, mirroring the effect of introducing two copies of *LFY* into *Arabidopsis* (Blázquez et al., 1997). Other possibilities include evolution in upstream flowering time genes or changes in the *cis*-regulatory elements at one or both *vcLFY* locus.

Conclusions and prospects—Our in situ hybridization data suggest that changes in the expression of *LFY* on the lineage leading to violet cress could have played a role in the evolution of early flowering (precocious expression) and bract production (diffuse expression). It should be stressed, however, that when trying to understand the genetic basis of species differences, gene expression provides only correlative evidence. Nonetheless, in situations where the genetic pathway is well understood evaluation of the expression of multiple interacting genes could help us identify the most likely cause of an interspecies difference (Kellogg, 1996). Therefore, it would be useful to study the expression of violet cress homologs of known regulators of *LFY*. For example, it would be desirable to study *TERMINAL FLOWER 1* because this gene influences *LFY* expression and has been hypothesized to be important in inflorescence evolution (Bradley et al., 1996, 1997).

Notwithstanding the potential value of gene expression data, transformation experiments could bring an added level of clarity (see Baum, 1998). For example, if the *vcLFY* locus was introduced into *Arabidopsis* and caused precocious flowering and/or bracteate flowers, one would have good reason to suspect that *vcLFY* (or its *cis*-regulatory elements) played a direct role in the evolution of rosette flowering. Such information would not only shed light on the genetic basis of inflorescence evolution in violet cress, but could also help us to understand the general mechanisms of inflorescence development and bract suppression in *Arabidopsis* and other Brassicaceae.

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RESEARCH ARTICLE

EgLFY, the *Eucalyptus grandis* homolog of the *Arabidopsis* gene *LEAFY* is expressed in reproductive and vegetative tissues

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The *EgLFY* gene cloned from *Eucalyptus grandi*s has sequence homology to the floral meristem identity gene *LEAFY (LFY)* from *Arabidopsis* and *FLORICAULA (FLO)* from *Antirrhinum*. *EgLFY* is preferentially expressed in the developing eucalypt floral organs in a pattern similar to that described previously for the *Arabidopsis LFY*. *In situ* hybridization experiments have shown that *EgLFY* is strongly expressed in the early floral meristem and then successively in the primordia of sepals, petals, stamens and carpels. It is also expressed in the leaf primordia of adult trees. The expression of the *EgLFY* coding region under control of the *Arabidopsis LFY* promoter could complement strong *lfy* mutations in transgenic *Arabidopsis* plants. These data suggest that *EgLFY* plays a similar role to *LFY* in flower development and that the basic mechanisms involved in flower initiation and development in *Eucalyptus* may be similar to those occurring in *Arabidopsi*s.
Key words: *Eucalyptus*, flowering, *LEAFY*, reproductive development.

***EgLFY*: O homólogo em *Eucalyptus grandis* do gene *LEAFY* de *Arabidopsis* é expresso em tecidos vegetativos e reprodutivos:** O gene *EgLFY*, clonado de *Eucalyptus grandis*, possui homologia de seqüência com o gene de identidade meristemática *LEAFY* (*LFY*) de *Arabidopsis* e *FLORICAULA* (*FLO*) de *Antirrhinum*. *EgLFY* é, preferencialmente, expresso nos órgãos florais em desenvolvimento de eucalipto, obedecendo a um padrão similar ao descrito para o gene *LFY* de *Arabidopsis*. Experimentos de hibridização *in situ* mostraram que o gene *EgLFY* é fortemente expresso em meristemas florais no início de seu desenvolvimento e, então, sucessivamente, durante a formação dos primórdios de sépalas, pétalas, estames e carpelos. Há expressão também nos primórdios foliares de árvores adultas. A expressão da região codificadora de *EgLFY* sob o controle do promotor do *LFY* de *Arabidopsis* pôde complementar mutantes *lfy* nulos em plantas de *Arabidopsis* transgênicas. Essas observações sugerem que o gene *EgLFY* possui um papel similar ao de *LFY* no desenvolvimento floral e que os mecanismos básicos envolvidos na iniciação e no desenvolvimento floral em *Eucalyptus* podem ser semelhantes aos de *Arabidopsis*.
Palavras-chave: eucalipto, desenvolvimento reprodutivo, *LEAFY*, florescimento.

INTRODUCTION

In the model species *Antirrhinum* and *Arabidopsi*s, the apical meristem switches from vegetative to floral development as plants enter the reproductive phase (Coen and Meyerowitz, 1991; Hempel et al., 1994).

In *Antirrhinum* and *Arabidopsi*s, the shoot apical meristem (SAM) initiates lateral primordia that develop into either shoots or flowers. The development of flowers instead of shoots is mediated by the action of floral meristem identity genes which include *LEAFY (LFY) in Arabidopsis* (Weigel et al., 1992) and its homologue *FLORICAULA (FLO)* in *Antirrhinum* (Coen et al., 1990). Inactivation of the *FLO* gene in *Antirrhinum* causes formation of indeterminate shoots in place of flowers and in *Arabidopsis lfy* mutants the structures that would normally develop into flowers develop into structures intermediate between shoots and flowers. FLO and LFY share 70% amino acid identity and each has a proline rich region and an acidic domain, which indicates their possible role as transcriptional activators (Coen et al., 1990). In *Arabidopsi*s, *LFY* has been found to activate homeotic genes, which regulate floral organogenesis (Weigel and Meyerowitz, 1993). Both *LFY* and *FLO* are expressed in the

floral meristem prior to initiation of floral organ primordia while expression at later stages of floral development in both species is less conserved (Coen et al., 1990; Weigel et al., 1992). In *Antirrhinum*, *FLO* expression is also observed in the leaf-like bracts which subtend the flower (Coen et al., 1990). *LFY* might act in suppressing bract formation in wild-type *Arabidopsis* since in *lfy* mutants lack of functional *LFY* RNA leads to ectopic bract formation (Weigel et al., 1992).

In contrast to what is observed for *Arabidopsis*, the apical meristem in eucalypts (*Eucalyptus* spp, Myrtaceae) generally remains vegetative. Lateral meristems, formed in the axils of the leaves, may give rise to a leafy shoot or to an inflorescence in response to inductive environmental conditions, such as day-length and temperature, if the tree is sufficiently mature (Drinnan and Ladiges, 1991). The *E. grandis* inflorescence is determinate and converts directly to a floral meristem(s). Both the inflorescence and flower meristems are completely enveloped by a pair of bracts which protect the primordia. While eucalypt flower buds and flowers are obviously structurally different from those of *Arabidopsis* and *Antirrhinu*m, the pattern and timing of organ development is similar in the three species (see figure 1). Within the bracts enclosing the eucalypt inflorescence (figure 1C), the flower is initiated on the sides of the floral meristem as four protusions, corresponding to sepals, which enlarge, elongate and rapidly fuse, forming the outer layer of the protective structure known as the calicine operculum (Pryor and Knox, 1971; Drinnan & Ladiges, 1991; Steane et al., 1999). The four primordia from the second whorl, which normally give rise to petals in *Arabidopsi*s, arise similarly in *Eucalyptus*, forming the inner (coroline) operculum (figure 1D). Stamen primordia, often in the number of several hundreds, arise in tightly packed whorls surrounding the central gynoecium and correspond to the third whorl of *Arabidopsis* and other plants. The gynoecium generally consists of four to five carpels in the innermost whorl (figure 1D). Early during reproductive development the bracts covering the flowers are shed. Depending on the *Eucalyptus* species, the calicine operculum also dehisce during early floral development (Steane et al., 1999; Drinnan & Ladiges, 1991). At anthesis, the coroline operculum is shed and the prominent stamens surrounding the single style are clearly visible (figure 1E).

As some common developmental features exist between the flower ontogenesis in eucalypts and model species such as *Arabidopsis* and *Antirrhinu*m, it may be suggested that the key floral regulatory genes, described for these model species, would be conserved in eucalypts (Southerton et al., 1998a,b). Nevertheless, it is expected that these genes would display some altered patterns of expression consistent with the unique structural features of the eucalypt flower.

Orthologs of FLO/LFY have been cloned and characterized in several woody perennial species such as Monterey pine *(Pinus radiat*a; Mellerowicz et al., 1998; Mouradov et al., 1998), *Populus trichocarpa* (Rottmann et al., 2000) kiwifruit *(Actinidia deliciosa*; Walton et al., 2001) and grape vine (*Vitis vinifera*; Carmona et al., 2002). Additionally, Southerton et al. (1998) described the cloning of a *LFY* homolog from *Eucalyptus globulus* and suggested that the biological function of *LFY* may be conserved in woody species. However, its specific role in the characteristic features of tree reproductive development has not yet been elucidated. Furthermore, partial or total *FLO/LFY*-like sequences have been reported from other basal angiosperms and gymnosperms (Frohlich and Meyerowitz, 1997; Frohlich and Parker, 2000), although in these cases functional information is not available.

We are currently studying genes involved in the early stages of floral development in woody tropical angiosperm trees. In this paper we describe the cloning of the *Eucalyptus grandis LFY/FLO* putative homolog (named *EgLFY*). We also describe and analyze its expression pattern during eucalypt reproductive and vegetative development. The *EgLFY* gene appears to be the functional homolog of *LFY* as deduced from data on its expression patterns during eucalypt reproductive development and from complementation experiments with *Arabidopsis lfy* mutants.

MATERIAL AND METHODS

Plant Material: Samples of vegetative and reproductive tissues of *Eucalyptus grandis* (var. Coffes Harbour) were collected in the fields of the Escola Superior de Agricultura Luiz de Queiroz, at the University of São Paulo (Piracicaba, SP, Brazil). Young expanding leaves were also used for isolation of genomic DNA. RNA-blot and *in situ* hybridization and SEM analyses were performed on plant tissues collected and fixed in different developmental stages during two growing seasons.

Cloning of EgLFY: Genomic DNA for PCR amplification, Southern analysis and construction of genomic libraries was isolated by the tradit ional CTAB-based method (Sambrook et al., 1989). Total RNA samples for cDNA library construction and Northern Blot were isolated from eucalypt leaves, vegetative apices and from a mix of inflorescences at different developmental stages using the Rneasy plant minikit (QIAGEN) following the supplier's instructions.

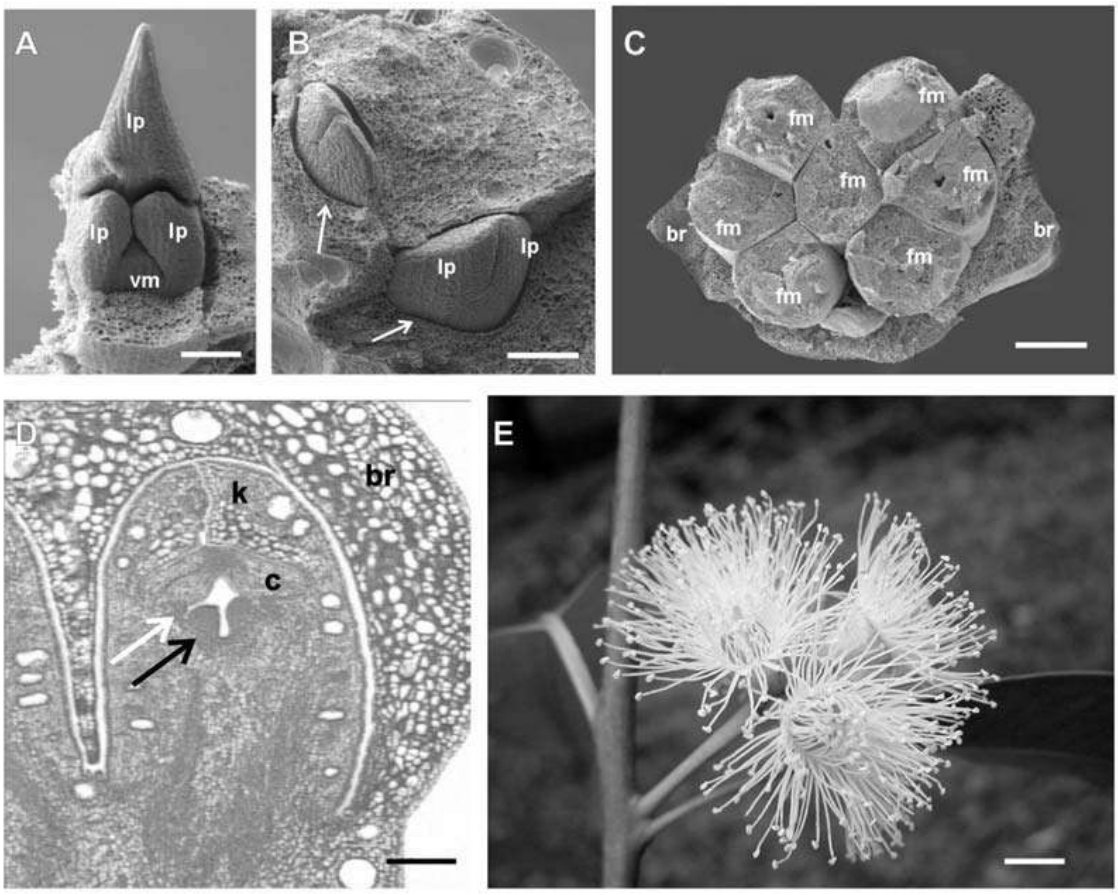

Figure 1. Development of *Eucalyptus grandis* shoots and floral buds. **A-C**: Scanning electron micrographs (SEM). **D**: longitudinal section in light microscopy. **A**: A terminal shoot with some of the leaf primordia (lp) removed to reveal the vegetative meristem (vm). The opposite and decussate phyllotactic pattern can be seen at the apex where two leaf primordia are forming opposite to each other and at right angles to the previous pair of leaves that have been removed. **B**: Young axillary vegetative buds (arrows) are forming opposite leaf primordia (lp). **C**: A developing axilary inflorescence, containing 6 flower meristems (fm) at roughly the same developmental stage. Bract primordia (br) that protect the inflorescence were removed. **D**: A longitudinal section though a developing flower. **k**: calicine operculum (fused sepals); **c**: coroline operculum (fused petals). The white arrow points to the site of stamen primordia development and the black arrow points to developing carpel primordia. **E**: Mature *E. grandis* inflorescence showing flowers at anthesis. Bars: A, B and F: 50 µm; C: 500 µm; D: 100 µm; E: 1cm.

The genomic clones of *EgLFY* were isolated by screening 165,000 plaques from an *E. grandis* genomic library (22 x 10^{-6} pfu) constructed with partially *Sau*3A-digested genomic DNA, using the Packagene Lambda Packing Systems (Promega). For this screening, we have used a biotin-labeled probe (North2South chemiluminescent system, Pierce) using the entire *Arabidopsis LFY* cDNA from plasmid pDW124 (Weigel et al., 1992) as a template. Two adjacent BamHI fragments (E28B with 2Kbp and E6B with 6Kbp) spanning the entire genomic *EgLFY* sequence were subcloned into pBluescriptKS (Clontech). Subclones were prepared by nested deletions (Zhu and Clark, 1995) and sequenced on an ABI Prism 377 (Perkin-Elmer/Applied Biosystems) automated sequencer using the DYEnamic ET terminator Cycle Sequencing Kit (Amersham/Pharmacia Biotec, USA) coupled with M13 reverse and forward primers following the manufacturer instructions.

A cDNA library was constructed using total RNA from a mix of *E. grandis* inflorescences at different developmental stages. The poly-A fraction of RNA was isolated and the first strand of cDNA was synthesized using the SuperScript cloning system (Life Technologies). The cDNA library screening was performed using a PCR-based strategy (Sussman et al., 2000) and the LFY-specific degenerated primers L1: 5′-CGGAYATIAAYAARCCIAARATGMGICAYTA-3′ and L4: 5′-CGGATCCGTGICKIARIYKIGTIG-GIACRTA-3′

(Frohlich and Meyerowitz, 1997). The insert size of the positive clones was determined by PCR using the M13 forward and reverse primers and the three longest clones were sequenced on both strands. The sequences of both genomic and cDNA versions of the *EgLFY* gene were deposited at GenBank databases with the accession numbers AY640313 and AY640314, respectively.

Southern and Northern Hybridization: Southern blotting was performed as described in Sambrook et al. (1989) using genomic DNA digested with XhoI and PstI and blotted on Hybond-N Plus membrane (Amersham). Northern experiments were performed using ten micrograms of total RNA extracted from leaves, vegetative apices and from a mix of inflorescences at different developmental stages, separated in a denaturing agarose gel (Sambrook et al., 1989) and hybridized to an *EgLFY* probe.

The *EgLFY* probe used in both Southern and Northern experiments was a 235bp PCR product obtained from the 3´ transcribed region of the gene, using primers E13:5'-TGGCGGAGCTTGGTGGGGACA-3' and E25:5'-CTTCCTCCTCCAAGTCCAATC-3', and an'*EgLFY* cDNA as a template. PCR reactions were performed in a final volume of 25 ïL with an initial 3 min denaturation at 96ºC, followed by 40 cycles of 96ºC for 40 sec; 45ºC for 30 sec and 72ºC for 2 min. The PCR product was purified using the Concert Kit (Gibco-Life Sciences). The probe was labeled with fluorescein using the DCP-Star GeneImage System (Pharmacia-Amersham). Hybridization conditions, washing stringencies and detection were those suggested by the kit manufacturer. As a control for gel loading in Northern experiments, the stripped membrane was re-hybridized with a heterologous probe for a constitutively expressed gene, under low stringency, using a cDNA for an *Arabidopsis* ubiquitin (Gen Bank accession AB5432) as a template.

*In situ hybridization:*Digoxigenin labeling of RNA probes, tissue preparation and hybridization conditions were performed as described before (Dornelas et al., 1999, 2000). The template for the *EgLFY* digoxigenin-labeled riboprobes was the 1,400bp fragment, containing the complete coding region, cloned in pGEM-T easy vector. The hybridized sections were viewed immediately and photographed under a Zeiss Axiovert 35 microscope.

Scanning electron microscopy (SEM) and light microscopy: The collected plant material was immediately fixed in 4% paraformaldehyde under vacuum for 24 h and dehydrated with absolute ethanol, where they were stored at 4ºC until needed. For light microscopy the dehydrated samples were embedded in Historesin (Leica, hydroxyetilmethacrilate). The resin polymerization was carried out at room temperature for 48 h. After polymerization, serial sections of 5-8 µm were obtained and stained with 0.05% toluidine blue (Dornelas et al., 1992). The histological sections were observed and photographed under a Zeiss Axiovert 35 microscope.

Alternatively, the plant material was initially dissected in absolute ethanol under an Olympus dissecting microscope. The resultant material was dried under CO_2 in a Balzer´s critical point drier and further dissected, when necessary. The samples were mounted in metallic stubs with carbon conductive adhesive tape, coated with colloidal gold and observed at 10-20kV using a ZEISS DSM 940 A or a LEO 435 VP scanning electron microscope, at the University of Sao Paulo (ESALQ-NAP/MEPA).

Sequence comparisons: The trimmed partial *EgLFY* genomic and cDNA sequences obtained were aligned using Clustal W (Thompson et al., 1994), before being checked for similarity with sequences already deposited in public databases using BLASTX (Altshul et al., 1997). Nucleotide and protein sequences of different *LFY* homologs were retrieved from GenBank (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) and aligned with Clustal W (Thompson et al., 1994). Distance matrixes were obtained from the alignments and comparative trees were built using TreeView (Page, 2000).

Complementation of the Arabidopsis lfy-26 mutant: The *Xba*I–*Sma*I *EgLFY* fragment, carrying the coding region of *EgLFY*, with its endogenous start and stop codons, was obtained from plasmid pEGLFY and blunt-ended using DNA polymerase I (Klenow fragment). An intermediate pDW132E vector was prepared by cloning the polished fragment described above into the SmaI site of pDW132, containing the *Arabidopsis LFY* promoter (Weigel et al., 1992). The correct orientation of the cloning process was checked by endonuclease digestion. The *Pst*I-*Spe*I fragment from the resultant pDW132E (*LFY::EgLFY*) vector was blunt-ended with Klenow and cloned into the plant transformation vector pSKI015 (a gift from D. Weigel, Salk Insitute, LA Jolla CA, USA), which contains the BAR gene, allowing selection with the herbicide Basta (Sylvet), constituting the pSKI015E vector. *Arabidopsis* plants (Columbia ecotype) transgenic for pSKI015E T-DNA were obtained by using *Agrobacterium tumefacien*s-mediated *in planta* transformation, as described by Bechtold et al. (1998). Putatively transformed seeds were selected upon germination on sand wetted with a Basta (Sylvet) solution at 500 µL.ml^{-1}. Homozygous (Basta-resistant) lines were obtained by selfing the primary transformants. The

segregation ratio of resistant:sensitive was used to estimate the number of transgene insertions. T2 lines, homozygous for the *LFY*::*EgLFY* T-DNA locus, were identified by sowing 200–300 T2 seeds, derived from different T1 plants under selective conditions. Transgenic and non-transgenic plants were grown in growth chambers at 23°C under illumination with fluorescent lights: long day (LD) conditions (16 h of light / 8 h of dark) or short day (SD) conditions (8 h of light / 16 h of dark). Finally, *LFY::EgLFY* transformants in the Columbia ecotype were crossed to the strong *lfy-26* mutant allele in the *Landsberg erecta* background (wild-type and mutant *Arabidopsis* seeds were obtained from the ABRC seed stock at the Ohio State University, Columbus, Ohio, USA). To genotype F2 plants at the *LFY* locus, CAPS (Cleared Amplified Polymorphic Sequences; Konieczny and Ausubel, 1993) markers that distinguished between Columbia and Landsberg were used (URL:http://www.salk.edu/LABS/pbio-w/caps.html). Transgenic and non-transgenic *Arabidopsis* flowers and inflorescences at different developmental stages were photographed under a stereomicroscope or analyzed by SEM.

RESULTS

The EgLFY *gene is an expressed Eucalyptus grandis homolog of* LFY: The *EgLFY* gene contains two introns (figure 2) and encodes a putative protein with high sequence similarity to FLO/LFY-like proteins (figure 3). The deduced protein sequence of *EgLFY* is 95.2% identical to the previously published *ELF1* gene, the *LFY* homolog in *E. globulus* (Southerton et al., 1998b). The *EgLFY* gene encodes a putative protein of 359 amino acids, which is 67% identical to *Arabidopsis* LFY and 71% identical to the FLO protein (figure 3). These three protein sequences are most similar in their C-

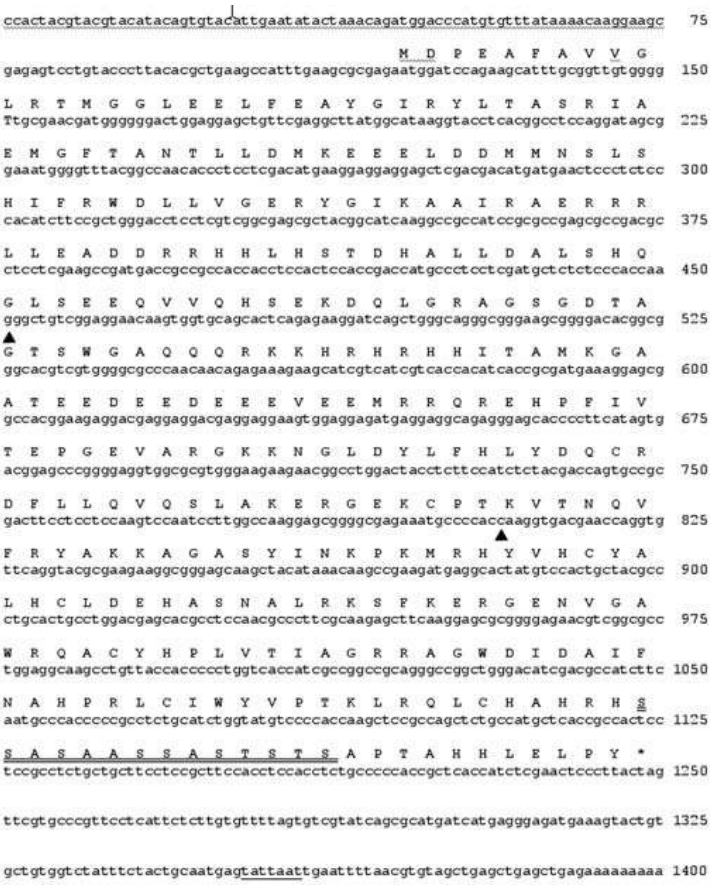

Figure 2. Nucleotide and deduced amino acid (single-letter code) sequence and structure of the *EgLFY* gene, an *E. grandis* homolog of *LFY*. The asterisk indicates the position of the stop codon. The arrowheads indicate the positions of the introns. The putative poly-adenylation site is underlined. The Ser-rich region at the C-terminal portion of the protein is double-underlined.

terminal regions. Beyond Arg-177, EgLFY is 80% identical to LFY and 84% identical to FLO. In this region, a stretch of 30 amino acids is identical in all three proteins, and a total of 156 amino acids in which virtually all changes are conservative replacements. N-terminal of Arg-177, the EgLFY protein is 55% identical to LFY and 58% identical to FLO. The EgLFY protein sequence contains a highly acidic region between glutamates 163 and 174, a short leucine zipper of leucines 45, 52 and 59, and a basic region between Arg-145 and His-153, all features observed in similar positions in the LFY and FLO sequences. EgLFY differs from LFY and FLO in that it lacks the proline rich region at its N-terminus and contains a serine and alanine rich region between Ser-335 and Ala-349.

The number of loci that hybridize with an *EgLFY* probe was investigated by Southern hybridization. This experiment was performed due to the report by Southerton et al. (1998) that *E. globulus* has a second *LFY*-like homolog that appears to be a pseudogene. Figure 4A shows a Southern blot of *Xho*I- and *Pst*I-digested genomic *E. grandis* DNA, probed with the *EgLFY* probe. Two hybridizing bands were detected at low-medium stringencies (washes in 2xSSC at 40ºC). Nevertheless, these additional bands could not be detected in Southern blot experiments when higher stringencies were used (0,1xSSC at 65ºC; data not shown). Thus, the presence of a second *LFY*-like gene in the *E. globulus* genome can not be ruled out. The Northern blot experiments (figure 4B) were always performed at high stringency and the cross-detection of transcripts of *LFY*-like loci other than *EgLFY* was unlikely. The Northern blot results (figure 4B) indicate that the expression of *EgLFY* is restricted to adult plants and that *EgLFY* is preferentially expressed in reproductive tissues.

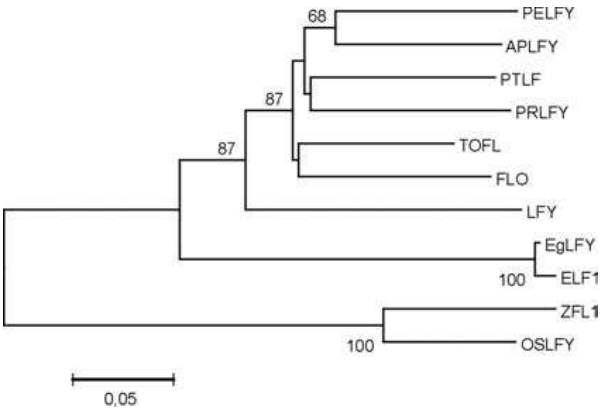

Figure 3. Phylogenetic relationships of EgLFY with other LFY/FLO homologs. The deduced amino acid sequence of EgLFY was compared with (accession nos. in parentheses): PTLF from *Populus balsamifera* (U93196); PRLFY from *Platanus racemosa* (AF106842), TOFL from tomato (AF197934), APLFY from apple (BAB83097); PEgLFY from pea (AAC49782), NTLFY from tobacco (U16172), ZFL1 from maize (AY179883), OSLFY from rice (AB005620), FLO from snapdragon (M55525), LFY from *Arabidopsis* and ELF1 from *E. globulus* (AF34806). Bootstrap support values (for 1000 replicates) are indicated when over 50.

EgLFY *is expressed in the tip of leaf primordia of adult trees and during floral organ development:* The expression pattern of *EgLFY* in vegetative and reproductive tissues was determined more precisely by *in situ* hybridization of longitudinal sections of vegetative and reproductive meristems of *E. grandis* (figure 5). No hybridization signal was detected in the shoot apical meristems of juvenile (6 months-old) plants (figure 5A), agreeing with the Northern blot results. In both apical and lateral vegetative meristems of adult (6 years-old) plants, the *EgLFY* transcripts were detected at the tip of the leaf primordia. No signal was detected in the shoot apical meristem itself (figures 5B and 5C). During reproductive development *EgLFY* expression was detected only in young floral buds, similar to the expression of the *FLO* and *LFY* genes in *Antirrhinum* and *Arabidopsis*, respectively. Eucalypt tissues tended to stain light brown during fixation, noticeably in oil glands and epidermal cells. However, the characteristic purple color generated from alkaline phosphatase substrates observed during the detection of the digoxigenin-labelled antisense probes was easily distinguished from the non-specific staining. No labeling other than background was observed in serial sections probed with sense probes (figure 5G). The patterns of *EgLFY* expression

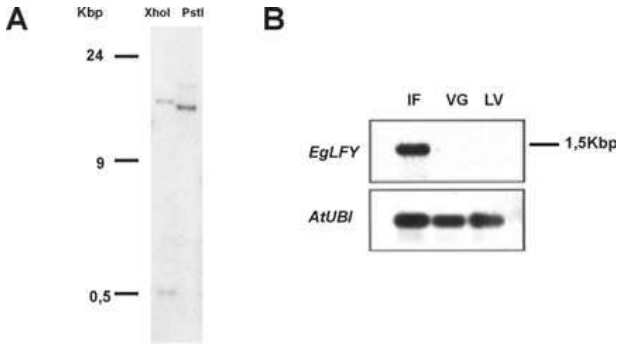

Figure 4. A. Southern blot of genomic DNA from *Eucalyptus grandis* probed with *EgLFY*. Lane 1, digested with *Xho*I; lane 2, digested with *Pst*I. **B**. Northern blot made with total RNA extracted from a mix of inflorescences in different developmental stages (IF), vegetative apices of juvenile plants (VG), young leaves of adult plants (LV) and probed with *EgLFY*. The same blot was re-probed with a heterologous *Arabidopsis* ubiquitin sequence (AtUBI) to show uniform loading and transfer of all lane contents.

in the floral buds of *E. grandis*, were similar to those described for *ELF1* expression in *E. globulus* and *E. macandra* (Southerton et al., 1998b) and a selection of the patterns observed at different floral stages are shown in figure 5. In developing flowers of *E. grandis EgLFY* was first detected uniformly in early floral meristems, before the onset of the floral organ primordia, (figure 5D). Later, the *EgLFY* hybridization signal was preferentially detected in areas corresponding to the developing floral primordia (figures 5E, 5H and 5I). Expression was briefly observed in sepal primordia and then in petal primordia (figures 5E and 5F). *EgLFY* expression declined in the sepals as they enlarged and fused, and was then observed in the petal primordia. As the petal primordia enlarged, expression became restricted to the center of the floral meristem, where the carpels form, and in the stamen primordia (figures 5H and 5I). Afterwards, expression declined in the petals and no hybridization signal was detected anymore in the operculum tissues. Expression was maintained during stamen development and in the region of the developing gynoecium, particularly in the developing ovules (data not shown). *EgLFY* expression was not detected in fully developed floral buds, but these tissues were extremely difficult to section and contained high levels of phenolic compounds and oils that interfered with proper *in situ* hybridization.

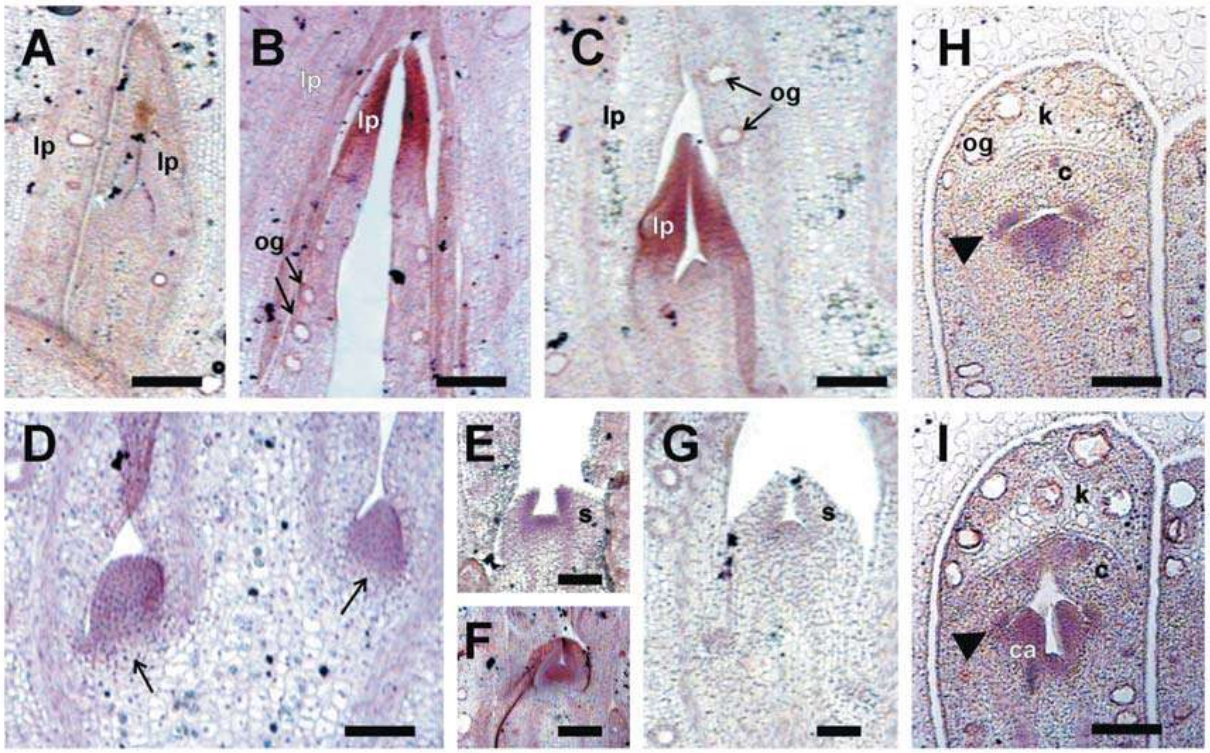

Figure 5. *In situ* localization of *EgLFY* transcripts during vegetative and reproductive growth of *E. grandis*. All sections are longitudinal. All hybridizations were done with the antisense probe, except when mentioned otherwise. The hybridization signal with the *EgLFY* probe was observed as a purple precipitate. **A**: vegetative meristem of a juvenile (6 months-old) plant. No signal was detected above background. **B**: *EgLFY* transcripts were detected at the tip of leaf primordia (lp) of the apical vegetative meristem of an adult (6 years-old) plant. **C**: Lateral vegetative meristem of an adult (6 years-old) plant showing no hybridization signal in the meristem and *EgLFY* transcript accumulation in the young leaf primordia. **D**: The early inflorescence meristems (arrows) expressed *EgLFY.* **E**: The *EgLFY* expression was detected in flower meristems and sepal primordia (s). **F**: Flower meristem hybridized with the *EgLFY* probe at a slightly later stage than that shown in **E**. **G**: Flower meristem at the same stage as that shown in **F** hybridized with an *EgLFY* sense probe. No hybridization signal was detected above background. **H**: Flower meristem at a late developmental stage, showing the fused calicine (k) and the coroline (c) opercula (**K**). This section is slightly oblique, so that the region of petal primordia fusion is not seen. The *EgLFY* expression was restricted to the site of stamen development (arrowhead) and at the center of the floral meristem. **I**: Flower meristem at a later developmental stage than that shown in **H**, the hybridization signal was more intense at the site of stamen formation (arrowhead) and in the carpel primordia (ca). **og**: oil gland. Bars: **A, B, C**: 50 μm; **D**: 25 μm; **E** and **G**: 20 μm; **F**: 15 μm; **H** and **I**: 100 μm.

The EgLFY *coding region can complement transgenic* Arabidopsis lfy *mutants:* When the *EgLFY* coding region was fused downstream to the *Arabidopsis LFY* promoter and introduced into the strong-phenotype *lfy-26 Arabidopsi*s mutant, complete restoration of the wild type development was observed (figure 6). The early arising (basal) flowers in the *Arabidopsis lfy-26* mutants were replaced by bracts adjacent to secondary inflorescence shoots, whereas later arising flowers were replaced by small bracts, in whose axils abnormal flowers developed (figures 6B and 6C; Weigel et al., 1992). These abnormal flowers contained sepals and carpels but no petals or stamens, these later being usually homeotically substituted by more sepals and carpels, respectively (figures 6C and 6D; Weigel et al., 1992). In contrast, wild-type flowers typically contain four sepals, four petals, six stamens, and two carpels. The *lfy*-26 floral phenotype was largely complemented by the *LFY::EgLFY* transgene. The main shoot of these plants developed flowers in both basal and apical positions, and most of these contained all four floral organ types (figures 6E and 6F).

DISCUSSION

We have isolated an expressed eucalypt *LFY* homolog named *EgLFY*. The *EgLFY* gene contains two introns that occur in identical positions to those found in all the described *LFY/FLO* homologs clones to date (Frohlich and Parker, 2000)

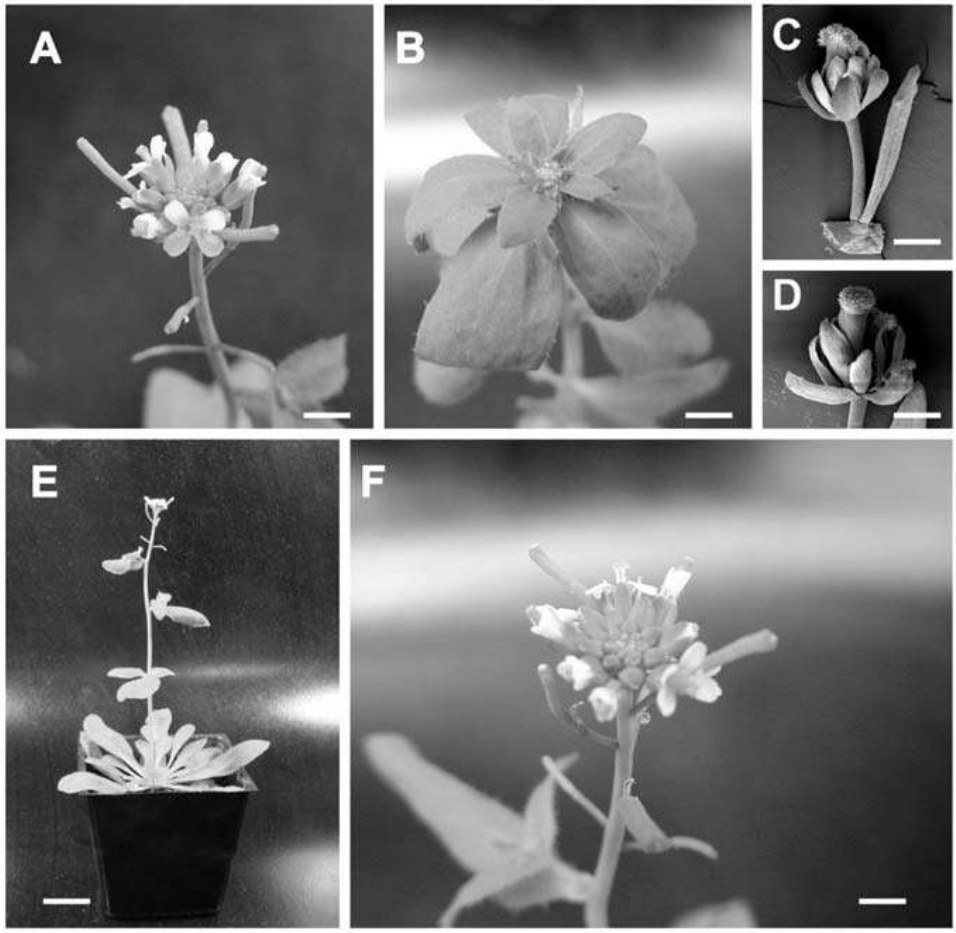

Figure 6: Complementation of an *Arabidopsis* strong *lfy* mutation by *EgLFY* expression driven by the *LFY* promoter. **A**: Wild type inflorescence (Columbia ecotype) showing flower buds at different developmental stages. **B**: main inflorescence axis of a *lfy-26* mutant. Solitary flowers are replaced by a cauline leaf (bract) adjacent to a lateral inflorescence axis or an abnormal flower. **C** and **D**: SEM images of abnormal flowers of the *lfy-26* mutant. Note the cauline leaf in **C** and the homeotic conversion of petals and stamens in sepal-like organs and carpel-like organs, respectively. **E**: A homozygous transgenic (LFY::*EgLFY*) *lfy-26* mutant showing a wild type phenotype, indicating the complementation of the *lfy* mutation by the expression of *EgLFY*. **F**: A higher magnification view of the inflorescence of the same plant shown in **E**. Bars: **A**, **B** and **F**: 3 mm; **C**: 300 μm; **D**: 250 μm; **E**: 3cm.

and its sequence and expression patterns are very similar to those described for most dicot *LFY*/*FLO* homologs in the literature. Expression of *EgLFY* driven by the *Arabidopsis* LFY promoter is able to restore the wild type phenotype of transgenic *Arabidopsis lfy*-26 mutants. These close structural and functional similarities strongly suggest that *EgLFY* is the functional eucalypt homologue of *LFY/FLO*. *LFY/FLO* homologues similar to *EgLFY* have also been isolated from other plants (Frolich and Parker, 2000). Weigel and Nilsson (1998) have reported that transgenic hybrid aspen *(Populus tremula x P. tremuloide*s) constitutively expressing the *Arabidopsis LFY* cDNA flowers precociously and shows similar phenotypes to *Arabidopsis* transformed with the same construct. Similarly, Peña et al. (2001) also reported the early flowering of citrus plants overexpressing a *LFY* homolog. These data add further weight to the hypothesis that floral regulatory mechanisms, and hence regulatory genes, are conserved among the angiosperms. The putative protein encoded by *EgLFY* shares a number of sequence motifs with other characterized LFY/FLO proteins (Frolich and Parker, 2000). The acidic domain is not conserved with respect to sequence and occurs in a region of relatively poor sequence conservation among the *LFY* homologs. The putative EgLFY protein, as well as its *E. globulus* homolog (Southerton et al., 1998) is shorter at the N-terminal end when compared to other LFY/FLO homologs and thus lacks the proline rich region suggesting that this motif may not be functionally significant. None of these protein sequence motifs has yet been demonstrated to be functionally important in any of the floral meristem identity genes. It is of interest to note that eucalypts probably have two *EgLFY*-like genes, although one of these is probably now inactive (Southerton et al., 1998). This duplication is probably a general phenomenon within the genus, and suggests that eucalypts may have experienced ancient genome duplications and many of their genes might be expected to be present in at least two copies (Southerton et al., 1998). In addition to being expressed in floral primordia in a pattern similar to *LFY* and *FL*O, th*eEgLFY* gene is strongly expressed in leaf primordia forming on vegetative meristems of adult plants, but not in the shoot apical meristem itself. The overall pattern of expression of *EgLFY* is, however, similar to other described *LFY/FLO* homologues (Coen et al., 1990; Weigel et al., 1992; Southerton et al., 1998; Peña et al., 2001; Carmona et al., 2002).

Experiments by Hempel et al. (1994) and Blázquez et al. (1997) using *in situ* hybridization and GUS reporter gene expression driven by the *LFY* promoter have now also established vegetative expression of *LFY* in both vegetative apices and young leaves of three different ecotypes of *Arabidopsis* grown under short day conditions. The *Arabidopsis LFY* gene is the earliest of the known floral identity genes to be expressed, and directly activates at least one of the later genes, *APETALA1* (Wagner et al., 1999). Plants carrying fusions of the *LFY* promoter to the *GUS* marker gene were used to demonstrate that *LFY* expression responds both to the long-day flowering pathway and to gibberelic acid (GA). Furthermore, deletion of a putative MYB transcription factor binding site within the *LFY* promoter prevented activation by GA, but not by the long-day pathway (Blázquez and Weigel, 2000). We have failed to identify any putative MYB transcription factor binding site within the *EgLFY* promoter (data not shown). However, exogenous application of paclobutrazol reduced the concentration of endogenous GA in apical tissues of different *Eucalyptus* species and enhanced the reproductive activity of grafted trees (Moncur and Hasan, 1994), suggesting that in *Eucalyptus*, high concentrations of GA inhibits the flowering process, as opposed to what is observed in *Arabidopsis*. It would be interesting to investigate whether paclobutrazol can interfere with *EgLFY* expression.

Although the available information suggests that overexpression of *LFY* is sufficient to promote the conversion of shoots into flowers in woody species such as *Populus* spp. (Weigel and Nilsson, 1995) and *Citrus* spp. (Peña et al., 2001), the role of the endogenous *FLO/LFY* homologs and their function during meristem development are poorly understood. Genetic studies in *Eucalyptus* are difficult because of the long time to flowering of trees and no characterized flowering mutants have been described in this genus. Nevertheless, recent advances in the transformation of *Eucalyptus* species (unpublished data from our own lab) and the large-scale cloning of a number of other floral gene homologues (https://forests.esalq.usp.br) may allow us to use reverse genetic approaches and to define more clearly the role played by *EgLFY* in *Eucalyptus* vegetative and floral tissues.

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GENETIC DIVERSITY OF *PINUS BRUTIA* IN SYRIA AS REVEALED BY DNA MARKERS

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ABSTRACT

Random amplified polymorphic DNAs (RAPDs) and amplified fragment length polymorphisms (AFLPs) were used to estimate the genetic diversity between and within 21 populations of *Pinus brutia* collected from five different regions of Syria. After screening 400 Operon Primers, only nine were able to detect polymorphism between the tree samples. The AFLP analysis also confirmed the low genetic variability. Even after digestion of monomorphic RAPD fragments with three restriction enzymes, no increased polymorphism between samples was revealed. The total number of identified polymorphic fragments (loci) between the 311 trees was 111 (74 RAPDs and 37 AFLPs). The highest level of genetic diversity was detected in the region of Latakia and the lowest one was detected in the region of Idleb. The genetic diversity detected within populations was higher than the one detected between populations. A dendrogram based on the results of the polymorphic RAPD and AFLP fragments reflecting the genetic distance between the analyzed *P. brutia* populations was developed. Our results showed that the general level of genetic variability in *P. brutia* populations collected in Syria was low. Relative uniformity of the landscape in the North Western part of Syria might add to the low genetic variability observed.

Key words: *Pinus brutia*, polymorhism, genetic variability, AFLP, RAPD.

INTRODUCTION

Pinus is the largest genus in the family *Pinaceae*, $2n = 24$ chromosomes with about 110–120 species. In the Mediterranean Basin the pine flora includes ten pine species. Although these Mediterranean pine forests cover only 5 % of the total area of the Mediterranean Basin, they comprise around 25 % of the forested area. Of these, *Pinus halepensis* Mill. and *Pinus brutia* Ten. are the most common species (BARBÉRO *et al.* 1998).

The natural distribution of *P. brutia* is confined mainly to the Eastern Mediterranean region covering NE Greece, the Black Sea, Turkey, Crete, Cyprus, Syria, Lebanon and Iraq. It tolerates a broad range of Mediterranean climates (EMBERGER *et al.* 1963), grows from sea level to 1500 m a.s.l. on a wide range of soil types (SELIK 1958; MIROV 1967), and is recognized for its adaptation to drought and alkaline soils (SPENCER 2001).

P. brutia is considered as a highly invasive species, occupying open disturbed sites e.g. through (fires). Due to the extreme Mediterranean climate and/or continuous human interaction, the relatively short-lived *P. brutia* trees are able to form stable pine vegetation associated with broad-leaved species (ZOHARY 1973; BARBÉRO *et al.* 1998). These criteria

have let to an increased interest in this species for commercial plantations during the last decennia, illustrated by breeding and provenance trails carried out in various countries in the Mediterranean region (PANETSOS 1981; ISIK 1986; BARITEAU 1992) and even outside (SOUVANNAVONG *et al.* 1995; SPENCER 1985 and 2001).

It is worrying however, that the *P. brutia* forests in Syria are not included in any of the above mentioned studies. Moreover, publications about these populations are scarce and have a general descriptive tendency (BARBÉRO *et al.* 1976; NAHAL 1983 and 1984). The Syrian forests of *P. brutia* play a vital role in the protection of the environment as they are part of it. Furthermore, it is a valuable seed source for the national reforestation program. Yet, the annual reoccurrence of fires and the continuing conversion of forest areas into agriculture have a negative impact on these natural resources.

Random Amplified Polymorphic DNA (RAPD) markers can be used for fast screening of nuclear genome variation (WILLIAMS *et al.* 1990; WELSH & MCCLELLAND 1990). Despite the frequent use of RAPD markers in crop species for various purposes, e.g. germplasm characterization (ZHANG *et al.* 1996; CHOUMANE *et al.* 1998; FERGUSON *et al.* 1998), the construction of genetic linkage maps (BAI *et al.* 1997; EUJAYL *et al.* 1998a; EUJAYL *et al.* 1999; HOQUE *et al.* 2002), and for the estimation of genetic diversity within and between species (RODRIGUEZ *et al.* 1999; MIGNOUNA *et al.* 1999; VIDAL *et al.* 1999), there are relatively few published reports of RAPD for forest trees (CARLSON *et al.* 1991, GRATTAPAGLIA *et al.* 1992; DALE *et al.* 1992, TULSIERAM *et al.* 1992; NELSON *et al.* 1993; KAYA & NEALE 1993; BINELLI & BUCCI 1994; GRATTAPAGLIA & SEDEROFF 1994; KAYA & NEALE, 1995; LU *et al.* 1995; ADAMS & TURUSPEKOV 1998).

The amplified fragment length polymorphism (AFLP) is another useful marker technology developed by VOS *et al.* (1995). AFLPs are robust and reliable because stringent reaction conditions are used for primer annealing. The AFLP technique combines the reliability of the restriction fragment length polymorphism (RFLP) with the power of the PCR techniques .This technique was widely used for germplasm characterization and genetic mapping in plants over the last five years (MAUGHAN *et al.* 1996; HILL *et al.* 1996; HE &PRAKASH 1997; HONGTRAKUL *et al.* 1997, EUJAYL *et al.* 1998a and b; ANGIOLILLO *et al.* 1999; GRACIA-MAS *et al.* 2000, TEULAT *et al.* 2000) but only few reports exist on its application in forest trees (ÅKERMAN *et al.* 1996,

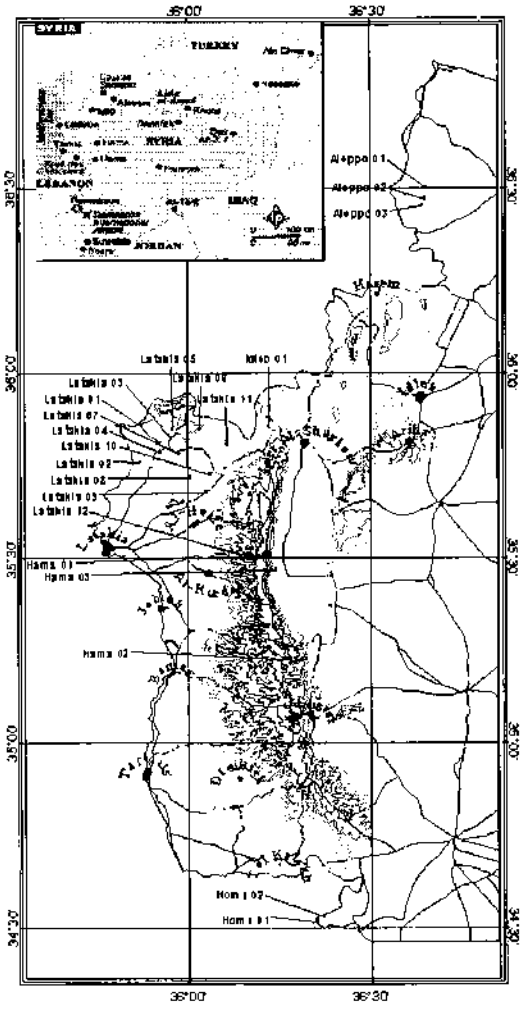

Figure 1. Collection sites of *P. brutia* on geographic map of Syria.

LERCETEAU & SZMIDT 1999, HAYASHI *et al.* 2001, YIN *et al.* 2003).

This study is, as far as we know, the first attempt to better understand the genetic processes operating within and between *P. brutia* populations in Syria, which is critical for successful preservation of the ecosystems they live in. For this purpose, RAPD and AFLP markers were used to evaluate the genetic variability within and between Syrian *P. brutia* populations.

MATERIAL & METHODS

Plant Material

Based on an ecogeographical survey, the distribution of *P. brutia* was mapped in Syria (IPGRI, unpublished). Five distinct and geographically separated regions with different climatic conditions were selected. They were Latakia, Aleppo, Hama,

Table 1. Number of populations*, regions and characteristics of the collection sites.

	Site characteristics[2]								Average tree characteristics[5]			
Site number	Latitude[1]	Longitude[1]	Altitude[1]	Slope (degree)	Exposure	PMQ[3]	m[4] (gegrees)	Description of collection sites	Region	Height (m)	Dbh[6] (cm)	Height first branch (m)
1	36.501	36.649	501	21	141	55.83	3	Area with extensive agriculture, pasturelands and large patches of pine forests	Aleppo	8.5	23.9	1.8
2	36.472	36.650	479	23	223.5	54.82	2.33		Aleppo	6.7	24.6	2.6
3	36.458	36.643	516	11	204	55.06	2.47		Aleppo	6.8	25.7	1.8
4	35.507	36.225	618	20	61.5	88.45	1.5	Isolated patch of pine forests bordered by maquis vegetation, pasture fields and agricultural lands	Hama	7.9	25.7	2.3
6	35.467	36.242	290	16	97.5	72.18	3.73		Hama	7.1	29.1	1.5
5	35.212	36.306	658	22	243	90.54	3.5	Isolated small *Pinus brutia* forest fragment bordered by maquis vegetation and some agricultural lands	Hama	7.2	21.7	1.1
7	34.517	36.344	1291	16	230.4	49.04	0.61	Isolated patch of *Pinus brutia* forest bordered by maquis and extensive pasture lands	Homs	7.2	34.8	1.9
8	34.527	36.368	1068	24	132	57.27	1.64		Homs	5.9	33.5	1.9
9	35.858	35.867	47	13	171	95.15	6.13	Mosaic of large *Pinus brutia* patches interspersed with agricultural fields,>800 km^2 which extends into Turkey	Lattakia	15.1	32.3	2.8
10	35.754	35.873	130	20	130.5	98.82	5.6		Lattakia	6.9	25.8	2
11	35.823	35.956	434	24	204.9	96.21	4.16		Lattakia	20	37.9	4
12	35.785	35.925	185	16	193.5	95.59	5.88		Lattakia	14.5	27.2	4.3
13	35.848	35.966	645	23	192.9	91.44	4.06		Lattakia	15.5	30.3	5.1
14	35.846	36.036	517	27	87	85.63	3.57		Lattakia	21.5	33.3	5.7
15	35.779	35.980	547	18	201.9	98.52	4.16		Lattakia	8.5	23.2	2.5
16	35.723	36.010	151	25	238.5	87.55	5.18		Lattakia	14.5	28.4	3.1
17	35.671	36.044	197	16	90	86.66	5.36		Lattakia	18.9	35	4.1
18	35.722	36.066	270	26	144	86.15	5		Lattakia	17.2	30.7	2.2
19	35.801	36.111	381	24	205.5	81.15	4.72		Lattakia	15.1	34	4.8
21	35.848	36.225	309	19	129	70.02	4.06		Idleb	10.1	29.6	2.9
20	35.510	36.142	689	25	222	100.18	3.79	One *Pinus brutia* fragment	Lattakia	10.9	28.9	3.3

* The term populations is being used for the trees sampled from the same geographical site.

[1)] Coordinates are of the weighted middle points of the 15 sampled trees.

[2)] Site characteristics were determined for each location of a sampled tree. The values given in this table are the averages.

[3)] PMQ is the pluviometric quotient.

[4)] m is the mean minimum temperature of the coldest month.

Homs and Idleb (names are derived from the province in which the largest part of the populations occur, Figure1).

In each of these regions, sample sites were selected with distinct environmental conditions (altitude, soil, associate vegetation, different population characteristics), or separated by effective gene flow barriers (distance, mountains), (Table 1). Minimum distance between the sampled trees was 150 meter.

DNA extraction

In total 311 samples of young needles were collected and lyophilized. Genomic DNA was extracted from individual plants. Needles were ground in liquid nitrogen and DNA extraction was performed according to the modified protocol of DOYLE & DOYLE (1990).

RAPD analysis

The protocol of WILLIAMS *et al.* (1990) for RAPD analysis was employed with minor modifications. A total of 400 decamer primers obtained from Operon Technologies, Alameda, Calif. USA (Kits A to X), were used to screen the DNA samples for polymorphism. All amplification reactions were performed using a DNA thermocycler Perkin Elmer 9600. The final reaction volume was 15µl and contained 10 pmol of the 10-mer Operon primers, 25ng of template DNA, 200 µM of each dNTPs, 1.5 mM $MgCl_2$ and 0.8 unit of *Taq* DNA polymerase. After initial denaturation for 4 min. at 94 °C, the reaction was subjected to 35 cycles of 30 sec. at 94 °C, 1 min. at 36 °C and 2 min. at 72 °C , followed by 10 min. at 72 °C. Amplification products were separated by electrophoresis on 1.5 % agarose gels made in 1X TAE buffer (40 mM Tris-Acetate, 1mM EDTA, pH = 8) and were visualized by ethidium bromide staining. Preliminary tests with one sample for each of the five different regions of Syria were conducted to select the RAPD (as well as AFLP) primers able to detect polymorphism.

AFLP analysis

The protocol for the AFLP assay was carried out as described by VOS *et al.* (1995) with minor modifications. 0.5 µg of DNA was digested with the restriction enzymes *Pst*I and *Mse*I. Pre-amplification and selective amplifications were performed as described in the original protocol. Thirteen primer combinations were tested to screen for polymorphism between the DNA samples. The primer combination P100/M301, where *Pst*I-100 has four selective nucleotides (5'GACTGCGTACATGCAG + AACC) and *Mse*I-301 has also four selective nucleotides

(5'GATGAGTCCTGAGTAA + TATA), was selected to analyze the 311 samples. The PCR profile for the pre-amplification program was: 30 sec. at 94 °C, 30 sec. at 60 °C, 1 min. at 72 °C, for 30 cycles. The pro-gram for selective amplification was the following: 30 sec. at 94 °C, 30 sec. at 65 °C, 1 min. at 72 °C, for one cycle. This was followed by 11 cycles over which the annealing temperature was decreased by 0.7 °C per cycle followed by 30 sec. at 94 °C, 30 sec. at 56 °C, 1 min. at 72 °C, for 23 cycles. The amplified fragments were electrophoresed on 6% polyacrylamide gels and stained with silver nitrate (BASSAM *et al.* 1991). The presence and the absence of the bands were visually recorded.

Data analysis

For each marker system (AFLP and RAPD), a matrix of all fragments (bands) scored in the 311 trees was generated using "1" when the band was present and "0" when the band was absent. Fragments of the same molecular weight were scored as identical. Each fragment was presumed to represent a single genetic locus. Genes diversity at each RAPD and AFLP locus and genic variation statistics for all loci were estimated by the software package POPGENE, version 1.32 (YEH *et al.* 1997). Analysis of genetic diversity (*GD*) was calculated following the formula of NEI (1987):

$$GD = n(1-\Sigma p^2)/(n-1)$$

where n is the number of samples and p is the frequency of one allele.

Gene diversity was calculated as follows:

$$H = (1-\Sigma p_{ij}^2) \qquad \text{(WEIR 1990)}$$

where p_{ij} is the frequency of jth allele generated with the primer i.

In the total population, gene diversity (H_t) was divided into the gene diversities within (H_s) and among (D_{st}) populations in the different regions, thus

$$H_t = H_s + D_{st} \qquad \text{(NEI, 1987).}$$

Genetic differentiation (G_{st}) relative to the total population was calculated using the coefficient of gene differentiation (NEI 1973; 1987):

$$G_{st} = D_{st}/H_t$$

G_{st} can take value between 0 (no differentiation between subpopulations) and 1.0 (complete differentiation between subpopulations.

The amount of gene flow between populations, Nm, where N is the population size and m is the fraction of individuals in a population that are migrants, was estimated using the following formula (BOEGER *et al.*, 1993):

$$Nm = 0.5[(1/G_{st}) - 1]$$

If $Nm < 1$, then local populations tend to differentiate; if $Nm > 1$, there will be little differentiation among populations and migration is more important than genetic drift (WRIGHT 1951).

A dendrogram of genetic distance between 21 populations was constructed using the Unweighted Pair Group Mean Average Method (UPGMA) (SNEATH & SOKAL 1973) of the software pakage Numerical Taxonomy and Multivariate Analysis System, version 2.01 (NTSYS-pc, ROHLF 1997).

RESULTS

RAPD analysis

Out of 400 Operon primers tested on five samples

Table 2. Number of polymorphic fragments in 21 *P. brutia* populations based on RAPD and AFLP data.

Population and region	# of trees	Number of polymorphic loci (fragments) per										Total number of polym fragments
		RAPD - Operon primers									AFLP	
		B-04	B-20	C-01	C-13	F-15	J-01	P-05	P-14	V-04		
1 Aleppo	14	2	7	6	2	4	6	2	7	7	19	62
2	15	2	6	7	3	4	7	2	6	7	22	66
3	15	2	6	9	2	4	6	4	6	8	11	58
Aleppo	44	2	7	9	3	4	7	4	7	9	25	77
1 Hama	14	3	7	7	2	6	6	2	6	8	15	62
2	14	2	4	8	2	6	7	3	6	7	17	62
3	15	2	3	4	2	3	5	2	5	5	11	42
Hama	43	3	7	9	2	7	7	3	9	9	23	79
1 Homs	15	4	5	8	2	7	7	3	9	7	17	69
2	14	3	7	5	2	2	6	3	7	6	16	57
Homs	29	5	8	9	2	7	8	4	10	8	23	84
1 Latakia	15	3	4	4	2	5	7	4	4	8	20	61
2	15	3	5	5	3	4	6	4	2	4	18	54
3	15	4	5	4	3	5	7	3	7	8	18	64
4	15	4	7	5	2	4	8	3	6	7	20	66
5	15	3	6	5	2	3	9	2	7	8	17	62
6	15	3	7	6	2	5	7	2	8	6	22	68
7	15	5	5	5	2	2	7	4	7	8	14	59
8	15	4	8	6	3	2	7	5	5	6	16	62
9	15	3	5	5	1	4	4	4	5	8	10	49
10	15	5	5	5	2	4	6	4	4	6	21	62
11	15	2	5	6	2	4	6	5	7	6	24	67
12	15	2	5	4	2	4	5	3	5	6	24	60
Latakia	180	7	9	9	4	6	9	6	10	10	32	102
Idleb	15	4	4	5	2	4	6	3	2	5	27	62
The whole collection	311	7	9	9	5	7	10	6	10	11	74	111

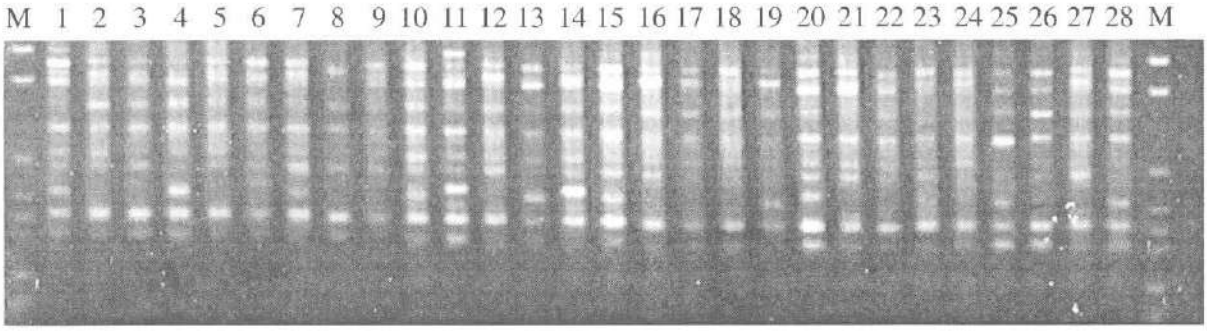

Figure 2. Genomic DNA from populations of *P. brutia* amplified with OPJ-01. Electrophoresis in 1.4% agarose gels. M: molecular marker VI, Mixture of PBR328 cleaved with *BglI* and PBR328 cleaved with *HinfI*, lane 1, lane 2-28 DNA samples of pine.

representing the 5 populations, only nine primers (OPB-04, OPB-20, OPC-01, OPC-13, OPF-15, OPJ-01, OPP-05, OPP-14 and OPV-04) were able to amplify clear and repeatable polymorphic fragments. These primers were then used to analyze the 311 plants representing the 21 populations from the 21 collection sites. The total number of polymorphic fragments was 74. Their molecular weight ranged from 230 to 2500 bp. Fragments with higher molecular weight were mostly monomorphic. The number of polymorphic fragments (polymorphic loci) detected per primer ranged from 5 (with OPC-13) to 11 (with OPV-04, Table 2), which represents an average of 8 polymorphic fragments per primer. The highest number of polymorphic fragments (71) was detected in population 11 of Latakia while the smallest number (44) was detected in population 3 of Hama. An example of amplification with OPJ-01 is shown in Figure 2.

In order to increase the level of polymorphism detected, amplified products of primers producing one or two monomorphic fragments (OPE02, OPF09, OPG16, OPH12, OPN02, OPN03, OPN05, OPS09, OPS13, OPT08, OPD20, OPA18, OPW12, OPW13) were digested with three restriction enzymes (*HinfI*, *EcoRV*, and *TaqI*). Some restriction enzymes cut the amplified fragments (Table 3), but none of these were polymorphic.

AFLP analysis

Distinct and polymorphic fragments (37) of the combination P100/M301 were used for the analysis of the 311 samples. Number of polymorphic fragments varied from 10 (in Population 9 of Latakia) to 24 (in the populations 11 and 12 of Latakia, Table 2). An example of the AFLP amplification is presented in Fig. 3.

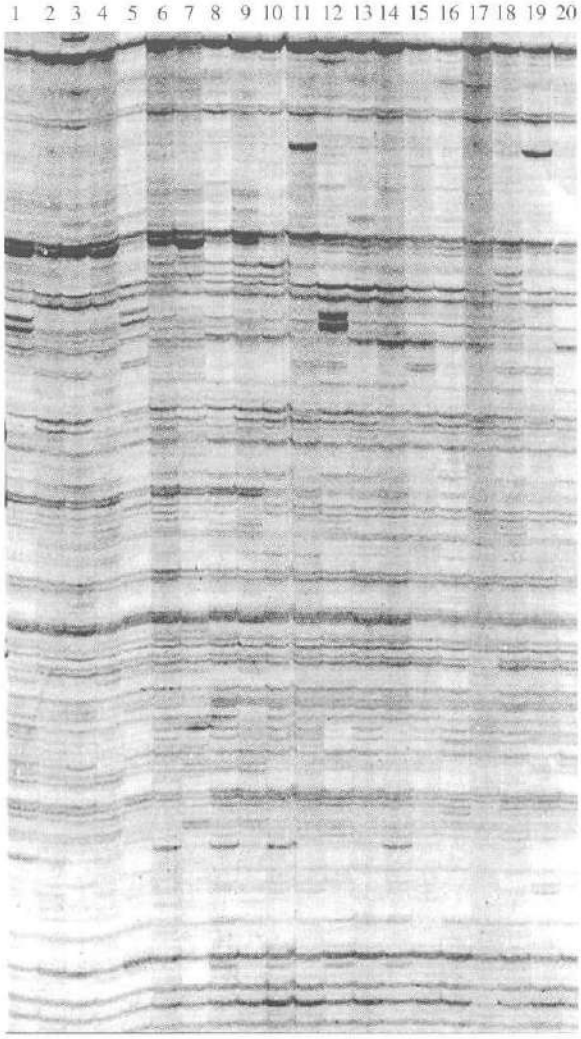

Figure 3. AFLP analysis of populations of *P. brutia* using primer combination P100/M301.

Genetic variability

For each population, the observed number of alleles (na), the gene diversity (h) and the percentage of polymorphic loci for RAPD, AFLP and RAPD +

Table 3. Digestion of monomorphic RAPD fragments and restriction enzymes used.

Operon primers producing monomorphic fragments	Restriction Enzymes		
	Sites for *EcoRV*	Sites for *HinfI*	Sites for *TaqI*
OPA-18	0	0	1
OPD-20	0	2	1
OPE-02	0	1	1
OPF-09	0	1	2
OPG-16	0	1	1
OPH-12	1	2	0
OPN-03	1	1	0
OPS-09	0	2	3
OPS-13	0	1	0
OPT-08	0	1	0
OPW-12	0	1	0
OPW-13	0	1	0

AFLP were calculated (Table 4). Clear difference between the data based on RAPD and those based on AFLP can be seen. In most populations, the level of polymorphism detected with RAPD marker was higher than the one detected with AFLP. The mean of polymorphic RAPD loci over all regions was 57.6%, where this mean was 48.8% for AFLP loci. We will focus on the results obtained for the combined data (RAPD and AFLP data) because they reflect in a better way, the genetic variability between the samples. The number of polymorphic fragments (loci) varied between populations. It ranged from 42 (in population 3 of Hama) to 69 (in population 1 of Homs) (Table 4). No region specific loci were detected. All loci were presented in all populations but with different frequency. The value of gene diversity varied from 0.15 in the population 3 of Hama to 0.26 in the population 11 of Latakia. The comparison between the 21 populations showed that the observed number of alleles varied from 1.38 in the population 3 of Hama to 1.61 in the population 6 of Latakia.

In order to understand the population structure and to estimate the genetic diversity within and between regions, the different populations were regrouped according to the collection regions (Latakia, Aleppo, Hama, Homs and Idleb) . For each region the values of the observed number of alleles (n_a), the effective number of alleles (n_e), the gene diversity (h), and Shanon's information index (I) were calculated (Table 5). The lowest values for observed and effective number of alleles were in the region of Idleb (1.5586 and 1.3831, respectively) while the highest numbers were in the region of Latakia (1.9189, and 1.4843, respectively). The high values of standard deviation (SD) observed in all parameters showed that the variations within the regions were more important than among the regions.

Total gene diversity (H_t) including the gene diversities within populations (H_s) and among populations (D_{st}), and the amount of gene flow, Nm, between populations, were estimated for each region and over the all regions (Table 6). The higher value of total gene diversity was detected in the region of Latakia (H_t = 0.2887, SD = 0.028) while the lowest value was detected in Idleb (H_t = 0.2183, SD = 0.0447). Concerning the amount of gene flow, the Nm values varied from 1.693 in Latakia to 3.266 in Aleppo, showing the little differentiations among populations in one region and over all regions.

The combined data of the 111 polymorphic fragments derived from RAPD and AFLP analysis was used for the estimation of the Nei's genetic similarity and genetic distance between the 21 populations (Table 7). The highest genetic similarity was detected between populations 3 and 4 from Latakia (0.9694) while the lowest genetic similarity (0.8508) was between population2 from Homs and population12 from Latakia, which means that the biggest genetic distance (0.1616) was between these two populations (Table 7) .

Cluster analysis

A dendrogram based on Nei's genetic distance using UPGMA is presented in Fig. 4. It showed that the 21 populations were clustered together into 2 distinct groups. The first group includes the populations 10, 11, and 12 from latakia with the unique population 21 from Idleb, while the second group

Table 4. The values of observed number of alleles, gene diversity and the percentage of polymorphic loci in the whole samples of Syrian *P.brutia*.

No. of populations	Regions	No. trees/pop	Results with RAPD markers				Results with AFLP markers				Results with RAPD and AFLP markers			
			n_a	h	No. of polym. loci	% of polym. loci	n_a	h	No. of polym. loci	% of polym. loci	n_a	h	No. of polym. loci	% of polym. loci
1	Aleppo	14	1.581	0.25	43	58.11	1.514	0.2	19	51.35	1.56	0.24	62	55.86
2		15	1.595	0.24	44	59.46	1.595	0.3	22	59.46	1.6	0.24	66	59.46
3		15	1.635	0.24	47	63.51	1.297	0.1	11	29.73	1.52	0.2	58	52.25
1	Hama	14	1.635	0.25	47	63.51	1.405	0.2	15	40.54	1.56	0.22	62	55.86
2		14	1.608	0.23	45	60.81	1.46	0.2	17	45.95	1.56	0.22	62	55.86
3		15	1.419	0.17	31	41.89	1.297	0.1	11	29.73	1.38	0.15	42	37.84
1	Homs	15	1.703	0.28	52	70.27	1.46	0.2	17	45.95	1.62	0.26	69	62.16
2		14	1.554	0.23	41	55.41	1.432	0.2	16	43.24	1.51	0.21	57	51.35
1	Lataki	15	1.554	0.22	41	55.41	1.541	0.2	20	54.05	1.55	0.23	61	54.95
2	a	15	1.487	0.18	36	48.65	1.487	0.2	18	48.65	1.49	0.19	54	48.65
3		15	1.622	0.25	46	62.16	1.487	0.2	18	48.65	1.58	0.24	64	57.66
4		15	1.622	0.25	46	62.16	1.541	0.2	20	54.05	1.6	0.25	66	59.46
5		15	1.608	0.24	45	60.81	1.46	0.2	17	45.95	1.56	0.22	62	55.86
6		15	1.622	0.25	46	62.16	1.595	0.2	22	59.46	1.61	0.25	68	61.26
7		15	1.608	0.24	45	60.81	1.378	0.2	14	37.48	1.53	0.22	59	53.15
8		15	1.622	0.24	46	62.16	1.432	0.2	16	43.24	1.56	0.22	62	55.86
9		15	1.527	0.21	39	52.70	1.27	0.1	10	27.03	1.44	0.17	49	44.14
10		15	1.554	0.22	41	55.41	1.568	0.2	21	56.76	1.56	0.22	62	55.86
11		15	1.581	0.25	43	58.11	1.649	0.3	24	64.86	1.6	0.26	67	60.36
12		15	1.487	0.21	36	48.65	1.649	0.2	24	64.86	1.54	0.22	60	54.05
1	Idleb	15	1.473	0.19	35	47.30	1.73	0.3	27	72.97	1.56	0.22	62	55.68
	Mean	14.8	1.58	0.23	42.6	57.60	1.48	0.2	18	48.76	1.55	0.22	61.1	59.82

Table 5. Summary of genic variation statistics for all loci over all the regions.

Regions	No. of trees		RAPD				AFLP				RAPD + AFLP			
			na*	ne*	h*	I*	na*	ne*	h*	I*	na*	ne*	h*	I*
Aleppo	44	Mean	1.7027	1.4482	0.2615	0.3884	1.6757	1.482	0.27	0.3937	1.6937	1.4594	0.2643	0.3902
		St. Dev	0.4602	0.3678	0.1944	0.2757	0.4746	0.403	0.2105	0.2962	0.463	0.3784	0.199	0.2814
Hama	43	Mean	1.7568	1.4247	0.2515	0.3807	1.6216	1.406	0.237	0.351	1.7117	1.4184	0.2467	0.3708
		St. Dev	0.432	0.3651	0.1873	0.2602	0.4917	0.374	0.2022	0.2906	0.455	0.3664	0.1916	0.2697
Homs	29	Mean	1.8243	1.4759	0.2903	0.4391	1.6216	1.41	0.237	0.3506	1.7568	1.4541	0.2726	0.4096
		St. Dev	0.3831	0.3088	0.1595	0.2252	0.4917	0.388	0.2047	0.2922	0.431	0.3369	0.1767	0.2517
Latakia	180	Mean	1.9459	1.4948	0.2937	0.4469	1.8649	1.463	0.2788	0.4239	1.9189	1.4843	0.2887	0.4393
		St. Dev	0.2277	0.3449	0.1666	0.2167	0.3466	0.339	0.1711	0.2335	0.2742	0.3417	0.1675	0.2216
Idleb	15	Mean	1.473	1.3428	0.1895	0.2751	1.7297	1.464	0.2759	0.4094	1.5586	1.3831	0.2183	0.3199
		St. Dev	0.5027	0.4126	0.2184	0.3095	0.4502	0.334	0.1864	0.2691	0.4988	0.3909	0.2115	0.3022
all regions	311	Mean	1.973	1.499	0.2972	0.4535	1.9459	1.486	0.2912	0.4425	1.964	1.4948	0.2952	0.4498
		St. Dev	0.1633	0.3413	0.1615	0.2066	0.2292	0.335	0.1672	0.2229	0.1872	0.3376	0.1627	0.2112

*na = Observed number of alleles
*ne = Effective number of alleles
*h = Nei's (1973) gene diversity
*I = Shannon's Information index

contained the 17 other populations. Three distinct subgroups were identified in the second group. The first subgroup contained two populations from Aleppo (Populations 1 and 2), the second contained three populations from Latakia (populations 7, 8 and 9), and the third subgroup contained 12 populations from 4 different regions (6 from Latakia, 3 from Hama, 2 from Homs and 1 from Aleppo). Within subgroup 3, the populations 1, 2, 3, 4, 5 and 6 from Latakia were closely regrouped together with the population 2 of Hama. The populations Hama 1, Aleppo 3 and Homs 1 formed another cluster while the populations Homs 2 and Hama 3 were relatively isolated in this subgroup.

DISCUSSION

Genetic diversity between and within *P. brutia* populations collected from five different regions of Syria was evaluated using DNA markers. The analysis of these populations with RAPD and AFLP markers showed different level of polymorphism and genetic diversity. The fact that out of 400 Operon primers tested only nine were able to detect polymorphism between the samples, suggests that the level of similarity of the detected loci between the samples was high.

Even the digestion of RAPD fragments with restriction enzymes failed to increase the level of polymorphism detected, confirming the high level of genetic similarity at the sequence level in the Syrian populations of *P. brutia*. Similar results were obtained by KAYA & NEALE (1995) where they found that

Table 6. Analysis of gene diversity in five regions of collections.

Regions	Number of trees		RAPD + ALFP						
			H_t	H_s	D_{st}	G_{st}	Nm	# of polymorph. loci	% of polymorph. loci
Aleppo	44	Mean St. dev.	0.2646 0.0398	0.23 0.033	0.0351	0.1328	3.266	77	69.37
Hama	43	Mean St. dev.	0.2477 0.0368	0.197 0.027	0.0507	0.2035	1.9568	79	71.17
Homs	29	Mean St. dev.	0.2718 0.0312	0.233 0.027	0.0388	0.1442	2.9681	84	75.68
Latakia	180	Mean St. dev.	0.2887 0.028	0.223 0.02	0.0657	0.228	1.6932	102	91.89
Idleb	15	Mean St. dev.	0.2183 0.0447	0.218 0.045	0.0003	0	α	62	55.86
All regions	311	Mean St. dev.	0.2952 0.0265	0.275 0.023	0.0202	0.0703	6.6121	107	96.40

H_t: gene diversity in total population
H_s: gene diversity within populations
D_{st}: gene diversity among populations
Nm^* = estimate of gene flow from G_{st}, e. g., $Nm = 0.5\ (1 - G_{st})/G_{st}$

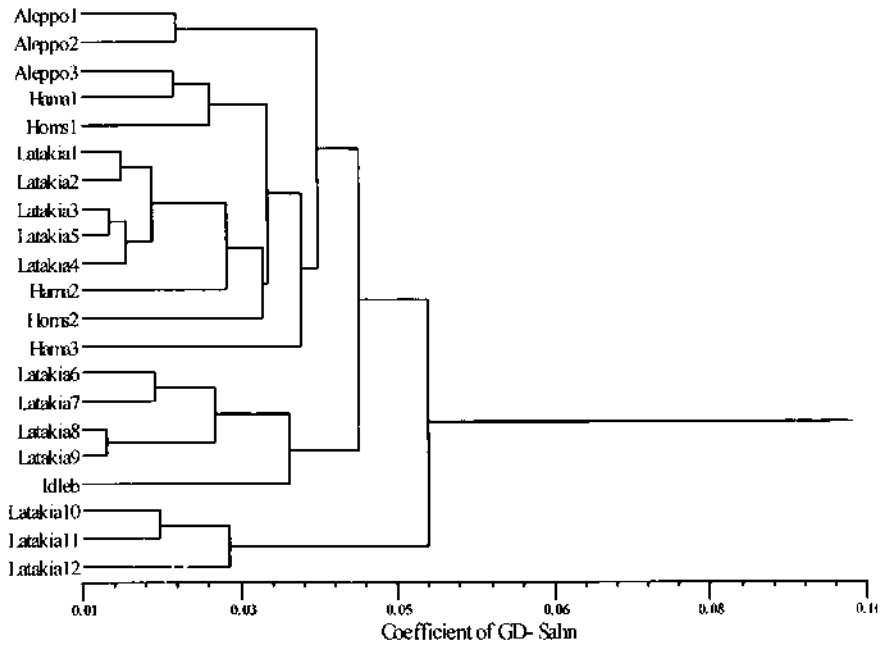

Figure 4. Genetic distance between trees from 21 Syrian populations based on AFLP and RAPD data.

high proportion of RAPD loci detected in Turkish red pine (*P. brutia*) was monomorphic. These results are in disagreement with a study based on isoenzymes carried out on accessions originating from islands of the Northeastern Aegean sea (PANETSOS *et al.* 1998). In that study, the level of genetic diversity detected was higher than the values detected in our populations of Syria. Previous studies on the species

Table 7. Values of genetic similarity (above diagonal) and genetic distance (below diagonal) between the 21 populations of *P. brutia*.

	AL1	AL2	AL3	HA1	HA2	HA3	HO1	HO2	LA1	LA2	LA3	LA4	LA5	LA6	LA7	LA8	LA9	LA10	LA11	LA12	Idleb
AL1	****	0.966	0.92	0.904	0.907	0.871	0.88	0.885	0.91	0.898	0.909	0.902	0.919	0.921	0.888	0.875	0.885	0.888	0.919	0.892	0.91
AL2	0.035	****	0.94	0.933	0.921	0.888	0.894	0.909	0.909	0.917	0.907	0.898	0.915	0.919	0.892	0.883	0.881	0.879	0.907	0.883	0.913
AL3	0.083	0.062	****	0.953	0.906	0.911	0.922	0.905	0.911	0.899	0.916	0.904	0.919	0.919	0.905	0.903	0.916	0.881	0.88	0.873	0.89
HA1	0.101	0.07	0.049	****	0.916	0.914	0.937	0.922	0.926	0.926	0.944	0.918	0.936	0.913	0.897	0.89	0.886	0.87	0.879	0.875	0.891
HA2	0.098	0.082	0.099	0.087	****	0.913	0.926	0.917	0.938	0.944	0.958	0.95	0.94	0.953	0.925	0.923	0.902	0.886	0.889	0.891	0.92
HA3	0.138	0.119	0.093	0.09	0.091	****	0.922	0.896	0.908	0.898	0.919	0.906	0.919	0.901	0.905	0.872	0.866	0.89	0.869	0.874	0.865
HO1	0.128	0.112	0.081	0.065	0.077	0.082	****	0.908	0.927	0.915	0.947	0.923	0.929	0.922	0.909	0.886	0.88	0.904	0.896	0.898	0.876
HO2	0.122	0.096	0.1	0.081	0.087	0.11	0.096	****	0.93	0.915	0.915	0.904	0.929	0.91	0.907	0.917	0.881	0.874	0.867	0.851	0.872
LA1	0.094	0.096	0.094	0.077	0.064	0.096	0.076	0.072	****	0.948	0.965	0.955	0.942	0.935	0.922	0.905	0.894	0.901	0.89	0.89	0.896
LA2	0.108	0.087	0.107	0.077	0.058	0.108	0.089	0.089	0.053	****	0.96	0.934	0.944	0.936	0.897	0.896	0.868	0.858	0.866	0.868	0.877
LA3	0.096	0.098	0.087	0.058	0.043	0.085	0.055	0.089	0.036	0.041	****	0.969	0.967	0.962	0.932	0.92	0.905	0.9	0.9	0.905	0.913
LA4	0.103	0.107	0.101	0.086	0.052	0.099	0.081	0.101	0.046	0.068	0.031	****	0.96	0.953	0.919	0.911	0.892	0.898	0.892	0.908	0.9
LA5	0.084	0.089	0.084	0.067	0.063	0.084	0.073	0.074	0.06	0.057	0.034	0.041	****	0.961	0.946	0.936	0.907	0.876	0.883	0.883	0.897
LA6	0.083	0.084	0.085	0.091	0.048	0.104	0.081	0.095	0.068	0.067	0.039	0.049	0.039	****	0.959	0.944	0.922	0.898	0.905	0.912	0.929
LA7	0.119	0.114	0.1	0.109	0.079	0.1	0.096	0.098	0.082	0.109	0.07	0.084	0.055	0.042	****	0.965	0.946	0.913	0.882	0.877	0.912
LA8	0.133	0.124	0.103	0.117	0.08	0.137	0.121	0.087	0.1	0.11	0.083	0.093	0.067	0.058	0.036	****	0.951	0.894	0.892	0.882	0.909
LA9	0.122	0.127	0.088	0.121	0.104	0.144	0.128	0.127	0.112	0.142	0.1	0.115	0.098	0.081	0.056	0.051	****	0.919	0.892	0.893	0.897
LA10	0.119	0.13	0.126	0.14	0.121	0.117	0.101	0.135	0.104	0.153	0.106	0.108	0.132	0.108	0.091	0.112	0.084	****	0.944	0.932	0.913
LA11	0.085	0.098	0.128	0.129	0.118	0.14	0.11	0.143	0.116	0.144	0.106	0.114	0.124	0.1	0.126	0.115	0.115	0.058	****	0.948	0.906
LA12	0.114	0.125	0.136	0.134	0.115	0.135	0.107	0.162	0.117	0.142	0.1	0.097	0.125	0.092	0.131	0.126	0.113	0.07	0.053	****	0.919
Idleb	0.095	0.091	0.116	0.116	0.084	0.145	0.132	0.137	0.11	0.131	0.091	0.105	0.108	0.073	0.092	0.095	0.108	0.092	0.099	0.085	****

of *P. brutia* based on morphological, anatomical, protein, allozymes and resin characteristic have revealed the existence of considerable variation of growth characteristic of this species (ARBEZ 1974; CALAMASSI *et al.* 1988; ISIK 1986; CONKLE *et al.* 1988). But, variation of most of these characteristics appeared to be related mostly to altitude and/ or climatic factors. In our study no relationship was identified between the variations in morphological characters (as crown shape, needles, angle of the stems, height to the first fork, height to the first branch, bends in the stem) and the altitude, the soil and mother rock, or climatic factors (data not presented).

Genetic diversity within and between populations in the same area

The gene diversity within and between populations in each region was estimated separately. The comparison of these values showed that this diversity varied from one population to another within the same region. For example, the difference in gene diversity values was higher between populations from Hama than those from Aleppo. The highest level of gene diversity was detected in the region of Latakia followed by Homs, Aleppo, Hama and the lowest value was in Idleb (Table 5). The detection of more diversity in Latakia populations could be due

to the high number of samples analyzed (180), which are collected from 12 different sites, and to the surface on which these collections were dispersed. On the other side, the variability within the populations was high which could be noticed from the high value of SD for each region (Table 5). This suggests that variation existing within populations and consequently within regions was more important than the values detected between regions.

Gene diversity between populations of different regions

The analysis demonstrated higher level of intra-population diversity compared to inter-populations. Region specific alleles were absent. All alleles detected in this study were present in all populations and all regions but with different frequency, some alleles (OPB-20.4 and OPC-13.4) were very frequent, they were present in 99.8% of the samples while others were rare (OPJ-1.10) and were present in 4% of the total samples (data not presented).

The majority of the gene diversity was detected within the *P. brutia* populations (93.2%, whereas only 6.8% exists between the populations (Table 6). This is in accordance with others studies based on different markers, although the percentages can vary considerably (KAYA & NEALE 1993; KARA *et al.* 1997; PANETSOS *et al.* 1998; KOROL *et al.* 2002).

The small value of genetic differentiation and gene flow in the different regions (0.1328 in Aleppo to 0.228 in Latakia, and 1.693 in Latakia to 3.266 in Aleppo, repectively) showed the little differentiation between populations from the different regions.

At the country level, the values of genetic diversity and genetic distance were calculated for the 21 population sites (Table 7). The highest value of genetic diversity was between populations Latakia 12 and Homs 2 and the smallest value was between populations Latakia 3 and 4.

The dendrogram based on the genetic distance (Fig. 4) showed that the 21 sites (populations) were clustered close to each other and regrouped into two distinguished groups. The most distant group comprises three sites of Latakia (sites 10, 11 and 12) which represent sites localized close to each other and in a relatively separated area of Latakia (Fig. 1) with one site of Idleb. The second group comprises three subgroups, the first one includes the populations 1 and 2 from Aleppo, the second one comprised the three populations from Lata-kia (7,8 and 9) while the other populations of Latakia were in the third subgroup with the populations from Aleppo and Hama and Homs. The populations 1, 2, 3, 4, 5 and 6 from Latakia were more close to each others than the others populations of Latakia. We could notice that in Latakia, the populations which were clustered close to each others in the dendrogram were collected from sites geographically close to each others (eg. Populations 1and 2, then 3, 4, 5, and 6, then 7, 8, and 9). The high level of similarity estimated between the population of Idleb and the populations 10, 11, and 12 of Latakia, and their clustering in the same group in the dendrogram could be due to the geographically close locations (Fig. 1). The situation is completely different for samples collected from Homs. Although the two sites in Homs are much more close to each other than to the other sites of Syria, the two populations analyzed have relatively high values of genetic distance (0.096) and have higher values of similarity with populations from distant sites (Table 7). For the regions of Aleppo, two of the three populations analyzed (1 and 2) were clustered in the same subgroup and were close to each other geographically. The third population, although it is geographically close to the other populations, was clustered with the population of Hama collected from a very distant place. The populations 1, 2 and 3 of Hama were the most dispersed populations. There were all in the same group but in three different subgroups (Fig. 4).

We could conclude from our analysis that although the genetic diversity within populations is higher than that between populations, the general level of genetic variability detected in the Syrian *P. brutia* was not high. Similar results were obtained on Turkish *P. brutia* and it was noted that the genotypes originated from distantly located geographic regions, with unfavorable ecological conditions for growth, had low value in proportion of polymorphic loci (KAYA & NEALE 1993, 1995). The low genetic variability seems to be a character of *P. brutia* where the same information was obtained using the isozymes analysis (KARA *et al.* 1997).

The genetic variability detected within populations of Syria is more important than the one detected between populations. These results confirm the results obtained with isozyme markers on *P. brutia* in the in the Aegean island, Greece populations (PANETSOS *et al.* 1998). Similar results were obtained through the analysis with SSR markers (ELHANS 2001).

The limited level of genetic diversity revealed by RAPD and AFLP markers in the Syrian populations of *P. brutia* could be explained by the fact that these trees were collected from relatively close areas. Seemingly they were subjected to similar conditions

of selection pressures imposed by common environmental influences. The recent development of SSR markers from species of *Pinus* like *P. halapensis* (KEYS *et al.* 2000, SHEPHERD *et al.* 2002) and even from *P. brutia* (ELHANS 2001) could help to reveal higher levels of variations in the Syrian populations. The detection of higher level of genetic diversity and the identification of specific population alleles would provide good information about the genetic structure of the Syrian *P. brutia* populations and could help in selecting the most variable genotypes to conserve and use them as a source for the national forestation program of *P. brutia*.

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CONSERVATION OF TROPICAL FOREST GENETIC RESOURCES: IPGRI'S EFFORTS AND EXPERIENCES

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Introduction

Long-term conservation of forest genetic resources (FGR) is a cornerstone of sustainable forest management. Through conservation and proper forest management, it is possible to maintain the evolutionary processes of species and the diversity of their genepools for use now and in the future. Traditionally, forest conservation has often been understood as a steady-state process, whose goal is to preserve natural forests in their original state. Forests, however, are dynamic systems. Human activities have modified forests and their biodiversity for millennia, even in seemingly pristine tropical forests (see review by McNeely 1994).

Among the different components of biodiversity, genetic diversity is the building block of the evolutionary process. Conservation of FGR, therefore, should accommodate evolutionary concepts to ensure continuous adaptation under changing environments (Eriksson *et al*. 1993). Several global and regional threats to forest ecosystems are contributing to profound changes in the patterns of distribution of tree genetic diversity. Global climate change is modifying the prevailing environmental conditions to which forests have adapted. Forest fragmentation, the introduction of invasive species and atmospheric pollution also are having adverse effects on forests. It has been predicted, however, that changes in land use will have a greater impact on biodiversity in the tropical terrestrial biome in the next 100 years than will changes in climate, nitrogen deposition, biotic exchange or atmospheric carbon dioxide (Sala *et al*. 2000).

As tropical deforestation is still continuing (Geist & Lambin 2002), it is clear that many conservation efforts in the tropics have not been effective. This lack of success can be attributed partly to inadequate participation by various stakeholders in natural resources management and conservation. Conservation strategies should take into account the needs of different stakeholders, as well as non-environmental policies that can indirectly affect the use of forests. Recent efforts to promote sustainable forest management in tropical forests are welcome, but may not be enough to safeguard biodiversity in these forests. It is difficult to achieve sustained timber yields from natural forests without degradation of habitats and subsequent loss of biodiversity (e.g. Bawa & Seidler 1998). However, sustainable forest management may indirectly support FGR conservation efforts in natural tropical forests.

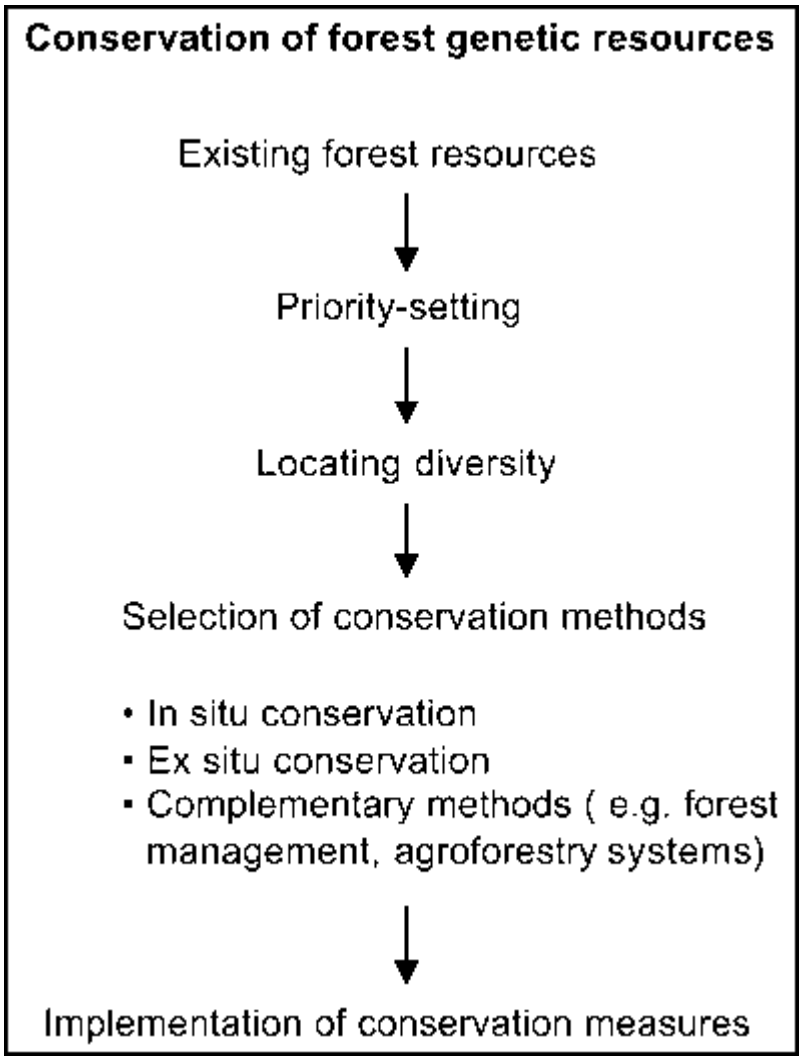
This paper gives an overview of the conservation of FGR and some of the related activities of the International Plant Genetic Resources Institute (IPGRI) and its partners. We discuss priority setting, locating genetic variation, conservation methods, implementation and the role of sustainable forest management in maintaining genetic diversity in tropical forests. Lastly we highlight some issues that are important for better conservation of FGR, particularly in a regional context.

Conservation of forest genetic resources as a process

Conservation of FGR is a multiphase process (Figure 1). Existing forest resources and the genetic diversity they contain provide a basis for conservation measures. The selection of priority species is one of the most important phases of the conservation

process because future activities will be based on priorities set in the planning phase. As soon as priority setting has been completed, genetic diversity must be assessed and located for subsequent conservation activities. The selection of conservation measures depends on how the objectives of a conservation programme are defined. These measures then provide a framework to implement practical conservation work.

Figure 1. Simplified schematic representation of the conservation process



Setting priorities for conserving forest genetic resources

Only a small number (fewer than 140) of the world's 50,000 or more tree species is being used in forestry, and current gene conservation or breeding efforts cover even fewer species (National Research Council 1991). Yet many more tree species are useful to man in terms of their non-wood products or environmental services. Hence long-term FGR conservation efforts, especially for tropical and subtropical tree species, should be strengthened (National Research Council 1991; Palmberg-Lerche 1999). In practice, however, conservation needs far outweigh the available human and financial resources, and relatively little is known of even the basic biology of most tree species. These constraints make priority-setting one of the most important tasks not only of conservation work but also of research.

The need to develop decision strategies for setting priorities is particularly urgent for countries that are trying to identify priority tree species and populations for inclusion in regional and national conservation programmes. The University of British Columbia in Canada has been developing a framework for prioritizing species, populations and conservation methods under an IPGRI-supported research project. In addition to developing this framework, the project aims to develop a general procedure to support more comprehensive strategies for conservation and management of FGR (IPGRI 1999, 2000).

The framework attempts to prioritize species or populations in a rational manner, on the basis of threats, potential or present values, and the means that are available for

conservation (see IPGRI 2000). The framework requires basic information on the status and dynamics of genetic diversity, as well as its values, threats and potential for conservation management. This range of information is rarely, if ever, available, but the framework provides a tool for compiling existing information and identifying research needs. This process produces a priority ranking or classification of species into priority groups that can be used by managers to select and implement necessary measures.

Testing the decision-making framework in the field

The framework has been tested at two sites in São Paulo, Brazil: a 2000ha forest reserve with 267 tree species, and a park of more than 35,000ha with about 300 tree species (Koshy *et al*. 2002). Field data for these two sites are readily available from permanent sample plots. The two-step prioritization process starts with specifying tree species and identifying their potential threats and values. Subsequently the species are ranked for further management options. The second step involves acquiring additional information and estimating how much this information costs.

In the first step, tree species are given a score of 1-5 based on threat and value evaluations by various stakeholders. In the field test, for example, the threat evaluation was based on exploitation intensity, fragmentation and fire. The value evaluation combines economic and biological factors. Economic factors include present and potential commercial values of wood, non-wood products and social and cultural values. Biological factors consist of the ecological functions of a species and its contribution to phylogenetic diversity. Both the threat and value scores are given a relative weight indicating the importance of a species to different stakeholders and a probability that reflects the certainty of a given score. The final threat and value scores are calculated as a sum of the three components, i.e. initial score, weight and probability, and the species are ranked for priority. The higher the score, the higher the priority for management.

In the second step, additional information is collected for high-ranked species at i) demographic, ii) non-genetic (phenology, pollination, seed dispersal, etc.) and iii) genetic levels. Additional information will either increase or decrease the probability of managing (i.e. whether conservation is needed or not). More precise information reduces the likelihood of making a 'wrong' decision because the range of probability distributions can be narrowed. The increased costs of obtaining additional information at the different levels are estimated and included in the analysis while a decision tree is being constructed.

Koshy *et al*. (2002) conclude that the decision-tree approach is an effective tool for rational decision-making in gene conservation, provided that the necessary information can be estimated (if it is not available), and that monetary values can be assigned with reasonable accuracy. The main challenge is accurately estimating population parameters, which increase the probability of making 'correct' management decisions. It is likely that further research on new methodologies and results will alleviate this problem in the future, thus making the developed framework more useful. Other advantages of the framework are that the collection costs of additional information can be compared directly with the expected returns of such a choice, and that the framework is amenable to sensitivity analysis to identify influential factors in decision-making.

Locating genetic diversity

The process of decision-making for conservation of FGR highlights the importance of information on the amount and location of genetic diversity. No matter how useful the decision-making framework is in practice, no conservation effort can be effective without adequate information on genetic diversity. At present, however, this kind of information is extremely scarce, especially in the case of tropical forests, and cannot be produced rapidly. Thus different layers of information, i.e. patterns of species and forest ecosystem distribution, threats and the amount and spatial distribution of genetic diversity, must be combined to assess adequately the state of genetic diversity in forest ecosystems.

Combining information produces a spatially explicit framework for conservation efforts by identifying areas to be ranked according to the level of threats and the genetic diversity they hold (Boffa *et al*. 2000). Once species distribution has been identified, more efforts can be made to evaluate genetic diversity. Detailed information on genetic diversity and threats is essential to designing effective conservation areas, which will safeguard intra-specific diversity and maintain evolutionary processes. Several conservation areas,

hosting a considerable portion of a species' genetic variation, may be needed. Modern tools, such as remote sensing and geographic information systems, can greatly facilitate this work.

Locating genetic diversity in Vietnam

The Research Centre for Forest Tree Improvement (RCFTI), under the Forest Science Institute of Vietnam, has been locating the genetic diversity of the threatened timber tree species *Pterocarpus macrocarpus*, *Xylia xylocarpa* and *Dalbergia oliveri* (Le & Nguyen 1999). The work carried out so far has focused on assessing tree species composition and natural regeneration potential, and the relations between the target species and other dominant species in the remaining forests. Land-use changes from 1973 to 1995 have also been evaluated in selected locations, and socio-economic surveys have been carried out to assess the importance of forests to local livelihoods.

This work has revealed that the species are found only in some national parks and nature conservation areas, and that they occur independently of each other (Le & Nguyen 1999). *P. macrocarpus* and *X. xylocarpa* occur in semi-deciduous or deciduous forests, whereas *D. oliveri* grows mainly in evergreen forests. All of the species are light-demanding and the potential for natural regeneration, except in *P. macrocarpus*, seems to be high. Over-exploitation, however, still endangers these species and conservation efforts must be further developed.

Remote sensing studies have found that forest cover in some areas decreased markedly (20-35%) between 1973 and 1995. Other areas saw only small reductions of 3-7%. Most of these changes were caused by the conversion of forest land into agricultural and residential land. A similar pattern has been observed in many neighbouring countries (FAO 1999). Socioeconomic studies have shown that, although paddy and milpa cultivation is the main source of employment for local people, forests are also important to livelihoods. For example, farmers surveyed around the distribution areas of *P. macrocarpus* use wood extensively for house building and cooking.

Socio-economic factors are sometimes neglected or seen as irrelevant when focusing on the conservation of forest genetic diversity. However, the success or failure of any conservation project depends heavily on how the needs of local people are taken into account during planning and implementation (Enters 2000; Isager *et al*. in these proceedings).

RCFTI is currently assessing the distribution of intra-specific genetic variation in *P. macrocarpus* using isozyme studies. These will provide information on genetic diversity within selected populations (located mainly in the Central Highlands of Vietnam) and enable sound planning of future conservation efforts. This work is also regionally important. To date the genetic diversity of *P. macrocarpus* has been assessed only in Thailand, where a west-east geographic pattern in genetic variation has been found (Liengsiri *et al*. 1995). For regional gene conservation purposes, it is important to know whether this pattern extends to neighbouring countries, i.e. Myanmar, Lao PDR and Vietnam (Liengsiri *et al*. 1995; Coles & Boyle 1999).

Methods for conserving forest genetic resources

A wide range of methods, from protected reserves to intensive management of breeding populations for production systems, can be used to conserve FGR. The choice of methods depends on available genetic material, selected time scale and specified aims. The method selected and the subsequent implementation of a conservation strategy also depend on the availability of both human and financial resources.

The two most commonly considered methods for conserving FGR are *in situ* and *ex situ* conservation. The term *in situ* refers to the continued maintenance of tree populations at their natural sites, in the environment to which they have adapted. *Ex situ* conservation takes place outside the natural habitat of a tree species, and may consist of activities such as establishing live collections or *ex situ* conservation stands, or storing seeds, pollen or tissue.

FGR can also be conserved and maintained by using tree species in forestry or other land-use systems such as agroforestry. It is likely, however, that forest management interventions reduce genetic variation in tree populations (Savolainen & Kärkkäinen

1992). Not even carefully planned and implemented forest management activities, therefore, can replace more active conservation measures with clearly specified objectives. The same caveat also applies to tree domestication and the use of trees in agroforestry systems.

In situ conservation

There is a general consensus among scientists and practitioners that no single conservation method is adequate, and that different methods should be applied in a complementary manner (e.g. Palmberg-Lerche 1999; Boffa *et al*. 2000). *In situ* conservation, however, has a number of benefits and so often forms the basis of conservation programmes. It allows evolutionary processes to be maintained, including the adaptation of tree populations to changing environmental conditions. This is particularly important for breeding programmes, since future human needs and environmental conditions are difficult to predict.

The reproductive biology and overall survival of many tropical forest species are dependent on complex ecological interactions. Thus long-term genetic conservation of such species is difficult, if not impossible, without *in situ* conservation. In addition, recalcitrant seed behaviour (i.e. inability to tolerate desiccation) also complicates long-term *ex situ* conservation efforts for many tropical tree species (see below). *In situ* conservation can also contribute to the conservation of biological diversity at higher levels, i.e. species and ecosystems.

Protected areas have often been established on the basis of ecosystem or species conservation, rather than gene conservation. Thus the design of *in situ* conservation programmes has been considered primitive (National Research Council 1991). In tropical forests in particular, the complexity of interactions and lack of scientific information have hampered the development of *in situ* conservation strategies. Uncertainty surrounds the adequate size of *in situ* conservation areas, the number of individuals to be included, how to select locations, and how genetic variation is distributed within selected areas (Palmberg-Lerche 1999).

Undisturbed tropical forest ecosystems are often taken as a starting point when planning *in situ* conservation programmes. Today, however, rural landscapes are a mosaic of disturbed and less disturbed patches of forest, ranging from seemingly natural and secondary forests to seriously degraded forests and other wooded fragments. The most obvious genetic effects of fragmentation are a loss of genetic diversity at both population and species levels, changes in the genetic structure of a population and increased inbreeding (Young & Boyle 2000). Clearly, *in situ* efforts cannot always rely on intact natural forests, and so it is essential to understand how the processes of genetic drift, gene flow, selection and mating affect genetic diversity in fragmented forests (Young & Boyle 2000).

IPGRI's partners at the Universities of Costa Rica, Alberta and Massachusetts have been studying the effects of fragmentation on the genetic diversity of *Enterolobium cyclocarpum* in Costa Rica. Studies of the reproductive biology of *E. cyclocarpum*, a tropical dry forest species, in continuous forests and forest fragments in pastures indicate that pasture trees are less likely to receive pollen and set fruit, and that their fruits bear less seed, than trees located in continuous forests (see IPGRI 2000). Also, outcrossing rates within the two groups of trees have been found to be similar, but progeny vigour among seedlings in continuous forest is higher than in pastures.

These results have immediate implications for the conservation and management of *E. cyclocarpum* and other species in similar habitats. Trees in pastures can aid the movement of pollinators between intact forest fragments and so contribute to gene flow and maintenance of genetic diversity among forest fragments. However, because seedlings in pastures have less vigour, seeds from these areas cannot be recommended for establishing plantations or rehabilitating degraded natural forests.

In southern India, sandal (*Santalum album*) forests have long been exposed to selective logging, poaching and changes in land use. The effects of disturbance on the genetic diversity of sandal have been investigated by the University of Boston, together with the Ashoka Trust for Research in Ecology and the University of Agricultural Sciences in Bangalore (IPGRI 1999, 2000). Results from two protected areas indicate that genetic diversity of sandal is highest in the core undisturbed park zone, whereas allelic diversity

is reduced in the outer buffer and disturbed zones. These findings suggest that present protection measures are adequate and that excessive logging will cause genetic deterioration in natural sandal populations. Allelic diversity, however, is similar in the two disturbed zones, which suggests that, contrary to general assumptions, no significant genetic segregation has resulted from different degrees of disturbance. This finding can be attributed to the fact that sandal is insect-pollinated and animal-dispersed, i.e. substantial gene flow still occurs despite varying degrees of disturbance.

Ex situ conservation

As mentioned earlier, *in situ* and *ex situ* conservation should be used in a complementary fashion to conserve FGR. Both are an integral part of the conservation process, and both can only be effective after genetic diversity has been located and conservation priorities have been set. The main purpose of *ex situ* conservation is to capture and maintain a representative sample of the existing genetic diversity of a species. For highly endangered tree species, *ex situ* conservation may be the only approach in the short to medium term. The main pitfalls of collecting germplasm samples for *ex situ* conservation are: i) limited coverage of genetic variation; ii) biases in the collected plant material; and iii) samples that are too large to deal with (Brown & Hardner 2000). Because *ex situ* conservation is more costly than *in situ* conservation, it is particularly important that sampling of populations and germplasm within populations is given special attention to maximize the use of limited financial and human resources.

The traditional approach to *ex situ* conservation of FGR is to establish conservation stands of a species outside its native habitat to facilitate gene management. However, long-term maintenance of the collected genetic variation in *ex situ* stands tends to be complicated by genetic drift and potential contamination by external gene flow. Another commonly used method of *ex situ* conservation is to store seeds collected from a range of natural populations. In the case of many tropical tree species, however, this method is limited by recalcitrant seed behaviour.

More than 70% of commercially valuable tropical tree species are estimated to have recalcitrant or intermediate seeds (Ouédraogo *et al*. 1999). In recent years, therefore, considerable effort has been expended in developing *in vitro* techniques for *ex situ* conservation of recalcitrant tree species. A notable example is cryopreservation (Benson 1998; Marzalina *et al*. 1999). Cryopreservation, the storage of cells or tissue at ultra-low temperatures, offers great opportunities for longer-term *ex situ* gene preservation. Its applicability, however, has been limited by difficulties in identifying protocols to reduce the water content of tissue before freezing. Tissue water content must be reduced to avoid lethal ice formation in cells. Different results from different laboratories engaged in drying the same species of seed are an additional obstacle (Walters 1999). Although it is likely that further research will ease these problems, the main application of cryopreservation seems to be in supporting tree improvement programmes and conserving biotechnically derived germplasm (Benson 1998), rather than as a widely applied *ex situ* conservation method to support *in situ* conservation of FGR.

Micropropagation, the use of tissue and organ cultures for organogenesis and somatic embryogenesis, can also be applied to maintain genetic variation in germplasm collected for *ex situ* conservation. Practical propagation applications are already available for several tropical trees, based on shoot tissue culture in broadleaved tree species and on somatic embryogenesis in conifers (see Luukkanen 1998 for a review of biotechnology in tropical forestry). Micropropagation does not necessarily require expensive laboratory facilities, and can therefore be used in less-developed conditions. Maintaining juvenile material, however, can be problematic. At present, the technology used in somatic embryogenesis is in most cases too expensive for cost-effective *ex situ* conservation.

Since 1997, the Danida Forest Seed Centre (DFSC) and IPGRI have cooperated in enhancing *ex situ* conservation methods for recalcitrant tropical forest trees. Project activities have focused on determining whether seeds are recalcitrant, intermediate or orthodox, and have included seed development research to assess optimal conditions for seed collecting, germination and storage. An international Forest Tree Seed Research Network has been established under the project to facilitate information exchange among scientists developing protocols for collecting, handling, testing and screening of tolerance to desiccation and optimal storage conditions. At present more than 20 countries worldwide are involved in the project. More than 50 tropical forest

species have been screened so far. Activities also include publications and training workshops to increase research capacity, particularly in developing countries.

Implementation of *in situ* conservation at an operational level

Putting FGR conservation strategies into practice at an operational level is a major challenge. Three important aspects of implementing *in situ* conservation must be considered, i.e. how to establish a network of *in situ* conservation areas, how to improve the usefulness of protected areas for FGR conservation, and how sustainable forest management can promote FGR conservation (see FAO/DFSC/IPGRI 2002).

Networks of in situ conservation areas

The key variables in planning and establishing a network of *in situ* conservation areas are location, number of areas and their size or the number of individuals they contain. The factors that should be considered when selecting areas for an *in situ* gene conservation programme can be summarized as follows (FAO/DFSC/IPGRI 2002):

- Abundance of priority species;
- Low risk and threat levels (including land tenure issues);
- Efficient management agency in terms of commitment and resources;
- Support from local people;
- Compact in shape and presence of forest buffer zone; and
- Opportunities to conserve other priority species.

Even if governments implement a conservation programme for FGR in state-owned forests, they must rely heavily on local people's participation to make conservation efforts successful. In many countries, local people have traditional or customary rights to use public lands for subsistence purposes, and any efforts that might prevent them from exercising these rights are likely to cause conflicts. Conservation efforts can only be successful if local people see such efforts as important to their livelihood and as a source of benefit.

The factors listed above are important in terms of practical implementation of *in situ* conservation efforts, but should not replace the original ideal of gene conservation. Populations for conservation programmes should be selected on the basis of known or expected distribution of genetic variation, so that conservation areas contain the maximum genetic diversity of a priority species. However, detailed data on the distribution of genetic diversity in forest ecosystems are often unavailable, especially in the tropics. In such cases, the populations of a species can be selected systematically across its distribution range. Alternatively, the distribution range can be divided into different ecological zones, and representative populations selected from each zone (FAO/DFSC/IPGRI 2002).

FAO/DFSC/IPGRI (2002) suggest, as a general guideline for the number of gene conservation areas required for any species, that between one and three areas in each major ecological zone are likely be adequate for widespread and highly outcrossing species. This figure reflects the fact that such species often have more or less continuous patterns of variation, and that a considerable amount of their genetic variation is found within populations. For inbreeding species or outcrossing species with scattered and discontinuous ranges, more than three conservation areas in each ecological zone are likely to be needed. The number of areas will also depend on the level of threat facing a given population, what resources are available to manage the areas, and the present or expected importance of a variant, i.e. its economic value and genetic distinctiveness (FAO/DFSC/IPGRI 2002).

How large should an *in situ* conservation area be? For many tropical tree species, it is difficult to estimate the minimum viable population size that will ensure long-term maintenance of adequate genetic diversity. For most tropical tree species, we lack detailed biological information on sexual systems, incompatibility mechanisms, flowering

patterns, pollination vectors and gene flow. However, general guidelines based on current scientific understanding do exist.

Kageyama and Reis (1993) offer guidelines for Brazil based on different abundance categories. For common tree species with high abundance (>5 trees per hectare) and a wide natural distribution range, a large number (20) of smaller gene reserves (500ha) is likely to capture most of the genetic variation within the species. For rare species (2-5 trees per 100ha), it is estimated that a very large area (5000-10,000ha) is required to maintain a viable population. In comparison, the guidelines of FAO/DFSC/IPGRI (2002) are based on population size, and recommend that each gene conservation reserve should include a minimum of 300 adult interbreeding trees.

The guidelines include assumptions, such as low threat levels and undisturbed ecological interactions (e.g. pollinators and seed dispersers), and so should not be adopted without further consideration. These assumptions may not hold in a given country or situation, and the actual number of individuals or the size of a conservation area may vary. Depending on the frequency of different genes in a population and the type of genes (i.e. dominant or recessive), the number of trees required to conserve adequate genetic diversity is likely to range from several hundreds to several thousands (FAO/DFSC/IPGRI 2002).

The role of sustainable forest management in gene conservation

Sustainable management of forests has received a great deal of attention during the past decade. Major developments took place after the Convention on Biological Diversity (CBD) was signed at the United Nations Conference on Environment and Development (UNCED) in 1992. Before this, the concept of sustainability in forest management was commonly considered in terms of sustained wood production. Now it is also understood to cover biodiversity at all levels. Sustainable forest management has been defined as "a process of managing permanent forest land to achieve multiple objectives to produce desired forest products and services without undue reduction of future productivity, and without undue undesirable effects on the physical and social environment" (ITTO 1992).

Several international agencies have been actively developing guidelines to enhance sustainable forest management. The International Timber Trade Organization (ITTO) has published guidelines for sustainable forest management of natural tropical forests, for the conservation of biological diversity in tropical production forests, and criteria and indicators for sustainable forest management of natural tropical forests (ITTO 1990, 1992, 1993, 1998). The Centre for International Forestry Research (CIFOR) has also been active in promoting the formulation of criteria and indicators for sustainable forest management (Prabhu *et al*. 1999). Field testing of various criteria and indicators is taking place in many countries. Recently, criteria and indicators have been developed for the conservation of genetic diversity in forest ecosystems (see Boyle 2000 for a review).

Conservation of FGR is an integral part of sustainable use of forests because it maintains short-term viability of individuals and populations, and their evolutionary potential, and ensures the present and future use of FGR (see Boyle 2000). Because only a tiny proportion of tree species and their germplasm is actively and adequately conserved, the maintenance of genetic diversity in most tree species depends on how natural production forests are managed to meet human needs. It is often suggested that accelerating the establishment of tree plantations and other tree-based production systems would alleviate the pressure on natural forests, especially in developing countries. However, the present and probable future use of FGR depend heavily on tropical natural forests as only 2% of tropical forests are plantations (FAO 1999).

There is a growing consensus among scientists and practitioners that sustainable management of natural tropical forests is technically feasible, but constrained by economic, social and political factors (e.g. Reid & Rice 1997; Bawa & Seidler 1998). Commercial logging has a major impact on FGR, not only by reducing population size, but also by causing structural alterations that are likely to affect the complex ecological interactions between trees and the various animals that maintain genetic processes (Bawa & Seidler 1998; Wickneswari & Boyle 2000). Logging also reduces regeneration potential by physically damaging remaining trees and seedlings. Collection of non-wood forest products can also have dramatic genetic effects, especially if it focuses on reproductive parts such as flowers and fruits (Wickneswari & Boyle 2000).

The concept of low or reduced-impact logging has evolved in response to the need to reduce damage to the residual stand. It incorporates a number of additional measures such as precutting of climbers, comprehensive timber harvest planning, directional felling, increased supervision, reduced roadside clearing and more careful road construction. The viability of low-impact logging as a standard forestry practice depends on how many additional, cost-increasing measures are included, and in what kinds of stands and sites they are applied (Ahmad *et al*. 1999; Hamzani *et al*. 1999). The concept has been developed mainly to meet carbon management, rather than gene conservation, objectives, although practices that maintain carbon stocks in natural tropical forests generally help to maintain FGR as well. Low-impact logging may not be the best way to conserve FGR in tropical production forests, but it is better than current concession-based logging practices which drastically reduce seed production and regeneration (Curran *et al*. 1999).

High-intensity logging is likely to cause an immediate reduction in genetic diversity and so lead to inbreeding. In a ridge forest in Peninsular Malaysia, a 56% reduction in basal area due to logging reduced genetic diversity by 5-23.4% (measured less than one year after logging) in five species with different life histories (Wickneswari & Boyle 2000). The long-term genetic consequences of logging, however, may not always be as dramatic because ample seed or seedling banks and gene flow from undisturbed areas may compensate for immediate losses in genetic diversity. Compared with an unlogged site in lowland dipterocarp forest in Peninsular Malaysia, no adverse changes in the genetic diversity of six species were detected in sites logged over 40 years ago with a reduction in basal area of 13.5% and 40.7% (Wickneswari & Boyle 2000). These findings suggest that low-impact logging has the potential to promote the conservation of FGR in tropical production forests, even though natural forest management often has a simplifying effect on biodiversity at higher levels (Bawa & Seidler 1998). More studies on the genetic aspects of sustainable forest management are urgently needed.

Networking and conservation of forest genetic resources in Asia and the Pacific

As we have discussed above, the conservation and management of FGR require information on complex phenomena and processes. This underscores the need for more holistic approaches, both regionally and at the national level. In addition, a lack of human and financial resources to integrate new research results into practical forest management is an obstacle in many countries. Research is often duplicated because previous results remain localized; their dissemination prevented not by selfish motives but by the lack of a suitable channel for distribution.

Through networking, it is possible to avoid duplicating research efforts and create synergies among collaborating institutions and other stakeholders. Networking promotes partnerships and more efficient use of limited resources. It can also enhance the dialogue between scientists, managers, policy makers and users, and increase interaction between different sectors at a national level. This is a precondition for sustainable forest management. A network with a holistic approach, therefore, has the potential to enhance conservation and management of FGR in the Asia-Pacific region.

The concept of networking on FGR is not new to the Asia-Pacific region. A number of species-specific networks already operate in the region. These include the International Neem Network, TEAKNET, the International Network on Leucaena Research and Development (LEUCANET), the International Network on Bamboo and Rattan (INBAR) and the International Centre for Research and Training on Seabuckthorn (ICRTS) (see Sigaud *et al*. 2000 for a review). In addition to these, there is the South Pacific Regional Initiative on Forest Genetic Resources (SPRIG), a network for several island states. The global Tropical Montane Cloud Forest Initiative, which focuses on biodiversity conservation, also operates in the region.

Most of these networks try to promote better management of the genetic resources of a single species or group of species. They frequently emphasize tree improvement. Improved management of the genetic resources of economically important species is important, but existing network activity mainly focuses on plantation forestry or agroforestry. The management of genetic resources in natural forests has received little networking attention.

This lack of attention is surprising, given that natural forests in Asia and the Pacific provide raw materials for many economically valuable goods and products, as well as

many important environmental services. Dipterocarps are a good example of this negligence. Timber and non-timber products derived from dipterocarps provide substantial revenues for many countries, particularly in Southeast Asia. Their importance at the local level is also considerable-lowland tropical rainforests, commonly dominated by dipterocarps, support a huge array of biodiversity and support the livelihood of rural people in numerous ways. Several institutions have been conducting research on dipterocarps and their genetic resources, but the main constraint to progress has been a lack of coordinated action with well-defined objectives and priorities (Bawa 1998).

In a recent regional seminar (26-27 March 1999), the member institutions of the Asia Pacific Association of Forestry Research Institutions (APAFRI) presented their visions and country-based research needs (APAFRI 1999a, 1999b; Hoon & Awang 2000). The seminar's recommendations on networking can be summarized as follows (APAFRI 1999b):

- Information support services at national and institutional levels should be upgraded so that the national, regional and global knowledge pool can be better utilized.
- Area and skills-based regional and global networking efforts should be strengthened.
- APAFRI should promote information exchange in rapidly developing areas of science such as biodiversity assessment and conservation, and biotechnology.
- APAFRI should also support the establishment of research networks to meet the needs of its members and to strengthen cooperation among researchers.

In addition to these recommendations, many papers and country reports presented at the seminar identified other needs for research and development closely related to conservation and use of FGR, for example sustainable management of natural forests, tree improvement and tree domestication (Hoon & Awang 2000). A need exists, therefore, to enhance information exchange and cooperation for more efficient conservation and sustainable use of FGR in the Asia-Pacific region. A formal FGR network could fulfil this need and APAFRI has the potential to facilitate any networking effort in cooperation with relevant international and regional institutions.

Conclusions

Conservation of FGR aims to maintain the evolutionary processes of forests and the diverse genepools they contain for present and future use. Conservation of FGR is a cornerstone of truly sustainable use of forests. It is widely recognized that conservation or sustainable forest management cannot be successful without a broad planning process involving different actors within and outside the forest sector, as well as an inter-sectoral policy dialogue. The capacity of national institutions to carry out FGR conservation should be strengthened, and FGR conservation programmes should be included in national plans for forestry and biodiversity conservation (National Research Council 1991; Palmberg-Lerche 2000).

Conservation of FGR should be viewed as a continuous process, which begins by selecting priority species. Priority setting should be done in consultation with various stakeholders, and should be based on threats, present or potential values, and the resources that are available for conservation. The subsequent selection of populations for conservation requires information on the amount and distribution of genetic diversity if conservation activities are to be targeted effectively. Compared with *ex situ* conservation, *in situ* conservation has certain benefits, but different conservation methods should be applied in a mutually supportive manner, and in accordance with the costs and objectives of conservation. Sustainable forest management appears to have the potential to support conservation of FGR, but the impacts of various new management guidelines on genetic diversity in forest ecosystems require further study. In addition, several attempts are being made to use genetic criteria in forest management and forest certification schemes, but empirical evidence of their impact is currently limited.

A number of species-specific networks already exist, but research institutions in the region have indicated that cooperation on many FGR-related issues should be

integrated and strengthened. There is a need, therefore, to enhance the exchange of information to facilitate conservation and wise use of FGR in the Asia-Pacific region. Regional networking is the primary means of strengthening the capacity of national institutions to plan and implement FGR conservation programmes.

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Food Security

The Brazilian Case

Weber Antonio Neves do Amaral
and Alessandro Peduto

2010

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Abstract

Chronic food insecurity remains one of the main challenges to developing countries' sustainable development and thus to the stability of both nations and global political and economic regimes. Trade plays a vital role in a nation's economic growth and has several links with food security. This report provides the Brazilian context in terms of food security and trade linkages. In less than three decades, Brazil has changed from a net importer of food to a net exporter due to increased production (incorporating new areas of the country) and productivity. Gains of scale achieved by large agricultural enterprises have helped lower production costs and thus increase access to food and to the global commodity trade without affecting the country's internal market. Thus, for Brazil, trade has not affected food security adversely. However, a more detailed analysis is needed to identify the relationship between food security and trade at the local and regional levels; however, this is a complex exercise and beyond the scope of this report.

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This study is part of a larger, multi-region TKN project that seeks to understand better the impacts of trade policy on food security. It includes country case studies and regional analyses from Latin America, Southern Africa and Southeast Asia. It was made possible through the generous support of the Swedish Environment Secretariat for Asia (SENSA) and the Norwegian Agency for Development Cooperation (NORAD). The project outputs are available on the TKN website.

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Abbreviations and acronyms

BRL	Brazilian real
FAO	Food and Agriculture Organization of the United Nations
ha	hectare(s)
kg	kilogram(s)
MDA	Ministry of Agrarian Development
MERCOSUR	Southern Common Market
TEC	Common External Tariff
USD	U.S. dollar

Executive summary

Chronic food insecurity remains one of the main challenges to developing countries' sustainable development and thus to the stability of global political and economic regimes. Trade plays an important role in a nation's economic growth and has several links with food security. Therefore, a proper understanding of the direct and indirect links between trade and food security is highly important. Addressing these linkages is particularly challenging, however, since food insecurity is the result of the interplay of a series of factors operating at different levels, only one of which is trade.

Brazil plays an important role in both food production and trade. The expansion of its agricultural sector over the last few decades has guaranteed an increase in food supplies to its national market and, significantly, to the global commodities trade. Trade (internally and externally) is considered to affect at least three of the pillars of food security: availability, access and, thus, stability. When appropriate stimuli are given to agricultural producers, trade might have a positive impact on food availability and access, as food production increases and prices decrease. However, when trade is not supported by the right combination of agricultural policies, food availability might decrease and prices increase. The past few years have been characterized by unprecedented challenges for both developed and developing countries, spurred by the increases in food and fuel prices in the period 2006–08—as measured by the Food and Agriculture Organization's Food Price Index, which shows a compound growth rate of 25 percent in this period—and by the financial crisis and the resultant 2008–10 global economic slowdown. These challenges were exacerbated by the planet's growing human population (although the rate of growth has slowed significantly since the 1960s) and by the various effects of climate change (variations in rainfall patterns and droughts, new crop and livestock diseases, heat waves, etc.), which have serious repercussions on the capacity of most vulnerable countries, households and individuals to address food insecurity.

At the national level, food availability in Brazil is more than sufficient for its entire population. Domestic production of food, plus imports and minus exports, results in food availability per capita (in grain equivalent) of more than 340 kg per capita per year, about one third more than per capita nutritional requirements. The market share values of Brazilian imports for the period 1998–2007 decreased for dry beans (from 10.82 to 3.25 percent), maize (from 2.38 to 1.02 percent), rice (from 6.1 to 2.39 percent), soybeans (from 2.15 to 0.13 percent), refined sugar (from 0.0003 to 0.0002 percent) and wheat (from 6.03 to 5.57 percent). In the same period, exports increased for dry beans (from 1.85 to 30.85 kilotons), maize (from 7.17 to 10,933.46 kilotons), rice (from 6.61 to 201.48 kilotons), soybeans (from 9,274.75 to 23,733.78 kilotons), refined sugar (from 3,575.27 to 6,915.80 kilotons) and wheat (from 4.19 to 104.48 kilotons). As can be seen from the figures for the period from the late 1990s to the late 2000s, Brazil played a significant role in the world commodities trade; for example, in 2007 exporting maize (10,933.46 kilotons and 9.97 percent of market share), refined sugar (6,915.80 kilotons and 29.95 percent of market share) and soybeans (23,733.78 kilotons and 31.90 percent of market share), while importing maize (1,095.54 kilotons and 1.02 percent of market share) and wheat (6,638.02 kilotons and 5.57 percent of market share).

The Guaranteed Price Policy is the main agricultural policy used in Brazil to ensure food security and is aimed at small and medium-sized holders. The primary aim of this policy is to ensure that purchase prices are compatible with production costs, plus reasonable levels of profits. The growing productivity per acre for the main staple foods in Brazil more than compensates for the reduced area under cultivation (specifically for rice and beans). This increased production is also associated with higher fertilizer usage. Perhaps the most important factor responsible for the rise of food prices over the period 2006–08, besides the increase in demand for food due to population growth and, more importantly, rising

incomes, was the price of the fertilizers, especially in Brazil. As Brazil is one of the largest producers of agricultural commodities, the use of fertilizers is significant and is an important part of the costs of production. Brazilian demand for the raw materials needed to make fertilizers grew by about 6.6 percent per year between 2000 and 2008, and currently the country is applying self-sufficiency policies for these inputs in order to reduce the quantity of imports, reduce spending on agricultural commodities and boost farmers' competitiveness. Fertilizer prices also showed a direct correlation with oil prices, which is the raw material for the production of the nitrogen used in fertilizers. Increases in the price of oil, therefore, increase the production costs of nitrogen, and thus of the fertilizers used by farmers. Besides the logistics issues, the fertilizer trade also faces several protectionist barriers set up by producers. For example, China, a major producer of fertilizers, in April 2008 raised the export tax on the raw materials used to make fertilizers by up to 135 percent in order to avoid a possible shortage in its internal market.

1. Introduction

Chronic food insecurity remains one of the main challenges to developing countries' sustainable development and thus to the stability of global political and economic regimes. Trade plays an important role in a nation's economic growth and has several links with food security. Therefore a proper understanding of the direct and indirect links between trade and food security is highly important. Addressing these linkages is particularly challenging, however, since food insecurity is the result of the interplay of a series of factors operating at different levels, one of which is trade. The root causes of food insecurity include poverty, war and civil conflicts; environmental degradation; national policies that do not contribute to and promote agricultural development; inequitable access to food; market protection and subsidies; and limited access to markets for trading purposes (since free market access would contribute to income generation and eventually to lower food prices).

Other factors operate at the household and community levels (low productivity of crop and livestock systems; limited or insufficient access to food because of poverty, physical barriers and gender inequalities, etc.) and the individual level (low levels of education, poor health status, inequitable intra-household distribution, etc.) (EC, 2010: 3). These factors will not be addressed in this report, but should be considered when examining the relationship between trade and food security.

Brazil has played an important role in global food production and trade. The expansion of its agricultural sector during the last two decades has guaranteed an increase in food supplies to its national market and, significantly, to the global commodities trade.

1.1 Definition of food security

The Food and Agriculture Organization of the United Nations (FAO) defines food security as 'a situation that exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life' (FAO, 2003).

In addition to this concept, several other definitions are important when considering the issue of food security (FAO, 2006):

- **Food availability:** This refers to the availability of sufficient quantities of food of appropriate quality, supplied through domestic production or imports (including food aid).
- **Food access:** This refers to access by individuals to adequate resources (entitlements) for acquiring appropriate foods for a nutritious diet. *Entitlements* are defined as the set of commodity bundles over which an individual has control, given the legal, political, economic and social arrangements of the community in which he/she lives (including traditional rights such as access to common resources);
- **Utilization:** This refers to the utilization of food through adequate diet, clean water, and sanitation and health care facilities to reach a state of nutritional well-being where all human physiological needs are met. This brings out the importance of non-food inputs to food security.
- **Stability:** To be food secure, a population, household or individual must have sustained (i.e. stable) access to adequate food at all times and should not be in danger of losing access to food

as a consequence of sudden shocks (e.g. an economic or climatic crisis) or cyclical events (e.g. seasonal food insecurity). The concept of stability can therefore refer to both the availability and access dimensions of food security.

Trade (both internally and externally) is considered to affect at least three of these pillars of food security: availability, access and, thus, stability. When appropriate stimuli are given to agricultural producers, trade can have a positive impact on food availability and access, as food production increases and prices decrease. When trade is not supported by the right combination of agricultural policies, food availability might decrease and prices increase. In this policy report, we provide aggregated information on food production in Brazil and the food trade, and discuss the role of government policies in securing food stability, especially for lower-income families. We conclude that despite its increasing participation in the global food trade, Brazil's food security is not being affected negatively.

1.2 The state of global food insecurity

According to FAO, the number people suffering from hunger grew between 1995–97 and 2004–06 in all regions except Latin America and the Caribbean, despite the progress made in the 1980s and in the first half of the 1990s.

In the first quarter of 2009 the number of chronically hungry people in the world was estimated to be about 1 billion: around 642 million in Asia and the Pacific, 265 million in sub-Saharan Africa, 53 million in Latin America and the Caribbean, and 42 million in the Middle East and North Africa.

Despite these trends, food security and agriculture have been generally neglected in recent decades by both developing country governments and parts of the international donor community. As a result, the relative share of funding for food security and agriculture has decreased (EC, 2010: 3).

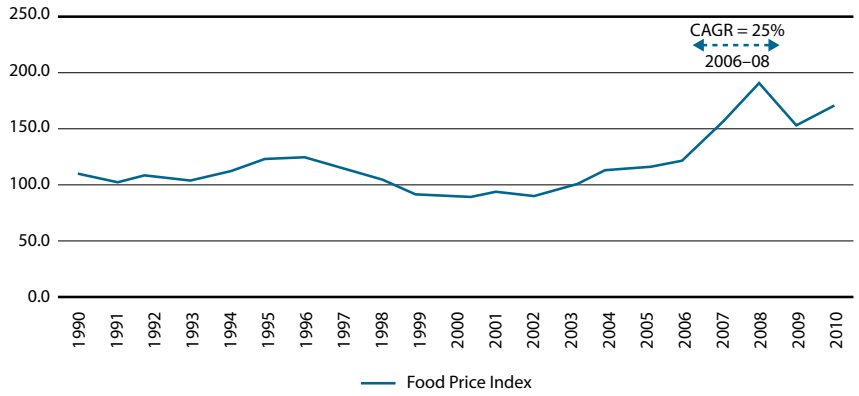
In addition, recent years have been characterized by unprecedented challenges for both developed and developing countries, spurred by the increases in food and fuel prices in the period 2006–08 (Figure 1)—as measured by the Food Price Index[1], which shows a compound growth rate of 25 percent over the period—and by the 2008–10 financial crisis and the resultant global economic slowdown (FAO, n.d.).

These increases marked the reversal of a decades-long trend of declining (real) prices for food on the global market and are likely to lead to a period of greater price volatility for food. These events have also created uncertainty about the ability of global markets to correct price distortions and triggered both speculation in food commodities and the new phenomenon of the large-scale acquisition of farmlands by richer food-deficit countries in poorer developing countries in Africa, Latin America, and Central and Southeast Asia (EC, 2010: 3).

Even though food prices, oil prices and financial crises affected developed, emerging and developing economies alike, their impacts varied significantly across regions, countries and population groups. In many countries the spike in food prices fuelled political instability and social unrest, which reignited the significance of food insecurity as a 'non-traditional' human security challenge.

1 FAO's Food Price Index consists of the average of six commodity group price indices weighted with the average export shares of each of the groups for 2002–04. In total, 55 commodity quotations are considered by FAO commodity specialists as representing the international prices of the food commodities in question and are included in the overall index.

Figure 1: FAO Food Price Index variation, 1990–2010



CAGR = compund annual growth rate

Source: FAO (n.d.)

The 2006–08 food crisis also had a direct impact on malnutrition figures. According to a World Bank estimate, the number of children suffering from irreversible after-effects of malnutrition increased by more than 40 million in 2008. For the poorest and most vulnerable countries, the effects of the crisis not only compounded the development challenges they face, but also put at risk the gains they had achieved in relation to the Millennium Development Goals, as growth stagnated, transfers of food were reduced and poverty increased (EC, 2010: 3). The main regions of hunger are located in less-developed areas of the world, i.e. Latin America (excluding Mexico), sub-Saharan Africa (excluding South Africa), South and East Asia (excluding Japan and South Korea), although several regions within developed countries also suffer from poverty and hunger.

These challenges are being exacerbated by the growing global population (although the rate of growth has slowed significantly since the 1960s) and by the various effects of climate change (variations in rainfall patterns and droughts, new crop and livestock diseases, heat waves, etc.), which have serious repercussions for the capacity of the most vulnerable countries, households and individuals to attain food insecurity (EC, 2010: 3).

2. The state of food insecurity and agriculture in Brazil

Brazil is the world's fourth-largest food exporter. Its agricultural sector is strong enough to meet all domestic needs and still generate foreign currency through exports. Even so, access to food is still a problem for millions of Brazilians.

Today, nearly a third of the Brazilian population is in a situation of food insecurity, meaning that they do not eat enough or well enough, with regularity or dignity, regardless of food trade policies.

The issue of food security has had a prominent place in Brazil's policy agenda for decades. On the national level, food availability in Brazil is in theory more than sufficient for its entire population. Domestic production of food, plus imports and minus exports, results in food availability (in grain equivalent) of more than 340 kg per capita per year, about one third more than the country's per capita nutritional requirements.

Brazil's average per capita calorie availability grew steadily over the last three decades at an annual rate of 0.7 percent, reaching 2,985 kilocalories in 2000 (FAO, 2009). However, due to the country's highly skewed income distribution, the lowest-income population segments are consuming less than their basic nutritional requirements (Meade *et al.*, 2004: 25).

Regarding international trade, Brazil's main agricultural export products are soybeans and soybean products, coffee, meat and meat products, frozen concentrated orange juice, sugar and sugar products, and tobacco. Agricultural exports totaled USD 24.8 billion in 2002 and have grown by 6 percent per year over the last two decades (AgraFNP, 2010b). Export earnings are used in part to finance grain imports such as wheat and corn, which are mainly used for feed in the rapidly expanding poultry sector (Meade *et al.*, 2004: 26).

The dramatic conclusion is that in Brazil, hunger and food insecurity are not due to any shortage of food because of the international food trade, but because people simply cannot afford to eat.

2.1 Brazil's major crops: Cultivated areas and productivity

During the last two decades the area of land under cultivation and the level of productivity have changed significantly in Brazil. In 2008 the total area under cultivation was approximately 64 million hectares (ha), of which soybean occupied an area of approximately 22 million ha, followed by corn (14.1 million ha), sugar cane (8.6 million ha) and beans (4.2 million ha). Tables 1 and 2, respectively, show changes in the area under cultivation and productivity for the most important crops in the country for the period of 2000/01–2008/09 and within-country variation among the country's major agricultural states. In this period, productivity improved significantly for corn (from 2.9 to 3.6 tons/ha), sugar cane (from 69 to 79 tons/ha), rice (from 3.2 to 4.3 tons/ha) and wheat (from 1.1 to 2.5 tons/ha), while it remained the same for soybean, cassava and beans in the last decade.

Table 1: Brazil's major crops, 2000/01–2008/09 harvests (production in millions of tons; area under cultivation in millions of ha; productivity in tons/ha)

Crop	Soy			Corn			Sugar cane			Bean			Rice			Cassava			Wheat		
	Prod.	Area	Pdtvy	Prod.	Area	Pdtvy	Prod.	Area	Pdtvy	Prod.	Area	Pdtvy	Prod.	Area	Pdtvy	Prod.	Area	Pdtvy	Prod.	Area	Pdtvy
Harvest																					
2000/01							344	5.0	69	2.6	3.9	0.7	10.4	3.2	3.2	22.6	1.7	13.5	1.7	1.5	1.1
2001/02	42	16	2.6	35.3	12.3	2.9	364	5.1	71	3.0	4.3	0.7	10.6	3.2	3.3	23.1	1.7	13.8	3.2	1.7	1.9
2002/03	52	18	2.8	47.4	13.2	3.6	396	5.4	74	3.2	4.4	0.7	10.4	3.2	3.3	22.0	1.6	13.4	2.9	2.1	1.4
2003/04	50	21	2.3	42.1	12.8	3.3	415	5.6	74	3.0	4.3	0.7	13.0	3.7	3.5	23.9	1.8	13.6	5.9	2.5	2.4
2004/05	52	23	2.2	35.0	12.2	2.9	423	5.8	73	3.0	3.9	0.8	13.4	3.9	3.4	25.9	1.9	13.6	5.8	2.8	2.1
2005/06	55	23	2.4	42.5	13.0	3.3	455	6.2	74	3.5	4.2	0.8	11.7	3.0	3.9	26.7	1.9	14.0	4.9	2.4	2.1
2006/07	58	21	2.8	51.4	14.1	3.7	516	6.7	77	3.3	4.1	0.8	11.3	3.0	3.8	26.9	1.9	14.1	2.2	1.8	1.3
2007/08	60	21	2.8	58.7	14.8	4.0	649	8.1	80	3.5	4.0	0.9	12.1	2.9	4.2	26.3	1.9	14.2	4.1	1.9	2.2
2008/09	57	22	2.6	50.3	14.1	3.6	687	8.6	79	3.5	4.2	0.8	12.6	2.9	4.3	26.3	1.9	13.9	6.0	2.4	2.5

Prod. = production; Pdtvy = productivity.

Source: AgraFNP (2010a) and authors' analysis

Table 2: Brazil's production (million tons) per major agricultural state and harvested area (million ha), 2001 & 2008 harvests

Production	RS		PR		SP		MG		GO		MS		MT	
Harvest	2001	2008	2001	2008	2001	2008	2001	2008	2001	2008	2001	2008	2001	2008
Soy	5.6	7.8	9.5	11.9	1.6	1.4	1.9	2.5	5.4	6.5	4.6	4.6	11.7	17.8
Corn	3.9	5.3	9.4	15.4	3.9	4.7	4.8	6.6	3.4	5.0	1.3	3.5	2.2	7.8
Sugar cane	1.1	1.4	28.1	51.2	212.7	390.2	18.2	47.9	11.7	33.1	8.6	21.4	12.6	15.8
Bean	0.1	0.1	0.6	0.8	0.3	0.3	0.5	0.6	0.2	0.2	0.0	0.0	0.0	0.1
Rice	5.5	7.4	0.2	0.2	0.1	0.1	0.2	0.1	0.2	0.2	0.2	0.2	1.2	0.7
Cassava	1.3	1.3	3.6	3.3	1.0	1.0	0.8	0.9	0.2	0.5	0.6	0.6	0.4	0.6
Wheat	0.9	1.7	0.6	1.9	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0	-	-
Area	**RS**		**PR**		**SP**		**MG**		**GO**		**MS**		**MT**	
Harvest	**2001**	**2008**	**2001**	**2008**	**2001**	**2008**	**2001**	**2008**	**2001**	**2008**	**2001**	**2008**	**2001**	**2008**
Soy	3.31	3.83	3.29	3.98	0.58	0.53	0.72	0.87	1.90	2.18	1.19	1.73	3.85	5.68
Corn	1.46	1.39	2.49	2.98	1.08	0.97	1.20	1.34	0.75	0.90	0.48	0.99	0.74	1.83
Sugar cane	0.03	0.04	0.36	0.59	2.66	4.54	0.28	0.61	0.15	0.40	0.11	0.25	0.18	0.22
Bean	0.16	0.10	0.53	0.50	0.22	0.18	0.43	0.42	0.53	0.10	0.02	0.02	0.03	0.09
Rice	0.99	1.07	0.08	0.05	0.04	0.02	0.10	0.07	0.11	0.09	0.05	0.04	0.44	0.24
Cassava	0.09	0.08	0.17	0.14	0.04	0.04	0.06	0.06	0.02	0.03	0.03	0.03	0.03	0.04
Wheat	0.56	0.85	0.78	0.82	0.02	0.04	0.01	0.01	0.01	0.01	0.06	0.03	-	-

RS = Rio Grande do Sul; PR = Paraná; SP = São Paulo; MG = Minas Gerais; GO = Goiás; MS = Mato Grosso do Sul; MT = Mato Grosso.

Source: AgraFNP (2010a) and authors' analysis

2.2 Brazil's trade market share of major food commodities

In the period 1998–2007 Brazil's commodity imports decreased for dry beans (from 211.03 to 96.27 kilotons), maize (from 1,728.90 to 1,095.54 kilotons), rice (from 1,513.30 to 720.77 kilotons), soybeans (from 828.23 to 97.93 kilotons) and refined sugar (from 0.05 to 0.04 kilotons). Only wheat imports increased (from 6,395.49 to 6,638.02 kilotons), although not significantly. Tables 3 and 4, respectively, show the imports and exports of the most important crops in the country for the period 1998–2007. Cassava was not included in the analysis since its trade values were not significant, as it is typically consumed locally.

The global market share values of Brazilian imports for the same period decreased for dry beans (from 10.82 to 3.25 percent), maize (from 2.38 to 1.02 percent), rice (from 6.1 to 2.39 percent), soybeans (from 2.15 to 0.13 percent), refined sugar (from 0.0003 to 0.0002 percent) and wheat (from 6.03 to 5.57 percent).

In the same period, exports increased for dry beans (from 1.85 to 30.85 kilotons), maize (from 7.17 to 10,933.46 kilotons), rice (from 6.61 to 201.48 kilotons), soybeans (from 9,274.75 to 23,733.78 kilotons), refined sugar (from 3,575.27 to 6,915.80 kilotons) and wheat (from 4.19 to 104.48 kilotons).

The market share values of Brazilian exports for this period also increased for dry beans (from 0.08 to 1.04 percent), maize (from 0.01 to 9.97 percent), paddy rice (from 0.006 to 0.007 percent), soybeans (from 24.41 to 31.90 percent), refined sugar (from 19.90 to 29.95 percent) and wheat (from 0.004 to 0.079 percent).

Table 3: Major Brazilian crop imports, 1998–2007 (imports in kilotons; global market share in %)

		Dry beans	Maize	Rice*	Soybeans	Sugar refined	Wheat
1998	kilotons	211.03	1,728.90	1,513.30	828.23	0.05	6,395.49
	% share	10.82%	2.38%	6.10%	2.15%	0.00%	6.03%
1999	kilotons	92.81	822.15	1,207.42	582.03	0.03	6,891.01
	% share	4.87%	1.05%	4.41%	1.39%	0.00%	6.11%
2000	kilotons	79.53	1,771.19	729.66	807.40	0.02	7,523.01
	% share	4.22%	2.16%	3.21%	1.67%	0.00%	6.43%
2001	kilotons	130.26	624.36	776.29	849.58	0.01	7,016.33
	% share	5.92%	0.76%	3.32%	1.48%	0.00%	6.22%
2002	kilotons	82.30	345.26	639.33	1,045.20	0.02	6,572.24
	% share	3.44%	0.39%	2.39%	1.84%	0.00%	5.44%
2003	kilotons	103.28	797.67	1,239.76	1,189.23	0.00	6,611.94
	% share	3.53%	0.89%	4.71%	1.81%	0.00%	5.99%
2004	kilotons	79.19	330.49	927.26	348.31	0.13	4,847.81
	% share	3.25%	0.40%	3.40%	0.60%	0.00%	4.16%
2005	kilotons	100.70	597.03	532.50	367.75	0.01	4,988.14
	% share	4.06%	0.68%	2.02%	0.55%	0.00%	4.13%
2006	kilotons	70.06	956.40	652.93	48.86	0.02	6,530.50
	% share	2.46%	1.01%	2.25%	0.07%	0.00%	5.26%
2007	kilotons	96.27	1,095.54	720.77	97.93	0.04	6,638.02
	% share	3.25%	1.02%	2.39%	0.13%	0.00%	5.57%
CAGR	kilotons	-7.55%	-4.46%	-7.15%	-19.23%	-2.63%	0.37%
	% share	-11.33%	-8.12%	-8.94%	-24.46%	-3.97%	-0.79%

* Rice includes broken, husked, milled and paddy rice.
CAGR = compound annual growth rate.

Source: FAO (2009) and author's analysis

In light of these figures, Brazil played a significant role in the world commodities trade in this period; for example, in 2007 exporting maize (10,933.46 kilotons and 9.97 percent of market share), refined sugar (6,915.80 kilotons and 29.95 percent of market share) and soybeans (23,733.78 kilotons and 31.90 percent of market share), while importing maize (1,095.54 kilotons and 1.02 percent of market share) and wheat (6,638.02 kilotons and 5.57 percent of market share).

In terms of share growth, maize showed a compound annual growth rate for imports from 1998 to 2007 of 108.12 percent. The increase can be explained by factors such as a favourable exchange rate, record harvests and low domestic prices from 2001 onwards (IEA, 2003).

Rice and beans play a very important role in the Brazilian population's diet, but they are not as significant as other crops in the country's trade statistics (720 kilotons of rice imports and 201 kilotons of exports in 2007), reinforcing two issues mentioned previously: food security in Brazil is not significantly associated with the trade in major food commodities aimed at foreign markets.

Table 4: Major Brazilian crop exports, 1998–2007 (imports in kilotons; global market share in %)

		Dry beans	Maize	Rice*	Soybeans	Sugar refined	Wheat
1998	kilotons	1.85	7.17	6.61	9,274.75	3,575.27	4.19
	% share	0.08%	0.01%	0.02%	24.41%	19.90%	0.004%
1999	kilotons	2.54	7.52	47.67	8,917.21	4,273.26	1.63
	% share	0.10%	0.01%	0.19%	22.13%	23.83%	0.001%
2000	kilotons	4.78	6.70	26.41	11,517.26	2,158.35	0.97
	% share	0.18%	0.01%	0.11%	24.31%	12.54%	0.001%
2001	kilotons	2.32	5,628.98	22.13	15,675.54	4,083.34	0.84
	% share	0.08%	6.72%	0.08%	27.52%	22.32%	0.001%
2002	kilotons	16.20	2,746.99	29.96	15,970.00	5,724.01	1.03
	% share	0.48%	3.14%	0.11%	29.23%	27.23%	0.001%
2003	kilotons	2.69	3,566.23	19.44	19,890.47	4,560.73	50.31
	% share	0.08%	3.93%	0.07%	30.58%	22.30%	0.046%
2004	kilotons	2.00	5,031.00	36.74	19,247.69	6,198.18	1,323.43
	% share	0.07%	6.08%	0.13%	33.39%	29.27%	1.113%
2005	kilotons	2.29	1,070.02	272.32	22,435.07	6,568.08	156.57
	% share	0.09%	1.18%	0.91%	34.31%	26.68%	0.130%
2006	kilotons	7.77	3,938.00	290.17	24,957.98	6,063.24	652.10
	% share	0.27%	4.13%	0.94%	36.77%	25.11%	0.516%
2007	kilotons	30.85	10,933.46	201.48	23,733.78	6,915.80	104.48
	% share	1.04%	9.97%	0.60%	31.90%	29.95%	0.079%
CAGR	kilotons	32.53%	108.12%	40.73%	9.85%	6.82%	37.95%
	% share	29.77%	100.65%	38.63%	2.71%	4.17%	35.30%

* Rice includes broken, husked, milled and paddy rice.
CAGR = compound annual growth rate.

Source: FAO (2009) and authors' analysis

2.3 Trends in the Brazilian population's food consumption patterns

According to Buainain and Da Silveira (2002), the calories available for daily consumption are, on average, sufficient to feed all Brazilians. The main issue is that extreme social and economic inequalities combined with household wastage/losses can reduce this amount to an unacceptably low value. This mainly affects poor people, who often simply cannot afford to buy sufficient food. Buainain and Da Silveira (2002) say:

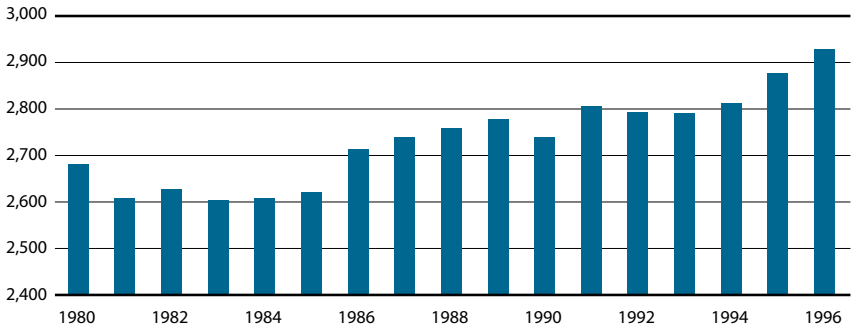
> A cursory analysis of the Brazilian food balance sheets shows several interesting facts. Food consumption in Brazil is changing, and the pattern of change is clearly perceptible. The average energy supply grew from 2,408 calories per day in 1970 to 2,938 in 1996, increasing at an annual rate of 0.77 percent .... This suggests a steady improvement in nutritional status for the average Brazilian. Mean individual requirements (account taken of gender-age composition) are about 2,200 calories/day, and the average supply in 1970 (only 2,408 calories) would seem insufficient once thought is given to household losses (some 10%) and inequalities between lower and higher income groups. A supply of 2,938 calories per day would seem safer (though of course a significant proportion of Brazilians is still unable to meet their needs).

Buainain and Da Silveira (2002) continue that food-energy consumption seems to converge towards the rate of population growth. Generally, Brazilians have started having a more diverse diet that includes more daily fat and protein consumption and a new mix of fruits and vegetables:

> Even in the presence of a better distribution of income, and consequently lower malnutrition at the bottom, average apparent consumption would not grow by much. With population growing at about 1.5% per year in the coming decades, calorie consumption would not grow faster than 1.9% per year. The rate of increase in calorie consumption per person would tend to diminish over time, possibly reaching very low values in 20 years. However, as per capita income grows food-consumption patterns change. In the case of Brazil between 1970 and 1996, whilst energy intake grew by 22%, and protein consumption correspondingly increased by 22.7%, fat consumption went from 46.1 to 81.5 grams per day, increasing by 76.7% in the same period. In protein consumption the composition of the protein mix varied significantly. The following table shows changes in some selected items between 1970 and 1996. The most striking increases in per capita domestic demand are undoubtedly those of poultry, beer, vegetable oil and milk. Also beef consumption has been on the rise, as well as vegetables and fruit other than citrus and banana. In fact, banana consumption has sharply diminished; intensive use of this product is typical of the traditional (mainly rural) Brazilian diet where it plays a significant role as a source of energy along with cassava, beans and rice.

Some food products have increased their part in the Brazilians' diet as a consequence of increased income and social changes associated with the process of urbanization. Food consumption habits in urban and rural regions are very different and notable differences exist among social groups in Brazil. For example, wheat consumption jumped from 36 kg/year in 1970 to 45–49 kg/year in 1980–96, while cassava consumption nearly halved from 98 to 50 kg/year. According Buainain and Da Silveira (2002), the rapid change of the urban–rural proportion in the Brazilian population, with the urban population already accounting for 80 percent of the total, is the main driver of these changes. However, this phenomenon might have less importance in the future.

Figure 2: Evolution of daily calories consumption by individuals in Brazil, 1980–96 (calories/day)



Source: Buainain and Da Silveira (2002)

2.4 Government policies and food security

The Brazilian government uses a guaranteed minimum price policy to stimulate agriculture. For example, in the 2007/08 harvest season, farmers facing several cost increases needed support policies to increase production or to reduce their loss of profit. In the first quarter of 2008, government was worried that increasing costs and growing world demand would put pressure on food prices, some of which were very important for Brazilians' diet and the country's agribusiness, such as rice, beans and maize. For example, the guaranteed minimum price of a 50 kg sack of rice was raised from BRL 22.00 to BRL 25.80 in the southern states of Santa Catarina and Rio Grande do Sul—typical rice producers—an increase of 17.27 percent. The price of a 60 kg sack of beans was raised from BRL 48.42 to BRL 80.00, an increase of 65.22 percent. The price of a sack of maize in South, Southeast, Mato Grosso and Goiás provinces and the Federal District was raised from BRL 14.00 to BRL 16.50, a 17.86 percent increase.

2.4.1 Agricultural programs and policies on food security: The Guaranteed Price Policy

The Guaranteed Price Policy is the main Brazilian agricultural policy for achieving food security and is aimed at small and medium-sized farmers. The primary idea of this policy is to ensure that purchase prices at least cover production costs, plus a certain level of profits. The government buys surplus crops, paying higher prices than those in the market. This mechanism reduces risks for crop producers during the harvest and is an important mechanism to reduce the price volatility that is intrinsic to the agricultural sector. When prices fall below the minimum level established by government, measures are taken such as buying crop surpluses from small farmers, prices equalization and financing the building up of stocks of selected crops. The minimum guaranteed price for crops considered to be regional and summer harvests is defined by government. This policy is aimed at achieving economic growth, increasing farmers' income and increasing the competitiveness of crop exports, when applicable.

The minimum price guaranteed is aimed at reducing or transferring to the wider society the uncertainty over prices faced by small-scale farmers. When fixed properly, minimum prices correctly anticipate market prices for producers, reducing the level of uncertainty for both producers and consumers and allowing the better allocation of productive resources (De Aguiar & Pinho, 1998). Guaranteed minimum prices policies directly influence producers' decisions on setting a targeted output for the next season, thus affecting the usage and intensity of production factors. Farmers take minimum price into account to decide on which crops they should plant. But they can also opt for the alternative of trading in the Stock and Futures Markets to protect themselves against future drops in price. The policy in theory guarantees a minimum return on the harvest, but this minimum price is not always enough to cover production costs.

During the food crisis, the government's minimum prices were adjusted as production costs of important crops increased and effectively eliminated or reduced profits for producers and/or impacted the final prices to consumers. In this context, the government presented the 2008/09 harvest plan, which contained changes in minimum prices of various products in order to adapt Brazilian production policy to commodity prices prevailing in the international markets.

2.4.2 Additional food policies

Due to the nutritional deficiencies of the poorest segments of the population and the country's social inequities, successive Brazilian governments have implemented a range of food assistance, anti-poverty, and welfare programs and other social policies over the past 50 years. These programs have concentrated

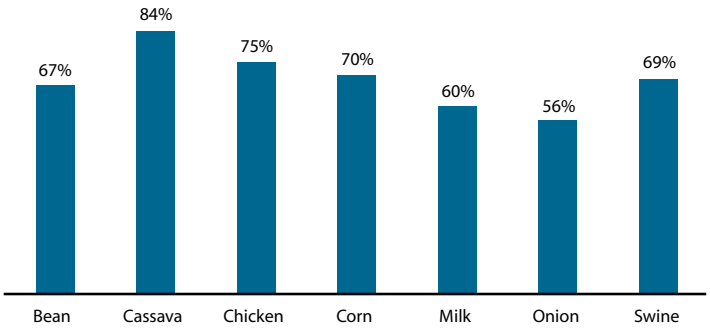
on investment in human resources and social assistance (pensions, health care, education, housing and basic sanitation), and programs to combat poverty (social welfare, programs to support peasant agriculture, agrarian reform, rural development and direct income transfers). During the 1990s (the so-called 'reform decade') various programs were implemented. From 1996 to 1999, government policies led to the formulation of the Alvorada project for poverty reduction in less-developed cities, the development of the Community Solidarity Program and the incorporation of the Bolsa-Escola project (a school fund project) as federal programs.

In January 2003, newly elected President Lula and his team of economic advisers launched Brazil's Zero Hunger Program ('Programa Fome Zero'), which constitutes the core of the social agenda of his administration. The program comprises 60 different initiatives with a goal of providing food access to 11.4 million families (or roughly 50 million people) within five years. The program is supported by agrarian reforms, producer incentives and the enactment of minimum agricultural income policies.

At the peak of the global food prices crisis, the Brazilian government's first response was the creation of the More Food Program (Programa Mais Alimento) by the Ministry of Agrarian Development (MDA), a program intended to equip, organize and strengthen small farms and counter the global food crisis and the recent increase in prices of agricultural commodities worldwide. There were great expectations for this program, since the government believed it would form one of the pillars of the country's attempts to try to withstand the crisis.

The MDA and the government hoped that this policy would achieve 18 million tons per year of excess production, focusing mainly on products that comprise the main Brazilian food basket ('*cesta básica*'): rice, beans, milk, wheat, coffee, fruit, poultry, onions, cassava and maize. A credit line of up to BRL 100,000 per farmer was established that could benefit nearly one million farmers by the year 2010. The advantage of this benefit to producers is clear: since approximately 70 percent of food arriving at Brazilian tables originates in family/subsistence farming (see Figure 3, which covers both the domestic and foreign markets).

Figure 3: Participation of family/subsistence farming in Brazilian food production, 2006



Source: IBGE (2006)

A fuller description of all major government programs and policies on food and agriculture appears in the Annex to this report.

3. Additional food security drivers

In addition to the main issues presented above, several other drivers should be considered when assessing the complexities of the issues affecting Brazil's food security, such as land use changes, competition for land and costs associated with food production, especially fertilizers and other agricultural inputs. These issues are discussed below.

3.1 Biofuels demand and land use changes

In Brazil, the recent growth of biofuels production has raised concerns about its sustainability, particularly the expansion of sugar cane production. It has been suggested that sugar cane cultivation increases Amazon deforestation and also reduces areas available for the production of other important foods such as rice, beans and maize. Addressing these concerns, the Brazilian government carried out a study in 2009 as part of the Agroecological Sugar Cane Zoning initiative to map out which areas are more conducive to sugar cane production. The initiative also prohibits cultivation of the Amazon forest, Pantanal and other protected areas, thus reducing the direct impacts of biofuel production on these ecosystems.

3.2 Competition for land use

Table 5 shows that Brazil has approximately 340 million hectares of arable land, of which less than 1 percent is used for sugar cane production, but almost 3 percent for soybean production and almost 40 percent for pasture. If proper development and law enforcement policies were put in place, such as land use zoning, increased agriculture productivity in Brazil might simultaneously reduce the pressure to develop new land areas for agricultural production and increase the amount of food produced without negative impacts on the environment or food security. By increasing mechanization and the use of fertilizers, large monoculture farms are producing more food, supplying internal needs at a higher productivity rate per hectare and thus contributing to global trade.

Table 5: Brazil's land area and its uses

Land area in Brazil	Land (M-ha)	% of total land	% arable land
Brazil	850		
Preserved areas and other uses*	510 (60%)		
Arable land	340 (40%)		
Cultivated land: all crops	63.1	7.40%	18.60%
Soybeans	20.6	2.40%	6.10%
Corn	14	1.60%	4.10%
Sugar cane	7.8	0.90%	2.30%
Oranges	0.9	0.10%	0.30%
Pastures	200	23.50%	58.80%
Available land (ag. livestock)	77	9.10%	22.60%

* These areas include the Amazon rainforest, protected areas, conservation and reforestation areas, cities and towns, lakes, and rivers.

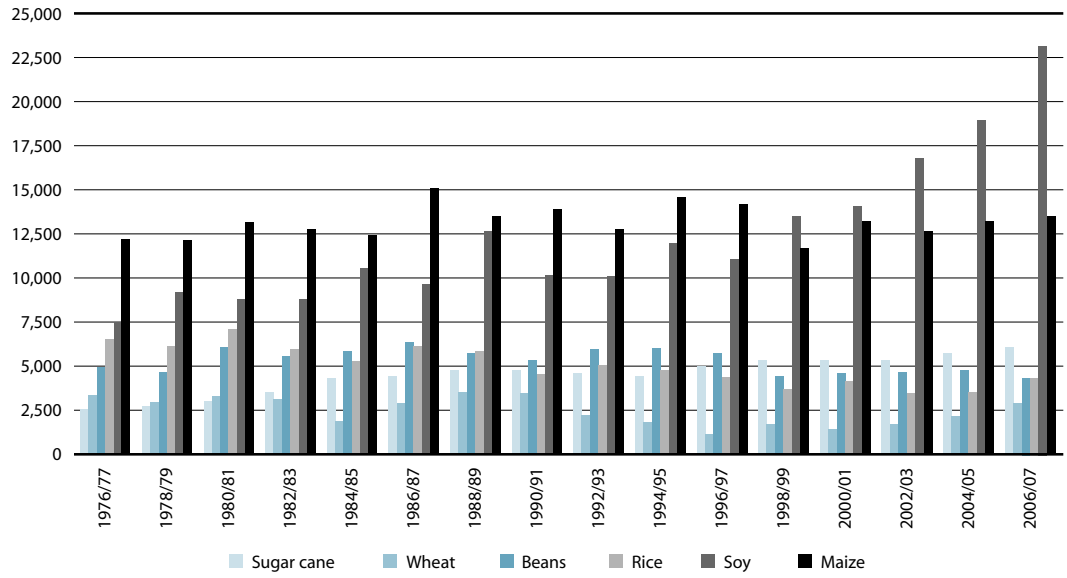
Source: Authors' analysis of data supplied by Instituto Brasileiro de Geografia e Estatística, Companhia Nacional de Abastecimento & União da Indústria da Cana de Açúcar

The growing demand for ethanol has increased the area of sugar cane under cultivation. Despite the government's efforts to monitor the dynamics of the evolving use of sugar cane to produce ethanol, these

initiatives are not sufficient to permit a detailed analysis of the impacts of sugar cane cultivation at the local level in terms of precisely describing the dynamics of crops changes, and the possible direct and indirect impacts on food prices and supply, and thus on global trade levels.

According to a study of the impacts of the sugar cane agribusiness in Brazil (IBASE 2008), changes in the total areas harvested of some crops does not mean that production has been reduced. Until 2006, growing productivity in the production of the main staple foods in Brazil more than compensated for the reduced area under cultivation (specifically of rice and beans). This production increment is also associated with increasing fertilizer usage, which between 1998 and 2007 grew by 68 percent, reaching 24.6 million tons and increasing Brazilian imports of fertilizers to about 17.5 million tons in 2007. This has meant that crops like beans that once were almost exclusive to small family farms are now being produced in the centre-west region in large, irrigated monoculture farms.

Figure 4: Evolution of areas of staple crops under cultivation, 1976/77–2006/07 (1,000 ha)



Source: IBASE (2008)

The total area of rice, beans and cotton harvested in the 2008 harvesting season showed a reduction when compared to the previous season (see Table 6). This was a small reduction at the country level, but possibly a significant one at the regional level. Some states might have become dependent on other states for their rice supplies or needed to import rice from overseas, although such a short period of analysis might result in misleading conclusions, and an assessment of long-term trends is required for an in-depth analysis.

Table 6: Variations in the harvested area of staple crops in Brazil and some centre-south states between the 2007 and 2008 seasons

Crop		Brazil	PR	SP	MG	GO	MS	MT
Sugar cane	Variation (ha)	964,182	108,502	433,400	124,662	144,880	68,423	32,901
	Variation %	11.5	20.1	9	19.2	36.2	35.7	14
Rice	Variation (ha)	-36,475	-7,480	-2,000	-18,629	-24,460	-7,109	-35,920
	Variation 5	-1.3	-13.8	-8.1	-21.7	-20.6	-16.7	-13
Beans	Variation (ha)	-26,466	-62,955	-13,850	26,041	-28,620	-3,189	46,622
	Variation %	0.7	-11.1	-7.2	6.6	-23	-15.5	108.5
Cassava	Variation (ha)	228,765	66,308	-15,710	4,172	80,820	-1,483	-5,706
	Variation %	2.4	5.1	-2.2	0.3	14.7	-1.5	-3.1
Maize	Variation (ha)	689,971	188,891	8,390	13,382	71,280	123,994	197,677
	Variation %	4.9	6.8	0.9	1	8.6	14.3	12.1
Soy	Variation (ha)	696,553	-30,130	700	-15,730	11,240	14,000	587,508
	Variation %	3.4	-0.8	0.1	-1.8	0.5	0.8	11.6
Cotton	Varitaiton (ha)	-54,499	-5,789	-19,380	-9,627	-10,770	-2,180	-21,252
	Variation %	-4.8	-47.2	-53.7	-31.7	-13	-4.7	-3.9

PR = Paraná; SP = São Paulo; MG = Minas Gerais; GO = Goiás; MS = Mato Grosso do Sul; MT = Mato Grosso.

Source: IBASE (2008)

3.3 Fertilizers and inputs

Perhaps the greatest reason for the rise of food prices in the period 2006–08, besides the increase in food demand due to population growth and, more importantly, increases in the incomes of consumers, was the price of fertilizers, especially in Brazil. As Brazil is one of the world's largest producers of agricultural commodities, the use of fertilizers is significant and has a considerable effect on the cost of production.

According to AgraFNP (2009), Brazilian demand for the raw materials used to produce fertilizers grew around 6.6 percent per year between 2000 and 2008, making Brazil the country with the largest growth during the analyzed period. However, as Brazil does not produce sufficient raw materials, the only alternative is importing what is required. Table 7 gives the proportion of imported raw materials for the Brazilian domestic fertilizer market over the last 20 years.

Table 7: The proportion of raw materials imported for Brazilian fertilizer production, 1990–2010 (%)

Year	Imported raw materials (%)
1990	36
2000	60
2003	64
2004	72
2005	67
2006	66
2007	73
2010 (forecast)	80

Source: AgraFNP (2009)

Fertilizer producers could in theory directly import the raw materials they require. However, for market reasons, this activity is controlled by large companies in the sector and the production chain is therefore limited to three companies, which account for 50 percent of Brazilian imports of potassium chloride

(AgraFNP, 2009). Also, if a company decides to import its own raw materials, only bulk buying permits gains of scale, stable supplies, better planning and dilution of risks. Table 8 shows Brazilian participation in world demand for the raw materials needed to produce fertilizers and the country's dependency on foreign markets and prices.

Table 8: Brazil's share of global demand for nitrogen, phosphorus and potassium, 2008 (%)

Nitrogen	Participation (%)	Phosphorus	Participation (%)	Potassium	Participation (%)
China	30	China	37	China	23
India	14	India	14	European Union	17
U.S.	12	U.S.	11	Brazil	13
Pakistan	3	Brazil	8	India	9
Brazil	2	Australia	3	France	3

Source: AgraFNP (2009)

Table 9 shows that Brazil is one of world's largest importers of the inputs used to produce fertilizers.

Table 9: Brazil's share in global imports of nitrogen, phosphorus and potassium, 2008 (%)

Nitrogen	(%)	Phosphorus	(%)	Potassium	(%)
U.S.	24	India	24	U.S.	19
India	10	Brazil	7	China	18
Turkey	5	U.S.	4	Brazil	14
France	4	Pakistan	3	India	9
Brazil	4	Mexico	3	Malaysia	4

Source: AgraFNP (2009)

Brazil's consumption of fertilizers has grown significantly from 12 million tons in 1994 to 25 million tons in 2007. However, this demand has shown some changes, for various reasons. The key ones were:

- farmers' capitalization and their capacity to obtain credit;
- the degree of technology used in production;
- the increase in the harvest area and the characteristics of newly cultivated soils;
- national agricultural policies; and
- changes in the exchange rate (prices on the international market are calculated in U.S. dollars).

Increases in the price of fertilizers have a direct impact on the cost of production, since they are the most relevant production input. The issue of fertilizer is a strategic concern for government in the context of the global food crisis and the security of the country's agricultural production because of Brazil's high dependency on foreign markets and foreign prices; its over-reliance on a small number of suppliers of local inputs, which can thus dictate raw materials prices, trade volumes and availability; increases in prices; and the fact that fertilizer costs form such a large part of production costs. Thus, the solution to this issue depends on solving many critical problems, a number of which are directly related to fertilizer imports, especially taxes on imports.

To try to prevent the spread of the food crisis, another government measure was a cut in the tariffs on fertilizer inputs imported from the Southern Common Market (MERCOSUR), particularly Argentina.

All such measures were related to MERCOSUR's Common External Tariff (TEC). Specific types of fertilizers were placed on the MERCOSUR TEC exception list and import tariffs for these products were decreased to zero.

Another approach was that of investment in infrastructure in Brazil itself for the production of fertilizer inputs. The government encouraged Vale and Petrobras to invest in research and technology development aimed at medium- and long-term exploration for deposits of several key minerals used to make fertilizer. It is thought Brazil has deposits that could make it virtually self-sufficient, except for potassium.

But even if the country were not self-sufficient in terms of these inputs, reducing the quantity of imports of such products would reduce spending on agricultural commodities. It would also reduce food prices for consumers and boost the competitiveness of farmers.

There is a direct correlation between fertilizer prices and oil prices, because oil is a raw material for production of nitrogen. Appreciation of the oil price therefore increases the production costs of nitrogen, one of the constituents of fertilizer. The price of oil has had another type of influence on world agriculture: the use of fossil fuels increases global warming, which in turn increases demand for renewable low-carbon fuels. This creates a greater interest in bioenergy, such as that produced from soybeans, corn and sugar cane. Thus, we might expect an expansion of the harvested area in the future, and thus higher consumption of fertilizers.

Besides the logistics issues, the fertilizer trade also faces several protectionist barriers established by producers. China, a major producer of fertilizers, raised the export tax on the raw materials for fertilizers by up to 135 percent in April 2008 in order to avoid a possible domestic shortage, causing prices in the international market to rise even more.

4. Conclusion

An assessment of the impacts of the policies adopted by the Brazilian government to deal with the 2006–08 global food price crisis shows that the crisis did not profoundly affect either Brazilian consumers or the country's trade performance.

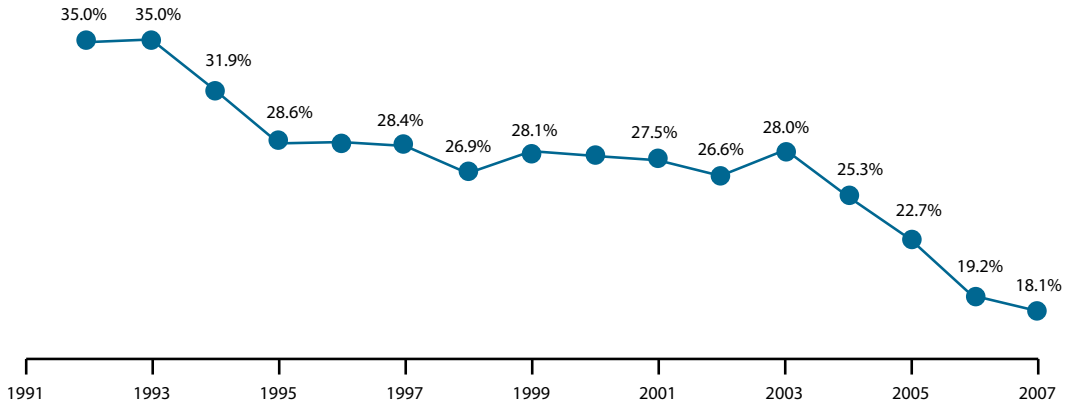
Brazil is a major food producer and sells a considerable share of its production in its domestic market, which allowed the government to put an array of policies in place to buffer Brazilians against the crisis and stimulate the domestic agricultural trade.

Moreover, during the last two decades there has been a considerable decrease in the percentage of the population living below the poverty line (defined as those with a monthly household income of below BRL 125). This means that a larger portion of the population has access to consumer goods, including food products.

In addition, in terms of the impacts of inflation on food prices (Figure 6), Brazil performed better than other countries, because it has an environment and a sophisticated economy that allowed it to fulfill domestic demand for food and expand its agricultural trade. During the food price crisis, Brazilians continued to consume at much the same level as previously, stimulated by socioeconomic improvements and higher incomes. At the same time, Brazil has played an important role in global food production and the global commodities trade. Its agricultural expansion during the last two decades has not only

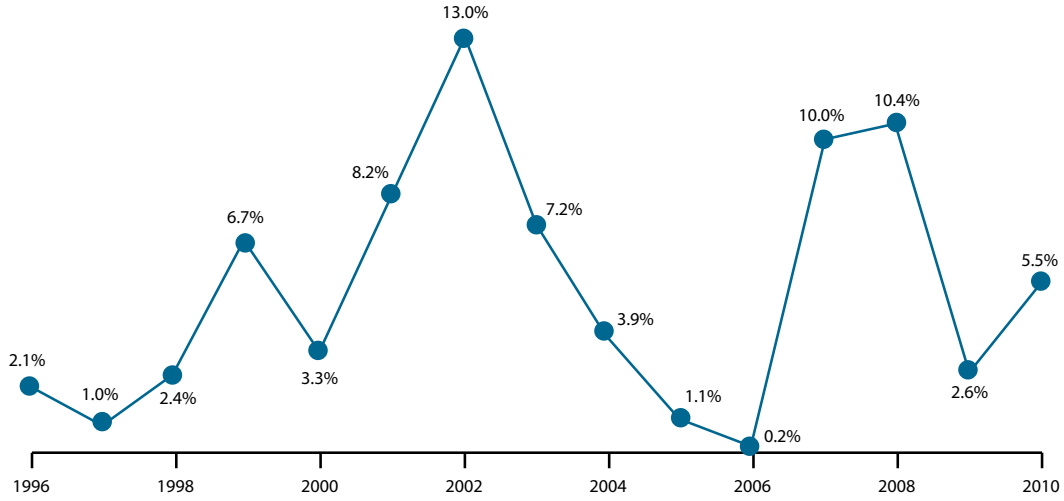
guaranteed increased food supplies to its domestic market, but, significantly, also to the global commodities trade, thus not adding additional pressure to global food prices.

Figure 5: Percentage of the Brazilian population living below the poverty line, 1991–2007



Source: DIAP (2010)

Figure 6: Variations in Brazilian food prices, 1996–2010 (%)



Source: Lacerda & Boteon (2009)

An analysis of complex issues such as food security and trade within and among countries and across regions requires a common and agreed methodological framework in order to draw comparable conclusions and make realistic assessments. This paper is not an exhaustive treatment of these issues as they relate to Brazil, but rather an attempt to raise issues to be considered when designing these frameworks, and, more specifically, to provide a reasonable understanding of the situation in Brazil in terms of food security and the trade in food during the last two decades.

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Annex: Major Brazilian government food and agricultural programs, 1994–2010

1994 – School Fund Program

The School Fund Program (Programa Bolsa Escola) was designed by the mayor of Campinas, José Roberto Magalhães Teixeira, for the provision of scholarships to families with young children and low incomes in order to stimulate regular school attendance. The program was implemented in 2001 on a national scale. It had benefitted more than five million families when in 2003 it was incorporated into the Family Fund Program by Luiz Inácio Lula da Silva, the current president of Brazil.

2001 – Food Fund Program

The Food Fund Program (Programa Bolsa Alimentação) was created on 10 August 2001 with the aim of promoting the health and improving the nutrition of pregnant women, nursing mothers, and children aged between six months to six years by supplementing family income. Individuals at nutritional risk belonging to low-income families who earned less than BRL 140 per capita monthly were potential beneficiaries of the program.

The benefits of the food fund were paid out by the Ministry of Health by means of magnetic card held by the mother or the adult responsible for a particular child. The benefit had a monthly value of BRL 15–45 per family. The program lasted for six months, but it could be extended for a further six-month period if beneficiaries remained at the same poverty level and had fulfilled a series of commitments to their health, such as undergoing pre-natal consultations/care, weighing and vaccinating children regularly, stimulating their physical and psychological development, and receiving guidance on diet and nutritional care.

2001 – Gas Assistance Program

The Gas Assistance Program (Programa Auxílio Gás) was an income distribution program implemented by the Brazilian federal government in 2001 to assist beneficiaries of the Social Protection Network.

The program provided a payment of BRL 15 every two months for families earning half the minimum wage to subsidize the purchase of gas canisters. Studies conducted at the time indicated that many needy families were suffering nutritional problems simply because they were unable to afford the gas needed to cook their food. The program intended to solve this problem. In 2002, it reached the 4.8 million families already served by the School Fund Program and in 2003 it was incorporated into the Family Fund Program.

2003 – Zero Hunger Program

In January 2003 newly elected President Lula and his team of economic advisers launched Brazil's Zero Hunger Program (Programa Fome Zero), which constitutes the core of the social agenda of his administration. The program comprises 60 different initiatives and was intended to provide additional food to 11.4 million families (or roughly 50 million people) within five years.

The program was supported by agrarian reforms, producer incentives and the enactment of minimum agricultural income policies. Other initiatives included the Food Coupon Program (inspired by the U.S. food stamp program), food vouchers to be exchanged at government-licensed food outlets, and food banks that redistributed surplus food from supermarkets and restaurants. Additional initiatives targeted low-income workers, while nutrition programs supplied food to pregnant women, new mothers and babies. The School Meals Program aimed to increase the quality of school meals using regional food resources. Existing school meals programs were expanded to cover siblings of children attending school and were extended into school vacations. Other related initiatives included food and nutrition campaigns to educate the population about healthy eating in order to prevent obesity and malnutrition.

2003 – Food Card Program

The Zero Hunger Program is defined as one of its most urgent strategies in the implementation of the Food Card Program (Programa Cartão Alimentação), which was aimed at fighting hunger and promoting food and nutrition security, understood as the guarantee of daily human access to food in sufficient quantities and of the required quality.

To implement the program and the other activities of the Zero Hunger Program, a partnership among the federal, state and local governments was essential. A food card was given to people with a family income per capita of less than half of the minimum wage. The card was intended to increase the financial resources of people facing food insecurity by giving BRL 50 per month to each family for a period of up to six months, extendable for a further two periods of six months. The holder of the magnetic card used to access these funds was preferably the woman who cared for the family.

The aim was to transform the living conditions of families by reducing their risk of experiencing food insecurity. Initially the program was mainly focused on the populations of cities identified as being in crisis. Later, the card was issued to families that collected garbage on rubbish dumps as a means of livelihood, mainly landless families, Maroon communities and indigenous people.

2003 – Family Fund Program

In the fall of 2003, the government merged all the existing income-transfer programs which up to that point had been administered by four different ministries—into one major program called the Family Fund Program (Programa Bolsa Família), which had a budget of BRL 5.3 billion (about USD 1.5 billion) in 2004.

This program was intended to provide direct income, under certain conditions, to families facing poverty (defined as those with a monthly income per person of BRL 70–140) and extreme poverty (those with a monthly income per person of less than BRL 70).

The program included the Zero Hunger Program, which has already been discussed above, and had three essential aims:

1. to promote the immediate relief of poverty through the direct transfer of income to families;

2. to strengthen the exercise of basic social rights in the areas of health and education, thus enabling families to break the cycle of intergenerational poverty; and

3. to coordinate complementary programs designed to develop families so that they overcame their vulnerability and poverty. Examples of supplementary programs included programs to generate jobs and income, teach adult literacy, and provide civil registration and the acquisition of other key documents.

2003 – Food Purchase Program

The Food Purchase Program (Programa de Aquisição de Alimentos) aimed to support small-scale farmers and involved the distribution of agricultural products to people facing food insecurity and the formation of strategic stocks of tools that enabled the restructuring and development of small-scale farmers. It was initiated each year when farm produce was sold after the harvest in order to cover farmers' costs (including the hiring of farm employees) and give them a fair profit, thus guaranteeing sufficient financial resources for the survival of their families with dignity.

Another objective of the program was to encourage the recovery and preservation of agro-biodiversity in different regions of the country by providing incentives for the work of organizations that supported and assisted small-scale farmers. In this sense, systems for the sustainable management of cultivation were encouraged in order to develop the plant species characteristic of each region.

The foods purchased directly from farmers or their associations and cooperatives formed part of governmental stocks from which food was donated to people facing food insecurity and inadequate nutrition who were being assisted by local social programs. Purchases were made directly by the National Provision Company (Companhia Nacional de Abastecimento), taking regional characteristics and local market conditions into account.

By ensuring that the production of small-scale farmers was purchased, the government gave them security and encouraged them to produce more good-quality food. The program therefore significantly improved the standard of living of the farmers and their families and promoted sustainable development in the areas that were assisted. By promoting the purchase of household production, agricultural activity became much more stable and was thus able to generate employment and income for producers on their own land, which encouraged them to stay on the land and not move to the already overcrowded cities to seek employment.

By storing food in public stocks the government sought to contain the rise in domestic food prices. To ensure the purchase and replenishment of stocks, the government held auctions before the planting season in order to signal to farmers the selling price they would receive for certain products, thus giving them more security. In addition to rebuilding depleted stocks, this measure aimed to ensure that crops would be sold at prices compatible with their production costs. A lack of sufficient public stocks would prevent the government from intervening more effectively in the market to curb the scale of food price increases, and this program was designed to avoid such a situation.

2008 – More Food Program

At the peak of the 2006–08 global food prices crisis the Brazilian government's first response was the creation of the More Food Program (Programa Mais Alimento) run by the MDA, which was designed to equip, organize and strengthen small-scale farms and thus counter the global food crisis and the recent worldwide increases in the prices of agricultural commodities. This program has already been discussed in section 2.4.2, above.

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Circular economy indicators for organizations considering sustainability and business models: Plastic, textile and electro-electronic cases

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Circular economy

ABSTRACT

Circular Economy is the optimal point of sustainability, given that it offers a set of practices capable of generating more sustainable operations, making sustainability feasible in organizations. To measure the innovations brought by Circular Economy, there is a recent need to develop circularity indicators, mainly for micro level (companies and products). Furthermore, the complexity of Circular Economy implies in a set of multi-dimensional indicators instead of a single one. This paper aims to develop a set of indicators linking Circular Economy principles, Circular Business Model and the pillars of Sustainability. The set of indicators was developed based in the hypothetic-deductive approach, following a number of iterations (cycles) and testing the theory in the empirical world. A mix of research methods (e.g. expert consulting, user's feedback, and case studies) was applied. The proposed indicators should be able to achieve the principles of the Circular Economy, and, at the same time, help to meet the specificities and needs of each circular business model. The main contribution of this paper is the development of a group of indicators, focused in the three dimensions of Sustainability (environmental (from material perspective), economic and social), applied in Circular Business Models to capture the innovations brought by Circular Economy that conventional indicators do not measure. Moreover, they will help any company to identify areas with high importance and potential for improvement, and thus increase Circular Economy performance in an efficient, clear and prompt manner. These indicators were applied in three Brazilian companies which have three different Circular Business Models. The results show that data from economic and social dimensions was not available or was diffused in the companies. It represents a barrier because most of the positive impacts gained with Circular Economy are presented in the social dimension, including job creation, mindset change, etc.

1. Introduction

Sustainability could be defined as the "balanced integration of economic performance, social inclusiveness, and environmental resilience, to the benefit of current and future generations" (Geissdoerfer et al., 2017, p.766). Some authors affirm that sustainability can help organizations to implement Circular Economy (CE) (Kravchenko et al., 2019; Sehnem et al., 2019). According to Sehnem et al. (2019), sustainability is a driver of CE and is mediated by innovation, Kravchenko et al. (2019) complement that CE is a stepping-stone towards sustainability.

The concept of CE arises with the objective of keeping the products, component, and materials useable and useful to return to the cycles. This economic model is based on restoration and regeneration (Ellen Macarthur Foundation, 2017). It is an economy based on the principles of design out waste and pollution, keeping products and materials in use and regenerate natural systems (Ellen MacArthur Foundation, 2018). A great differential of CE is not minimize negative impacts, as shown by eco-efficiency, but to optimize positive impacts, highlighting by eco-effectiveness (Niero et al., 2017).

Korhonen et al. (2018a) showed a definition for CE based on the

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pillars of sustainability (environmental, economic and social). The environmental goal of CE is to reduce the use of raw material and energy inputs, in addition to minimize waste generation and emissions. The economic goal of CE is to reduce the costs, risks and taxation from environmental pillar as well as to innovate new product designs and market opportunities for businesses. The social goal is the sharing economy, increased employment, participative democratic decision-making and increase a collaborative culture. CE, as an economic system, facilitates sustainable development (Korhonen et al., 2018b; Prieto-Sandoval et al., 2018).

Some authors affirm that a Circular Business Model (CBM) is a type of sustainable business model (Bocken et al., 2016). However, other see that not all CBM consider the dimensions of sustainability (environmental, social, and economic) (Mentik, 2014). CBM may be defined as "the logic of how an organization creates, delivers and captures value within closed circuits" (Mentik, 2014) and also "create, capture, and deliver value to improve resource efficiency by innovation" (Frishammar and Parida, 2019). Defining a business model is complex and requires that all the dimension of a business model be taken into account. Moreover, even moderate transformation of a mature organization's business model to include CE and sustainability can have positive environmental, economic and social effects (Frishammar and Parida, 2019).

Osterwalder and Pigneur (2010), developed the business model mapping tool named Canvas, which is divided into 9 components. The customer relationship component establishes the relationship that the organization has with its market segment. The value proposition describes how the organization creates value for its customers. The costs involved in operating the business model are also analyzed. The revenue source refers to the money that the organization generates. Key resources and key activities are those needed to operationalize the business model. The channels describe how the organization reaches its customers through communication, distribution and sales. The partners refer to the suppliers' network (Osterwalder and Pigneur, 2010).

Kiron et al. (2017) conducted a study to assess how organizations are contributing to sustainability. In the survey, 60,000 entrepreneurs were interviewed around the world. The results show that 90% of executives see sustainability as important, but 60% have a sustainable strategy (Kiron et al., 2017). In addition, 50% of organizations have changed their business models in response to sustainable opportunities. In this context, the change to a CE requires organizations to innovate their business models (Ellen Macarthur Foundation, 2017). Business Model Innovation (BMI) is essential to ensure companies competitive advantage and capabilities regarding to circularity and sustainability (Pieroni et al., 2019). BMI is "designed, novel, nontrivial changes to the key elements of a firm's business model and/or the architecture linking these elements" (Foss and Saebi, 2017, p.201). The innovation in CBM may facilitate the transition to CE (National Confederation of Industry, 2018). The use of indicators to measure circularity performance is essential to improve and assess CBM. However, the measurement and assessment of circularity performance is not yet a common practice in companies (Sassanelli et al., 2019).

According to The British Standard Institution (BSI) BS 8001:2017 (BSI, 2017), there are six types of Business Model which have the potential to fit within the circular economy system. They are based on-demand, dematerialization, product life cycle extension/reuse, recovery of secondary raw materials/by-products, product as service/product-service system (PSS), and sharing economy and collaborative consumption. Table 1 presents a brief description of each one.

CE could be applied in three levels (Yuan et al., 2006): micro (e.g. companies and products), meso (e.g. industrial symbiosis) and macro (e.g. countries). In this paper, we operate at the micro level of CE. Moreover, British Standards Institution published the BSI standard 8001: 2007 "Framework for implementing the principles of circular economy in organizations" (BSI, 2017), to assist in the principles, strategies, implementation and monitoring of the CE in companies (Pauliuk, 2018). However, there is still a need for specific standards and metrics (Saidani et al., 2019; Tecchio et al., 2017).

Circularity might be defined as a fraction of a product that comes from used products (from closed or open-loop cycles) (Linder et al., 2017). But there are some other important aspects like environmental burdens and social gains, which could be included in CE scope. Thus, CE could be experimented in an ecosystem working, sharing values for all stakeholders involved (Zucchella and Previtali, 2019). Moreover, several works emphasize the need to create CE metrics in micro level (Elia et al., 2017; Linder et al., 2017; Lonca et al., 2018), including the link with sustainability (Geng et al., 2012; Mesa et al., 2018).

Previous papers show that Circular Economy aims to reach sustainability (Franklin-Johnson et al., 2016; Mesa et al., 2018). But these indicators only addressed the material aspects (Virtanen et al., 2019). The majority of studies also involving specific CE indicators focused in end of life strategies (Di Maio and Rem, 2015; Figge et al., 2018; Jensen et al., 2019), and eco-efficiency (Laner et al., 2017; Yin et al., 2014; Zhou et al., 2018) instead of economic (Di Maio et al., 2017; Scheepens et al., 2016), environmental (Huysman et al., 2017) and social indicators (Geng et al., 2012). Some existing CE indicators are described below, including the advantages and disadvantages, according to their applicability, practicality, and CE principles, Table 2.

CE indicators are in the initial stage of development (Giurco et al., 2014). Traditional indicators could not express CE in its totality, because they are not designed for the systemic, closed-loop, feedback features that represent CE (Geng et al., 2013). Besides, the complexity of CE implies a need for a set of multidimensional indicators instead of a single one (Griffiths and Cayzer, 2016). Thus, there is a need to propose indicators to assess different Business Model economically, environmentally and socially (Pieroni et al., 2019), in this way, this work tries to fill this gap. Therefore, the research question investigated in this paper is: *How organizations can measure Circular Economy performance considering Sustainability and Business Model perspective?* So, this work aims to develop a set of multidimensional indicators, applied to Circular Economy, in the three dimensions of sustainability: environmental (from material perspective), economic, and social.

The paper is structured in four sections, where Section 2 outlines the Research Method, Section 3 presents the Results and Discussion and Section 4, the Conclusions.

2. Research method

According to Yin (2015) an exploratory study aims to explore a problem and collect information about the subject to build the hypothesis. In addition, an explanatory study identify and explain the roots of a problem; explaining the reality (Yin, 2015). Usually, exploratory studies offer a more detailed view of the subject. Thus, our research is exploratory because we formulated the indicators requirements based on literature and we tested our theory through empirical sections.

The set of indicators was developed based on a hypothetic-deductive approach (Gill and Johnson, 2002), following a number of iterations (cycles) and testing the theory through an empirical work (Kjaer et al., 2018). A mix of research methods (e.g. expert consulting, user feedback and case studies) was applied (see Fig. 1).

Pre-step:

In the pre-step, indicators requirements were formulated (see section 2.1) based on literature review.

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Table 1
Description of each circular business model.

Circular Business Model	Description
On-demand	"Producing a product or providing a service only when consumer demand has been quantified and confirmed" BSI (2017) p. 47.
Dematerialization	"Replacing physical infrastructure and assets with digital/virtual services" BSI (2017) p. 47.
Product life cycle extension/reuse	"New products are designed to be durable for a long lifetime (durability). Design improvements might be needed to also facilitate easier repair, particularly by third parties" BSI (2017) p. 47.
Recovery of secondary raw materials/by-products	"Value optimization by creating products from secondary raw materials/by-products and recycling (e.g. polyethylene depolymerization, steel, bio-based materials), whether open or closed loop" BSI (2017) p. 48
Product as service/product-service system (PSS)	"Company delivers product performance or defined results rather than the product or service itself" BSI (2017) p.49.
Sharing economy and collaborative consumption	"Lending or "collaborative consumption" amongst users, either individuals or organizations, but where some form of transactional arrangement (which could be financial) is provided" BSI (2017) p. 50.

Cycle 1:

In this step, we conducted personal semi-structured interviews in companies that were potential users of the indicators (see section 3.1). These interviews provided information about the companies and their strategies through CE. With this information and the indicators requirements, the first version of the indicators was developed through expert consulting. The first version of the indicators was sent to the companies and a workshop was conducted to provide improvement opportunities.

Cycle 2:

Through refinement and consolidation, a second version of the indicators was developed and sent to the companies. In this step a multiple case study was conducted with three Circular Business Models selected (see Table 3). A case study is an empirical research on a contemporary phenomenon in its context, evidencing the importance of doing a multiple case study to support replicable results and reliability (Yin, 2005). The companies collected the data and return the results (see section 3.1). These data were analyzed and after that, the final version of the indicators was confirmed (see section 3).

2.1. Indicators requirements

Sustainability is a goal to achieve in CE, so the indicators applied in many studies are usually multidimensional (Cook et al., 2017; Domingues et al., 2015; Mapar et al., 2017). The set of indicators proposed in this paper assumed three dimensions: environmental (from material perspective), economic, and social, with qualitative and quantitative indicators.

Based on the BSI 8001:2017, we identify that a requirement for a CE indicator is the ability of this indicators to achieve the CE principles at the same time that help to meet the specificities and needs of each CBM. Moreover, we identified through the organization's practices that the CE indicators must address issues related to resources, production and consumption; economics factors and social issues. All these requirements should be linked to the indicators applicability, thus we built the connections among the requirements using an intensity level, based on the BS 8001:2017 levels of circularity maturity and CE principles, to define the relations among the indicators, the CE principles and the CBM.

We used the CE principles proposed by the standard BSI 8001:2017 (BSI, 2017):

1. Systems thinking – a holistic approach to understand the interactions between individuals and activities within the wider systems they are part of;
2. Innovation – continually innovate to create value by enabling the sustainable management of resources through the design of processes, products/services and business models;
3. Stewardship – manage the direct and indirect impacts of their decisions and activities within the wider systems they are part of;
4. Collaboration – collaborate internally and externally through formal and/or informal arrangements to create mutual value;
5. Value optimization – keep all products, components and materials at their highest value and utility at all times;
6. Transparency – organizations are open about decisions and activities that affect their ability to transition towards a more circular and sustainable mode of operation and are willing to communicate these in a clear, accurate, timely, honest and complete manner.

The symbols used in the tables represent the intensity levels based on BSI 8001:2017 maturity model:

● represents a **strong relationship** regarding to the optimizing level.

◍ represents a **median relationship** regarding to the improving level, and.

○ represents a **weak relationship** regarding to the unformed and basic level.

These levels of intensity mean that the indicators with strong connections are very efficient to achieve the defined requirements presenting ways for doing business and creating additional circular values. The indicators with median connections could help in the achievement of the requirements but in the proposition of circular solution regarding to the product/service or process. The indicators with weak connections could be applied in the initial stages of the CE journey once they are useful to explore the opportunities. This are important to guide the organizations in the indicators implementation and the definition of strategies to improve the organization's CE performance. Organizations which apply CBM could gain benefits from this connection to direct CE efforts to the business model and principles to achieve CE.

In Table 4, we present the connections related to the environmental (from material perspective) dimension. Table 5 shows the connections related to the economic dimension and Table 6 to the social dimension.

3. Results and discussion

Literature showed that most indicators in CE focus on material flows (Moraga et al., 2019; Virtanen et al., 2019) and end of life strategies (Gigli et al., 2019; Jensen et al., 2019). But there is a need to create multidimensional indicators (Griffiths and Cayzer, 2016) to measure Circular Economy in its totality (Geng et al., 2013), including the sustainability dimensions. The innovations brought by CBM need to be measured by companies to set targets to

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Table 2
Indicators in literature.

Indicator	Advantages	Disadvantages	Reference
BIM-based Whole-life Performance Estimator (BWPE)	Evaluation of the performance of civil construction projects.	Restriction of the CE only in the scope of the reuse and recycling.	Akanbi et al. (2018)
Building Circularity Indicators (BCI)	Application of some CE principles in the context of civil construction, taking into account the types of materials used.	Need for prior technical knowledge about the circularity indicators used. Availability of the Excel spreadsheet dependent on the author.	Verberne (2016)
Circular Economy Index (CEI)	Possibility of assessing recycling linked with economy.	Restriction of the CE only in the scope of recycling, besides the difficulty of applying the because of the absence of a template.	Di Maio and Rem (2015)
Circular Economy Indicator Prototype (CEIP)	Developed according to CE principles. Ease of use due to a spreadsheet developed for calculation.	Availability of the spreadsheet dependent on the author.	(Cayzer et al., 2017; Griffiths and Cayzer, 2016)
Circular Economy Measurement Scale (CEMS)	Developed to measure CE practices in the civil construction sector through a questionnaire on a Likert scale.	Lack of platforms (templates or software) that make the calculations viable.	Nunez-Cacho et al. (2018)
Circular Economy Performance Indicator (CEPI)	Based in Life Cycle Assessment (LCA)	Need for prior technical knowledge about LCA and lack of templates that make calculations feasible.	Huysman et al. (2017)
Circular Economy Toolkit (CET)	Based on life stages of products and services. Ease availability and applicability.	It does not indicate which improvements could be needed to aim circularity.	Evans and Bocken (2013)
Circular Pathfinder (CP)	Based on CE practices, such as: extend, upgrade, reuse, repair, recondition, remanufacture, recycle and biodegrade. Easy access and use.	It provides enhancements to the product only for the redesign or design stages of the product.	ResCom (2017a)
Circularity Calculator (CC)	Developed according to practices of CE focused on the PSS and provides an adequate graphic vision of material and financial flows. In addition to providing quantitative output value for circularity, captured value, recycled content and reuse index.	Financial investment required for unlimited use of the indicator. Free limited use. Moreover, it does not clearly provide the calculation procedures for obtaining the outputs (circularity, captured value, recycled content and reuse index).	ResCom (2017b)
Circularity Index	Practical formulas for calculating the indicator.	Restriction of the Circulating Economy only in the scope of the recycling.	Cullen (2017)
Circularity Potential Indicator (CPI)	Developed through CE Building Blocks. Practical interface for users.	Availability depends on the author.	Saidani et al. (2017)
Ease of Disassembly Metric (eDiM)	Indicator focused on important CE practices such as disassembly of products.	Availability of the spreadsheet dependent on the author.	Vanegas et al. (2018)
Eco-efficient Value Ratio (EVR)	LCA based and economic information.	High complexity for application. Need for prior knowledge of LCA.	Scheepens et al. (2016)
Economic-Environmental Indicators (EEI)	LCA and LCC based.	High complexity for application. Need for prior knowledge of LCA and LCC.	Fregonara et al. (2017)
Economic-environmental remanufacturing (EER)	It allows to couple environmental and economic aspects to remanufacturing.	Restriction of the CE only in the scope of remanufacturing.	van Loon and van Wassenhove (2018)
End-of-Life Recycling Rates (EoL-RRs)	Practical indicator whose calculation procedures are available.	Restriction of the Circulating Economy only in the scope of the recycling.	Graedel et al. (2011)
Input-Output Balance Sheet (IOBS)	Based on information on the quantity and quality of resources used (renewable and non-renewable, recycled, permanently recycled and recyclable, biodegradable and compostable).	Difficulty in making the indicator available, since the authors belong to a private company.	MarcoCapellini (2017)
Longevity and Circularity (L&C)	Two indicators developed to measure product use and durability under some CE practices, such as reconditioning	Difficulties in obtaining some input data such as recycling efficiency, as well as the lack of a practical interface (such as a spreadsheet) available for calculation.	Figge et al. (2018)
Material Circularity Indicator (MCI)	Ease of access and use of the indicator. Quick and practical application, if all the input data is obtained.	Restricting CE in only some practices such as reuse and recycling. Difficulty in obtaining input data, such as: destination of the product after use and efficiency of the recycling process.	Ellen MacArthur Foundation and Granta (2015)
Material Reutilization Part (C2C)	Qualitative indicator that indicates the level of reutilization has the product.	Certification by an outsourced company, restricting CE to recycling practices.	(C2C, 2014)
Mine site MFA Indicator (MI)	Set of indicators for mining based on the economic and environmental dimension.	CE restriction only on waste management. Lack of tools to measure indicators.	Lèbre et al. (2017)
Multidimensional Indicator Set (MIS)	Multidimensional quantitative indicator applied to electrical and electronic waste.	CE restriction on recycling only. Lack of tools to measure the indicator.	Nelen et al. (2014)
Product-Level circularity Metric (PCM)	Relation between recirculation of materials and economic value.	Restricting CE in only some practices such as reuse, remanufacturing and recycling.	Linder et al. (2017)
Recycling Indices (RIs)	Indicator that expresses in percentage the recycling of a product by means of its elements.	Restriction of CE only in the context of recycling. Availability of the tool dependent on the author.	van Schaik and Reuter (2016)
Recycling Rates (RRs)	Indicator that takes into account the open loop and closed loop recycling, besides the rates of waste collection.	Required prior knowledge of MFA. Restriction of CE only in the context of recycling.	Haupt et al. (2017)
Resource Duration Indicator (RDI)	It illustrates the amount of time a material remains in the product system. That is, quantification of an important practice for EC, even the longevity of the product.	Difficulty in obtaining some input data, such as the recycling efficiency.	Franklin-Johnson et al. (2016)
Reuse Potential Indicator (RPI)	Indicator that takes into account the technology available to treat the waste.		Park and Chertow (2014)

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Table 2 (*continued*)

Indicator	Advantages	Disadvantages	Reference
		Difficulties in obtaining input data, such as the quality of the material used in the specific application and reasons for substitution.	
Set of Indicators to Assess Sustainability (SIAS)	Group of indicators in three dimensions (economic, social and environmental) focused on the practice of remanufacturing.	Lack of computational tools to facilitate calculations. Restriction od CE only in the context of remanufacturing.	Golinska et al. (2015)
Sustainability Indicators (SI)	Group of indicators focused on EC practices such as design and product modularity, including functionality, reconfiguration, reuse, recyclability.	Difficulties in obtaining data, especially in relation to end-of-life aspects of products.	Mesa et al. (2018)
Sustainable Circular Index (SCI)	Index based on the dimensions of sustainability and circularity.	Lack of computational tools to facilitate calculations. Lack of examples for practical application.	Azevedo et al. (2017)

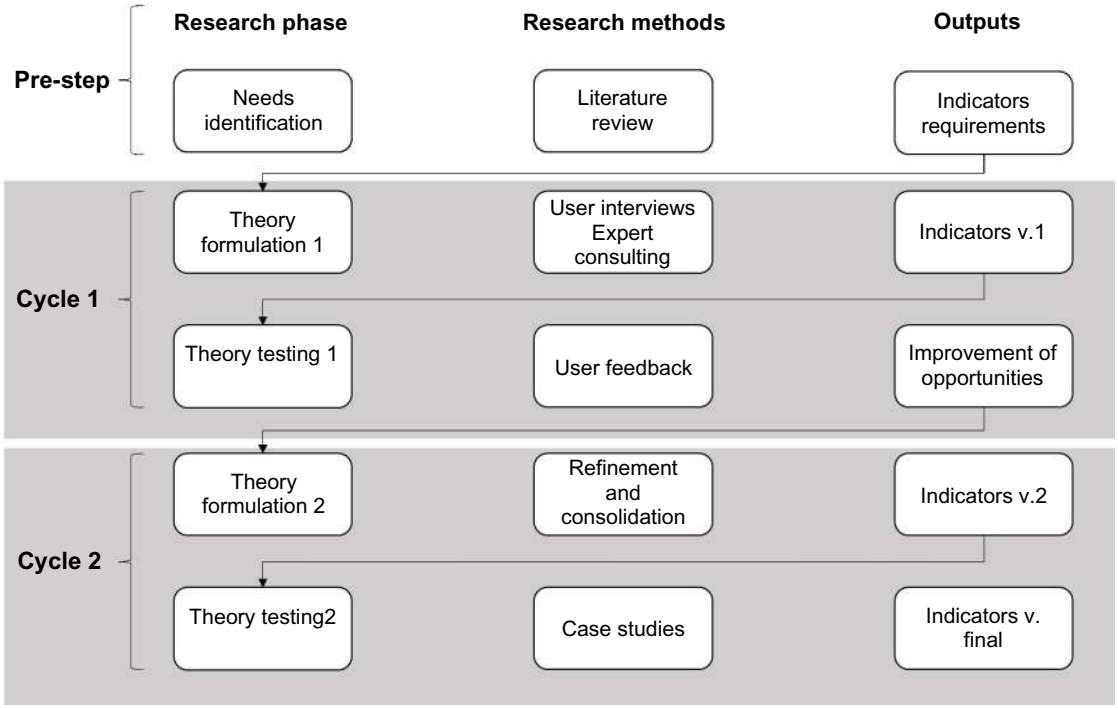

Fig. 1. Development process indicators following the hypothetic-deductive approach (adapted from Kjaer et al., 2018).

Table 3
Characteristics of the three Circular Business Models.

	Circular Business Model	Sector	Size
HP Brazil	Product as service/product-service system (PSS) and Recovery of secondary raw materials/by-products	Electro-electronic	Big
Malwee	Recovery of secondary raw materials/by-products and Product life cycle extension/reuse	Textile and fashion	Big
CIMFLEX	Recovery of secondary raw materials/by-products	Plastic	Small

improve practices and results. They could also propose essential aspects to companies follow to accelerate the transition to CE. The requirements of the indicators proposed in this paper link CE principles, CBM and sustainability.

Eighteen indicators were proposed and described in Table 7. The indicators were grouped considering the pillars of sustainability (environmental (from material perspective) - I, economic - II and social - III). In the environmental pillar we consider indicators for materials eg. reduction of raw materials, recyclability and reduction of toxic substances, in economic pillar were analyzed costs, revenues and taxes, and in social pillar were described employment, market and stakeholder's aspects. The indicators were described according to their applicability and forms of measurement. In addition, for some indicators sub indicators were established in order to clarify their application.

Fig. 2 shows the relations between the Canvas components and the indicators. At first, these novel indicators were classified in all components of Canvas, instead of the conventional indicators, which focused in key activities and resources as shown in Section 1. The indicators related to financial results (II1, II2 and II3) were classified respectively in cost structure and revenue streams. The indicator III4 was classified in customer's segments because is related to the market and the targets that the company want to achieve. The indicators I9, III1 III2. III3 and III5 are classified in key partners because they measure the characteristics of the stakeholders involved in the Circular Business Models, including the

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Table 4
Relationship between CE principles and material indicators proposed

Principles	Systems Thinking	●	●	●	●	●	●	●	●	●
	Innovation	○	●	●	○	○	●	●	●	●
	Stewardship	●	●	●	●	●	●	●	●	●
	Collaboration	◍	◍	●	◍	●	○	○	○	●
	Value Optimization	●	●	●	●	●	●	●	●	○
	Transparency	◍	◍	◍	◍	◍	◍	◍	◍	●
Indicators	**Dimension**	**Material**								
	Control Variables	***Reduction of raw materials***	***Renewability***	***Reciclability***	***Reduction of toxic substances***	***Reuse***	***Remanufacturing***	***Refurbishment***	***Product Longevity***	***Stakeholder structure and diversity***
Circular Business Models	Product as a Service	●	○	○	○	●	○	●	●	●
	Sharing Economy	●	◍	◍	◍	●	◍	◍	◍	◍
	Product life extension	●	○	●	●	●	●	●	●	●
	On-demand	●	○	○	○	◍	◍	◍	◍	◍
	Recovery by-products	●	◍	●	●	●	●	●	●	●
	Dematerialization	●	◍	◍	◍	◍	◍	◍	◍	◍

Table 5
Relationship between CE principles and economic indicators proposed

Principles	Systems Thinking	○	○	◍
	Innovation	○	○	●
	Stewardship	●	○	◍
	Collaboration	○	●	◍
	Value Optimization	●	○	●
	Transparency	○	◍	◍
Indicators	**Dimension**	**Economic**		
	Control Variables	***Financial results***	***Taxation or regulatory milestones***	***Circular investment***
Circular Business Models	Product as a Service	●	●	●
	Sharing Economy	●	◍	●
	Product life extension	●	◍	●
	On-demand	●	○	○
	Recovery by-products	●	●	●
	Dematerialization	●	◍	●

employees, the supply partners and its structure. The indicators I5, I6 and I7 are both classified in key activities and value propositions, because they show values the company want to deliver to keeping products, components, and materials valuable and useful to return to the cycles and practical strategies to close these loops. In the same way, indicators I1 and I3 are both classified in key resources and value proposition because these strategies aim to decouple economic growth from resource consumption. The I2 and I4 are classified in key resources because they are prior opportunities in Circular Economy, and according to create restorative and regenerative cycles. Finally, I8 and III6 are indicators related to value propositions because they show intrinsic values to incorporate in products and services to accelerate the transition to Circular Economy.

The development of the indicators was refined from user's feedback. It was consisted by meetings, interviews and workshops within the companies. The measure of the indicators was also clarified and in some cases new indicators were created. At first, the users found difficulties in understand and collect data for indicators. Thus, Table 7 was created to explain and detail the metrics for the companies. After, the application of the indicators brought new opportunities to the companies plan their CBM, values, strategies and also supply chain, stakeholders and product design. These indicators were applied in three Brazilian companies and the

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Table 6
Relationship between CE principles and social indicators proposed

Principles	Systems Thinking	●	●	●	●	●	●
	Innovation	●	○	◍	◍	●	●
	Stewardship	○	●	◍	●	●	○
	Collaboration	●	○	●	○	●	●
	Value Optimization	○	●	●	○	○	●
	Transparency	◍	◍	●	◍	●	●
Indicators	**Dimension**	**Social**					
	Control Variables	**Job creation**	**Income generated by jobs**	**Employee participation in the circular business model**	**Market characterization**	**Involvement of stakeholders in decision-making processes**	**Mindset / cultural change**
Circular Business Models	Product as a Service	●	●	●	●	●	●
	Sharing Economy	◍	◍	◍	●	●	○
	Product life extension	●	●	●	●	●	○
	On-demand	●	●	○	●	●	●
	Recovery by-products	●	●	●	●	●	○
	Dematerialization	◍	◍	●	●	●	○

respective results are shown in section 3.1.

3.1. Case studies

3.1.1. HP inc. (HP Brazil)

The electro-electronic sector is composed of industrial automation industries; electrical and electronic components; industrial equipment; generation, transmission and distribution of electricity; computing; electronic installation material; and household utilities (Abinee, 2018). Two business model were studied: PSS and Recovery of secondary raw materials/by-products. In this study, the focus is the PSS based on HP Brazil Managed Printing Services (MPS). The Recovery of secondary raw materials/by-products includes Sustainability indicators related to HP Brazil Circular Economy Ecosystem, with occasional global data.

In 2016, the total revenue of the sector was US$ 31,098 million. In 2017, the company exported US$5844.2 million of electrical and electronic products and imported US$ 29.663,1 million. In January 2017, the total number of employees in the sector was 234,586 employees and in January 2018 the total was 236,882 employees, an increase of 2294 jobs in a year (Abinee, 2018).

According to the Brazilian Association of the Electronic and Electronics Industry (Abinee, 2018) the perspective of Brazilian GDP growth is 2.5% in 2018 and the electronics sector is expected to grow by around 7% considering sales and production. Furthermore, 76% of the companies expect an increase in their activities, showing the importance of this sector for the Brazilian economy. The products that should be at the forefront of this growth in the sector are those in the areas of information technology and telecommunications. The expectation of growth of sales of electrical and electronic products abroad in the year 2018 will be 3% compared to last year, that is, these sales are expected to add up to US $ 6 billion. Regarding to importation, the expectation is also 5% (about US $ 31.4 billion) in 2018 compared to 2017. In relation to employment, it is estimated that there will be an increase of 241 employees, a growth of 2% in 2018 compared to 2017 (Abinee, 2018). Considering this context, HP's business model is described in Table 8.

HP has a global commitment to transform its business model for a more efficient, circular, and low-carbon economy spans across and beyond its value chain. It presents several initiatives to drive CE on products and solutions' design and recovery. Such as developing solutions that keep products and materials in use at their highest state of value for the longer time; reducing the resources required to manufacture and ensuring the materials in products are properly repurposed at end of life. HP presents several business models globally and the PSS includes, HP Device-as-a-Service, HP Subscription Services, HP Managed Print Services (MPS) and HP Instant Ink. Considering Recovery of secondary raw materials/by-products business model, HP offers repair, reuse and recycling programs in more than 60 countries that support responsible collection and processing to recover and re-use as much as possible. HP is considered an organization with circular practices, because they changed their business model to a product as a service whose reflected in changes in the product design, which was measured by the indicator "product longevity", and in the use of recyclable material, which was measured by the indicator "recyclability". Thus, HP's CBM is classified as product as a service and recovery of secondary raw materials/by-products. Table 9 presents the results for the company referring to the application of CE indicators.

3.1.2. Malwee Malhas LTDA (Malwee)

The textile and fashion sector in Brazil presented a revenue of US$29 billion, and generates approximately 1,494,000 jobs in more than 29,500 companies. Therefore, the selected company presents a great development in CE, having inserted in its model of management operations several types of business models as circular inputs and resources recovery for the product, and life cycle extension of manufacturing equipment as remanufacturing.

In informal market the reuse of product is also a strategic operation. Malwee is one of the leading fashion companies in Brazil and one of the most modern in the world. This company is deeply concerned about topics related to sustainability, owning a Strategy Sustainability. This strategic plan aims to look at the future of sustainability related to business model, products and operations and engaging its stakeholders to develop a sustainable value chain. According to this context, the organization's business model is described in Table 8. Hence, Table 9 presents the results for the company referring to the application of CE indicators.

Malwee has circular practices since they no longer consider cotton waste as useless and uses it as raw material again, using in

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Table 7
Set of circularity indicators proposed in this paper.

Dimension	Indicator	Sub indicator	Measure	Description
I)Material	1)Reduction of raw materials	a)Manufacturing	Quantity of raw materials reduced in the manufacturing	This indicator measures the reducing quantities of raw materials in the process of manufacturing (e.g. water, carbon dioxide, etc)
		b)Product	Quantity of raw materials reduced in the product	This indicator measures the reducing quantities of raw materials in the product itself, making it lighter
	2)Renewability	a)Renewable energy	Percentage of renewable energy sources in relation to the total energy used in manufacturing processes	The aim is to measure the quantity of renewable energy consumed in the manufacturing
		b)Renewable raw materials	Percentage of raw material from renewable sources in relation to all the materials used in a product	The aim is to measure the quantity of renewable raw materials used in the product
	3)Recyclability	a)Recycled materials	Percentage of recycled materials in the composition of the product	This indicator measure the use of recycled materials in the product
		b)Recyclability potential	Percentage of the product that may be recycled after use	This indicator measure the potential of recyclability of the product after use
	4)Reduction of toxic substances		Quantity of reduction of toxic substances	It aims to quantify the reduction of the use of toxic substances considering RoHS (Restriction of Certain Hazardous Substances).
	5)Reuse	a)Manufacturing process	Quantity of material reused in the supply chain	It aims to quantify the reused materials in the supply chain
		b)Product	Quantity of reused material in the product	It aims to quantify the reused materials in the product
	6)Remanufacturing		Quantity of remanufactured products	It aims to quantify the remanufacturing products
	7)Refurbishment		Quantity of the total recovery or parts (components) of the product, without necessarily going through all stages of the remanufacturing.	This indicator is expressed by the specification and quantity of the products and refurbished parts
	8)Product longevity		Quantity of time added in the lifespan of the product	This indicator may be obtained from consumer information, and/or from the company itself from product return information, average lifetime, replacement or purchase of new product, or time to replenishment
	9)Stakeholder structure and diversity	a)Structure	Qualitative	This indicator may be obtained qualitatively, for example on the structures and synergies or symbiosis of the business of a company with others associated with its supply chain
		b)Stakeholder	Qualitative	It aims to map stakeholders in the circular value chain
II)Economic	1)Financial results	a) Cost reduction	Monetary value from circular business model provided by cost reduction from raw materials, energy, etc	This indicator aims to show the cost reduction of the manufacturing because of the acquisition of less raw materials and energy
		b) Revenue generation	This indicator could be measured by: a) Competitive advantage: percentage of market share of the circular business model compared with the competitors. b) Risks: map the risks associated with the circular business models. c) New revenues: new revenues from circular business models.	This indicator aims to show the billing percentage generated by circular business model
		c)Profitability	Net profit of the Return On Assets (ROA) and Return On Equity (ROE)	This indicator measure the net profit
	2)Taxation or regulatory milestones		Qualitative	This indicator aims to specify the taxation or regulatory milestones that subsidize the circular business model
	3)Circular investment	Innovation	Quantify investments from the innovation process	This indicator aims to quantify in monetary values the financial resources invested to change the business model, from strategic and management actions to capacity building, operational and maintenance
III)Social	1)Job creation		Quantity of job creation from circular business model	This indicator aims to quantify the job creation from circular business model, e.g. quantity of job creation from reverse supply chain activities (maintenance, reverse logistics, reuse, remanufacture, refurbishment, etc)
	2)Income generated by jobs		Monetary value the income generated by job creation from circular business model	It aims to quantify in monetary values the income from new jobs creation from circular business model
	3)Employee participation in the circular model		Percentage of jobs in the company related to circular economy	It aims to quantify the percentage of jobs of the organization and its hierarchical level related to the circular economy
	4)Client mindset	a)Client	Qualitative	It aims to identify the client characterization, e.g. social level, geographical regions, age group, among others, according to the uses of the

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Table 7 (*continued*)

Dimension	Indicator	Sub indicator	Measure	Description
				product. Also includes the motivation and intention of the client.
		b)Value	Qualitative	This indicator aims to map perceived and captured values for each type of client, i.e. benefits generated for clients.
		c)Communication	Qualitative	Collection of data or information from consumer surveys, Customer Service Call Centers (CSCC), and other channels, identifying correlations with information on adoption of circular practices or sustainability, which are communicated or available to consumers who make purchasing decisions at the information available to them
	5)Involvement of stakeholders in decision-making processes		Qualitative	It aims to characterize qualitatively the stakeholders who participate in the general business model and those who effectively participate in the organization's decision making. Stratify the stakeholders according to each element of the business model: strategy and management, economic, operational and innovation.
	6)Mindset/cultural change		Qualitative	It aims to describe the process of change resulting from the implementation of the circular business model in the company. Especially cultural and mindset change.

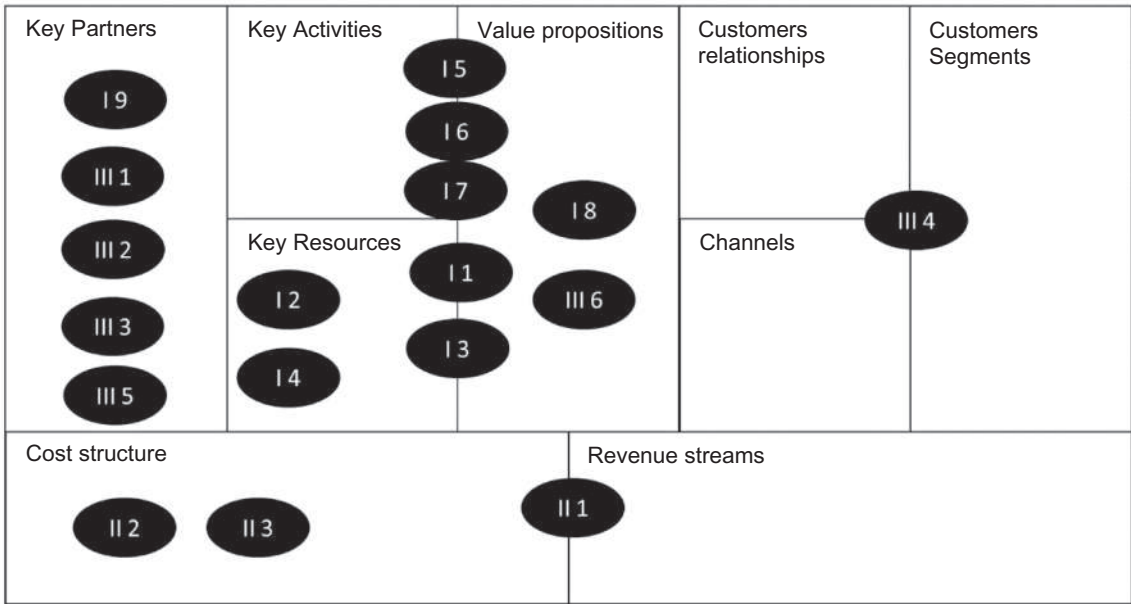

Fig. 2. Relation between indicators and CANVAS components.

this way circular inputs in the production. Moreover, Malwee keeps their manufacturing machines in use for as long as possible through remanufacturing. To identify these real changes we used the indicators "reduction of raw materials" and "remanufacturing". Thus, Malwee's CBM is classified as recovery and product life cycle extension. Hence, Table 9 presents the results for the company referring to the application of CE indicators.

3.1.3. *CIMFLEX indústria de comércio de plástico Ltda. (CIMFLEX)*

The plastic sector in Brazil has a gross billing of US$ 20 billion, generates approximately 320,000 jobs in plastics materials transformation and recycling industries in more than 12,300 companies (Abiplast, 2017). CIMFLEX was founded in 2004 and aims to offer products to the construction and plastics processing industries, meeting the requirements of these customers through the use of raw material with low environmental impacts. CIMFLEX's CBM is classified as Recovery of secondary raw materials/by-products. The company recycles agrochemicals and lubricating oils packaging (all packaging from high-density polyethylene - HDPE) and transforms them into resins and products used in civil construction, e.g.: ducts and corrugated conduits. CIMFLEX produces, per year, approximately 2000 tons of ducts and corrugated conduits. Moreover, they use reverse logistic to close the loops of agrochemicals and lubricating oils packaging. CIMFLEX operates in a business model to recover plastic resources, we used the indicator "recyclability" to measure its effectiveness as a circular organization. According to this context, the CIMFLEX's business model is described in Table 8. Thus, Table 9 presents the results for the application of CE

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Table 8
Description of circular business models of HP Brazil, Malwee, and CIMFLEX.

Component	HP Brazil	Malwee	CIMFLEX
Value proposition	HP Managed Printing Services provide convenience and performance benefits while reducing capital costs by managing maintenance of the technology fleet, freeing up valuable employee time and resources, increasing product longevity, reducing waste and ensuring product repair, reuse, and recycling at the end of service life.	The company has two value propositions: use of a pet knitwear (polyester yarn made from recycled PET bottles), use of biodegradable polyamide in the manufacture of gym clothes, and the remanufacture of the machines that are used to the clothes confection	The company visualized an opportunity to act in the closed loop of agrochemicals and lubricating oils packaging, by recycling them and using as an input to manufacture Tubes, Ducts and Corrugated Conduits to use in civil construction.
Customer Segment	HP Brazil MPS were firstly adopted by large companies as a Business to Business model. Today the public sector, small and medium business are also adopting the model, which can be expanded to other customer segments.	Clients are retail stores that sell clothes (Business to Business)	Clients are large companies (Business to Business).
Relationship with customers	The company identifies customer needs in terms of printing (volume, performance, etc.) and provides printing services and support in accordance to customer's demand, stablishing a service-based relationship.	The company product expresses the identity of the consumer.	The company seeks to meet customer requirements through direct verification.
Channels	Communication with customers and potential customers are made through mailing, events, direct engagements, personal contact, social media, website, among others.	Communication is the key to reaching different audiences. The Malwee Group is committed to disseminating its initiatives and projects, as well as sharing information and content so that people have the opportunity to know and contribute to a more conscious society.	The channels of communication is personal, telephone and internet.
Key resources	The essential resources are human, material and financial.	The main resources are people since the Malwee Group believes that development occurs by people and to the people. With respect to material resources, the Malwee group is active in the search for raw material and processes that have a lower socio-environmental impact. And the financial a structure resources are necessary to the development of the projects related to the sustainable plan.	The essential resources are human, material and financial.
Major partnerships	Sales Channels and recycling partner.	The Malwee Group recognizes that to the construction of a more sustainable value chain is necessary the exchange of knowledge and experiences among its participants. For this, has a partnership with universities and research centers, suppliers and retailers.	The main partner is waste collection cooperatives.
Cost and revenue structure	The customer pays a flat fee per printed page.	Conventional cost and revenue structure	Conventional cost and revenue structure.

indicators.

Considering the three cases, the companies showed difficulties in publishing data from economic and even in social dimensions which were not available or were diffused in the companies. It is a barrier because most of the positive impacts gained with CE are presented in the social dimension, including job creation, mindset change, and others. According to Schröder et al. (2019) social issues are not yet integrated into the concept of CE. There is uncertainty about how to measure the transition from a linear to a CBM and how CE can help sustainability (Schröder et al., 2019). Thus, our paper helps to fill this gap, as it points to develop indicators that measure CE performance considering sustainability in a systemic view.

It is important to emphasize that the indicators could not be applied individually. The set of indicators must be analyzed together because if there is an evolution in one single indicator it not necessarily means an evolution in the company performance in Circular Economy. For example, if the company produced with less raw materials but with low longevity, it is not a representative gain regarding Circular Economy principles.

Regarding the research question "How organizations can measure Circular Economy performance considering Sustainability and Business Model perspective?", we confirmed that the proposed indicators could be applied in companies with CBM to improve their performance according to CE principles and Sustainability. The developed indicators can be used to analyze and assess the current state of the CBM performance, evaluate the achievement of CE goals, and improve benefits for all stakeholders in the value chain (especially with social indicators).

Finally, the novel indicators presented have advanced in relation to those found in the literature because they have a simple and intuitive format and could be applied in various sectors and business models. Also, they not restrict the scope of CE, as conventional indicators do (Table 2), but they cover all CE principles. Besides, they were developed with the companies so they are understandable and easy to be used.

4. Conclusions

According to the results, the paper contributes in the application of CE in Business Models. The novel set of indicators expresses the complex dimensions of sustainability needed, including environmental (from material perspective), economic, and social. The main contribution of this paper is the development of a group of indicators applied in CBM to capture the innovations brought by CE whose conventional indicators do not measure. These innovations include systems thinking, mindset change, diversity, effectiveness, resilience and long term for all stakeholders.

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Table 9
Indicators result for HP Brazil, Malwee, and CIMFLEX.

Dimension	Indicator	Sub indicator	HP Brazil	Malwee	CIMFLEX
I)Material	1)Reduction of raw materials	a)Manufacturing	The company didn't buy over 681 thousand boxes of cardboard on 2017 The company saved about 7500 trees by reusing pallets from 2015 to 2017. The company decreased in 8% the intensity of use of materials for personal systems in 2017 (global data). The company decreased in 6% the intensity of use of materials for printers in 2017 compared to 2016 (global data). The water footprint decreases 1% in 2017 compared to 2016 for supply chain, operations and products and solutions (global data). The customer pays a flat fee per printed page.	Reduction of losses in the production processes, and consequently in the consumption of virgin raw materials, and use of recycled raw materials of approximately 7%.	• Reduction of above 75% of raw materials. • Reduction of water use in the milling line of the flow process for lubricants due to the use of hot water by cold water - lower energy use and reduced evaporation rate. • Reduction of water use in the COEX material milling line - changing milling with wash for dry milling. • Reduction of 18% of energy consumption in manufacturing processes. • Reductions in the incorporation of additives in the formulations of several products: 1) Reduction of 33.33% compatibilizer in COEX material; 2) 60% reduction of impact modifier in the PVC conduit; 3) Reduction of 100% in the amount of lubricant incorporated to the conduit PVC DN 20 mm.
		b)Product	Since 2010, the power consumption of HP's personal system products has decreased by 43% on average (global data).	Reduction of 0.06 kg/Piece in 3 years.	Reduction in weight of several products • Corrugated duct DN 63 mm - Reduction of 9.52%; • Corrugated duct DN 90 mm - Reduction of 27.27%; • Corrugated duct DN 110 mm - Reduction of 4.54%; • Corrugated duct DN 160 mm - Reduction of 27.77%; • Reinforced corrugated duct DN 40 mm - Reduction of 29.42%;
	2)Renewability	a)Renewable energy	In 2017, 50% of the energy used in global operations comes from renewable sources (global data).	Use of 100% renewable energy in production processes (still has natural gas and diesel oil in case of emergency); reduction between 12 and 15% of electricity consumption in the last 3 years. Replacement of GMP oil with natural gas since 2000.	100% of the energy comes from renewable sources (Small Hydropower Plants, wind energy, solar energy and thermoelectric plants from the burning of sugarcane bagasse).
		b)Renewable raw materials	Not available.	Not available.	The reuse of the water is done in the washing of the mills - being a closed circuit inside the company.
	3)Recyclability	a)Recycled materials			

(*continued on next page*)

Table 9 (*continued*)

Dimension	Indicator	Sub indicator	HP Brazil	Malwee	CIMFLEX
			The HP printer Deskjet GT 5822 manufactured in Brazil has up to 12% of recycled material on average in its composition by weight, in 2017. It has the potential of using recycled material up to 32%, also by weight.	Waste generated is recycled and projects under development to close the cycle (100% of the recycled textile waste).	Percentages of recycled inputs in the products: • Corrugated duct - 97.50%; • Corrugated conduit - 97.50%; • Corrugated drain - 97.50%; • Sewage Tube - 100%;
		b)Recyclability potential	In Brazil, 95% of all the company's waste is recycled, the other 5% destined for cogeneration of energy. Nothing is sent to landfills.	Reduction of 5–15% of the generated production residues, depending on the type of product produced. Process for drying the ETE sludge, which reduces the volume to 1/7 of normal. Parts may contain 12–50% of their composition, of recycled material (PET or defibrated)	All the products present recyclability potential of 100%.
	4)Reduction of toxic substances		The company does not use toxic substances.	There are no national regulations for such a measure, however, the 2020 Plan provides for a restriction target under international law.	Percentage of dry waste (class I) destined for landfills in relation to the quantity received of lubricating packages is 13%.
	5)Reuse	a)Manufacturing process	Equipment and end-of-life printing supplies plastics are recycled and transformed into new parts and pieces of locally produced printers and packaging.	Water reuse program in the production process since 2003, which has a nominal reuse capacity of up to 200 million liters of water per year (representing 25% of the total water used), and operate in the normal regime with 75% of the nominal capacity of the system.	Not applicable to the company.
		b)Product	The HP printer Deskjet GT 5822 manufactured in Brazil contains up to 12% of recycled material in 2017.	There is no product reuse per se, however, 100% of the waste generated is sent to recycling companies in the region.	Not applicable to the company.
	6)Remanufacturing		Not available.	Not applicable to the company.	Not applicable to the company.
	7)Refurbishment		Products with 10/10 reparability scale (global data) in iFixit.	Not applicable to the company.	Not applicable to the company.
	8)Product longevity		Printers' life time increases, on average, from 3 to 5 years due to the good conditions of operation and maintenance when on MPS model.	As for the consumption link, the products have a useful life of approximately 30 washing cycles and may be extended according to the product line.	The average lifetime of the products is 30–40 years - about 70%–80% of the life expectancy of the virgin materials.
	9)Stakeholder structure and diversity	a)Structure	Since 2008, the company works along with its manufacturing partners to build an end-to-end supply chain.	Not available.	Not available.
		b)Stakeholder	Not available.	Involvement to create businesses associated with the use of textile waste.	Creation of a network of companies associated with the collection, sorting, sorting and recycling of packaging. Involvement with ABIPLAST and with the PICPlast - Plastic Chain Incentive Plan, a partnership between ABIPLAST

						and Braskem to train employees in the transformation and recycling industries of plastic materials and to show the benefits of the material to society. For more information visit: www.picplast.com.br.
II)Economic	1)Financial results	a) Cost reduction	The recycled plastic resin is in average 15%–30% cheaper than virgin plastic resin.	The launch of products is focused on best practices and with low environmental impact in development, such as jeans and knitwear. Association for Global Best Practices (Sustainable Apparel Coalition) and ABVTEX certification schemes.	Confidential.	Activities in development for the organization of the sector - National Chamber of Recyclers of Plastic Materials - CNRPLAS - horizontal policies, training of cooperatives, quality seals - SENAPLAS - National Seal of Recycled Plastic - certification for companies and soon certification of recycled plastic resins
		b) Revenue generation	Confidential.	Products that excel for the quality and durability in relation to the average of its competitors.	Confidential.	Constant development of new applications for recycled products.
		c)Profitability	Confidential.	Not available.	Not available.	
	2)Taxation or regulatory milestones		Monthly, the company is taxed in ISS 2% + PIS/Cofins 9.65% + eventually INSS where they have people allocated by contract on MPS.	Tax regime as a limiting factor for the adoption of practices and circular processes. (e.g. double taxation on waste)	Tax and tax policies are a problem for circular business model involving the plastics chain. Fiscal asymmetry between States does not foster competitiveness. Suggestion of the adoption of credit granted to the sector.	
	3)Circular investment	Innovation	Confidential.	Innovations in water use since 2002, TECNOBIO, with optimization of the effluent treatment system. Use of new natural fibers and natural inputs of this 2011. New treatment systems using ozone in the production of denim jeans. Replacement of harmful chemicals. Use of biodegradable polyamide, helping at the end of the product life cycle.	Confidential.	
III)Social	1)Job creation		Confidential.	Not available.	Confidential.	
	2)Income generated by jobs		Not available.	Not available.	Confidential.	
	3)Employee participation in the circular model		Not available.	Not available.		
	4)Client mindset	a)Client	Confidential.	Not available.	Not available.	
		b)Value	Confidential.	Not available.	Not available.	

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Table 9 (*continued*)

Dimension	Indicator	Sub indicator	HP Brazil	Malwee	CIMFLEX
		c)Communication	Confidential.	Since its foundation, the company has adopted practices associated with longer-lasting business models. The process of internal transformation has already occurred in several areas of the company. Having pioneered water management, reducing impacts on dyeing and printing processes, and the use of raw materials with sustainable bias. The 2020 Plan, drawn up in 2014, mapped the entire value chain and defined 14 goals for the Group in 5 main areas.	Confidential.
	5)Involvement of stakeholders in decision-making processes		Not available.	Not available.	Not available.
	6)Mindset/cultural change		Not available.	Not available.	Not available.

CE aims to seek sustainability, in this work the proposed indicators are intrinsic related to Sustainable Development Goals and help to achieve: Clean water and sanitation (Goal 6), Affordable and clean energy (Goal 7), Decent work and economic growth (Goal 8), Industry, innovation, and infrastructure (Goal 9), Responsible consumption and production (Goal 12) and Climate action (Goal 13).

This paper investigates how companies could measure their performance in CE and proposed a multi-dimensional set of indicators to demonstrate benefits to measure at the micro level. These indicators were developed based on a hypothetic-deductive approach and were applied in a multiple case study. The main point is to create simple and clear indicators (Folan and Browne, 2005) to be applied in diverse sectors and contribute with this area of research.

New findings in both theory and practice aspects were achieved, because the indicators in literature were analyzed and with the user's feedbacks were possible to improve and develop this novel set of indicators. The link between empirical data and theory could enrich the research, since CE emerges from the practice (Agrawal et al., 2019).

4.1. Implications for theory and practice on sustainability

Theory is a way of establishing a conceptual order to the empirical complexity of the phenomenal world (Suddaby, 2014), for the author the true knowledge arises from different assumptions about the proper use of a theory. Moreover, abstract reasoning is a way of constructing knowledge. Additionally, theory proposal clearly presents why its development, which brings again to the area of knowledge and the relationship between theory and practice (Whetten, 1989). So, this paper contributes theoretically, because it presents a list of CE indicators of and sustainability not yet identified in the literature. Traditional indicators could not express CE in its totality, because they are not designed for the systemic, closed-loop, feedback features that represent CE (Geng et al., 2013). As well, the novelty of this paper remains to join sustainability, CBM and performance measurement for CE.

At the practical side, the results will help any company to identify areas with high importance and potential for improvement, and thus increase the CE performance in an efficient, clearly and prompt manner. Besides, the indicators allow companies to create their own improvement targets according to their defined CE strategy. Measuring CE and sustainability performance through the proposed indicators can help organizations find improvements and consequently operational, business model and strategy innovations.

4.2. Limitations and future work

The limitation of this work remains in the confidentiality of data involving the economic indicators and the availability of social indicators. Furthermore, the indicators were applied in only three case studies.

For future work, there is a need to apply these indicators in other sectors and companies for measuring also positive impacts from Circular Economy, for example, involvement of stakeholders in decision-making processes and the mindset, as core indicator. Also, it will be important to create an index with involves all the indicators and a scale to set targets for Circular Economy principles.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Metodologias ativas de ensino-aprendizagem

Uso da metodologia CANVAS em processos de ensino e aprendizado em Engenharia Florestal e Gestão Ambiental

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Resumo

Este trabalho descreve o processo de utilização da metodologia CANVAS, ou modelo de geração de negócios, co-criado por Osterwalder & Pigneur (2010), para os cursos de graduação em Engenharia Florestal e Gestão Ambiental da ESALQ, USP, especialmente em disciplinas dos últimos anos destes cursos. A escolha desta metodologia teve como objetivo proporcionar aos alunos, em etapas avançadas de seus cursos de graduação, uma oportunidade de converterem ideias e conceitos adquiridos no componente teórico da disciplina em planos de negócios, criando um ambiente coletivo de discussão das melhores soluções para problemas enfrentados pela sociedade.

Introdução

Um dos grandes desafios do ensino de graduação, particularmente dentro das disciplinas das etapas avançadas de Cursos de graduação, é o de conciliar uma sólida formação academia e profissional com as transformações constantes que ocorrem na sociedade e nos mercados, aumentando a empregabilidade destes profissionais, e tornando-os mais aptos a se adaptarem as estas mudanças. O conjunto de ferramentas dentro do modelo CANVAS traz a flexibilidade necessária dentro destes processos complexos que envolvem a formação de futuros profissionais e podem ser uteis como metodologia complementar para ensino-aprendizagem.

Descrição

A necessidade de atualização das práticas de ensino-aprendizagem demanda a busca de novas ferramentas para transmissão de conhecimento, troca de experiências e vivencias em sala de aula, entendimento das demandas da sociedade e em especial para o formação de profissionais melhor capacitados e formados. Este artigo descreve o processo de trazer para a sala de aula, uma metodologia (CANVAS) adotada amplamente por empreendedores, empresas e organizações do terceiro setor, a qual foi adaptada para o ensino de disciplinas dos Cursos de Engenharia Florestal e Gestão Ambiental, da ESALQ, USP.

Metodologia

As disciplinas, onde a metodologia CANVAS foi adotada, eram estruturadas em trêscomponentes principais, os quais refletiam também na estrutura das aulas: 1) componente teórico; 2) componente do estudo de caso; 3) componente do modelo CANVAS. Em todas as aulas, estes três elementos estavam presentes, sendo que a cada semana, os alunos, individualmente reportavam por escrito, dentro do modelo CANVAS, o progresso feito na elaboração do modelo de negócios para os temas previamente escolhidos. Nas últimas quatro semanas de aulas, os alunos, apresentavam uma vídeo curto (máximo de 5 minutos) sobre um tema central que unia os modelos de negócios individuais, baseados em roteiro com o formato de "contar estórias", além de uma apresentação individual dos planos de negócios, estruturados no formato do CANVAS.

Resultado

Os resultados obtidos nos últimos três anos com adoção da metodologia CANVAS nas disciplinas de graduação dos Cursos de Engenharia Florestal e Gestão Ambiental da ESALQ, USP, puderam ser qualificados: a) pela motivação e engajamento dos alunos com os

problemas sugeridos e as soluções de negócios propostos pelos menos; b) envolvimento direto dos alunos com os problemas escolhidos durante todo o semestre; c) pelo retorno obtido dos alunos após terem cursado a disciplina sobre a aplicabilidade do método em diversas outras situações das vidas profissional dos ex-alunos; e pelo uso da própria metodologia pelos egressos de ambos Cursos na criação de projetos e novos negócios.

Conclusões

A adaptação de ferramentas para o desenvolvimento de estratégias e modelos de negócios (CANVAS ou modelo de geração de negócios), como metodologia complementar de ensino-aprendizagem em disciplinas de graduação, mostrou-se útil para: i) proporcionar uma visão interdisciplinar para a resolução de problemas; ii) para explicitar as relações e conexões entre vários temas e abordagens adotadas em outras disciplinas dos dois cursos de Graduação, onde esta metodologia foi testada; iii) expor os alunos a uma ferramenta adotada pelo mercado e pelo terceiro setor para criação coletiva de modelos de negócios, aplicados a diversas áreas da economia e empreendedorismo.

Metodologias ativas de ensino-aprendizagem

Laboratório de Excel para Visualizar Conceitos de Cálculo e Física: Comparação com um Semestre Convencional

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Resumo

Utilizamos atividades de laboratório computacional com Excel para introduzir conceitos de cálculo e física para alunos de Biologia antes de introduzir tais conceitos de maneira formal. Comparamos o interesse e nível de aprendizado dos alunos de um semestre convencional com um semestre com a metodologia aqui apresentada. Apresentamos também as formas como o aprendizado fica enriquecido pelo uso do Excel.

O Trabalho

No ensino de ciências exatas para alunos calouros, conceitos simples podem ser de difícil compreensão quando a linguagem matemática acaba funcionando como uma língua estrangeira desconhecida. Um exemplo é a identificação dos pontos críticos de uma função usando o cálculo.

Neste trabalho discutimos as diferenças encontradas no aprendizado de turmas antes e depois da introdução da planilha Microsoft Excel como ferramenta auxiliar. O Excel foi escolhido por ser uma ferramenta disponível em praticamente qualquer computador. Dois semestres em anos subsequentes são avaliados, sendo o primeiro deles sem uso do Excel.

A comparação quantitativa dos dois semestres é feita pela normalização das notas dos alunos por notas de uma avaliação de 16 questões sobre matemática básica, aplicada no início de cada semestre.

Para cada tema da disciplina, antes de introduzir métodos analíticos convencionais utilizamos práticas de laboratório usando o Excel. Essas práticas acontecem na semana anterior às aulas teóricas onde o tópico é desenvolvido com mais rigor. Os cálculos numéricos e visualização gráfica proporcionados pelo Excel são capazes de fazer o abstrato se tornar acessível.

A metodologia foi empregada na disciplina Fundamentos de Física e Matemática para calouros de Biologia na FFCLRP (USP Ribeirão Preto), cuja carga horária é de 4 horas semanais. Para os laboratórios, a turma de 70 alunos é dividida em duas turmas de 35 alunos. O tópico é apresentado de forma intuitiva usando o Excel como ferramenta de visualização, e ao