Crop improvement the biotechnology option

Prakash P. Kumar
Department of Biological Sciences, National University of Singapore
10 Lower Kent Ridge Road, Singapore 117543

Abstract

Plant biotechnology involving genetic modification has been rather controversial. However, the major issues related to safety are being addressed by continued improvements in technology. Some of the related facts will be highlighted to set the tone for a scientific discussion on the possibilities of using the technology for crop improvement. Our main research interest is to understand the molecular regulation of shoot bud regeneration in plant tissue culture, which is essential for crop improvement by biotechnology. We have isolated and characterized some genes that are associated with adventitious shoot regeneration. These include a MADS-box cDNA (*PkMADS1*) from *Paulownia kawakamii*, which regulates vegetative shoot development and in vitro shoot regeneration from leaf explants. Another gene we have characterized from petunia codesfor a cytokinin binding protein (*PETCBP*). Preliminary functional analysis of this gene indicated that this also affects adventitious shoot bud initiation. Also, the antisense suppression of this gene in petunia causedexcessive branching. Results from our work and selected other publications will be used to highlight the possibilities of manipulation of such genes to improve crop species.

Plant tissue culture technique is widely used for basic research as well as for biotechnological improvement of plants. The extensive use of genetically modified (GM) crops has been slow in many parts of the world (including Europe and Australia)because of the controversies regarding the environmental and nutritional safety. However, recently several countries (e.g., China, India, Brazil) have approved GM crops for cultivation with the technological improvements such as the ability to produce marker-free transgenic plants and favorable environmental safety reports.

There are two major pathways of development in plant tissue culture, namely, organogenesis and somatic embryogenesis. The path of differentiation will be determined by the specializationof cells or tissues leading to regeneration of organs (roots, shoots) or somatic embryos from cultured explants. Study of regeneration *in vitro* has been approached by one of two techniques: 1. physiological/histological analyses or 2. genetic/molecular analyses of organogenesis-defective mutants. Using such analyses, various genes presumed to play critical roles in organogenesis *in vitro* are being identified. Future experiments on organogenesis can be based on these genes as reference points.

Mutants defective in phytohormone biosynthesis or perception demonstrate their importance in regulating plant growth. Often, alteration of final organ size is one of the consequences of modified phytohormone signaling in these mutants. We know that some signals may regulate cell division, growth and expansion to determine final organ size. Mutationsin *Arabidopsis* that interfere with the ethylene-signaling pathway alter organ size (Ecker 1995). For example, *ethylene-overproduction1* (*eto1*) and *constitutive triple response1* (*ctr1*) (mutations resulting in constitutive activation of

ethylene signaling) mutant plants have smaller than normal organs(reduction in cell size & cell number). Conversely, leaves and floral organs of *ethylene receptor1* (*etr1*) and *ethylene-insensitive2* (*ein2*) mutants are larger than those of the wild type. Furthermore, heterologous expression of dominant negative mutant ethylene receptor gene *etr1*has been shown to be effective in conferring delayed senescence phenotype. We have used the dominant negative mutant *ers1* gene from *Arabidopsis* to confer resistance to ethylene and hence extended shelf life for coriander plants (Wang & Kumar 2004).

Auxin plays a key role in organogenesis as well as cell proliferation and organ size. *ARGOS*, a novel *Arabidopsis* gene is highly induced by auxin. This gene is involved in callus formation and organ size control. Further, organ enlargement in plants overexpressing *ARGOS* is blocked by the loss of function of *AINTEGUMENTA (ANT)* (Yuxin et al., 2003). *ARGOS* participates in auxin signal transduction to regulate cell proliferation and organ growth through *ANT* during organogenesis. Loss of *ant-1* function reduces mature organ size and cell number, while overexpression of *ANT* leads to organ size increase in *Arabidopsis* (Mizukami & Fischer, 2000). Several other genes regulating organogenesis have been reported in the literature.

We established an *Arabidopsis* root culture system for flower induction from seedling roots of transgenic LEAFY-GR (steroid inducible LFY expression in a *lfy6* mutant background). Flowers and floral organs arise directly from root explants when they are treated with dexamethasone. This system was used with flower-specific cDNA microarray analysis to study LFY-dependent gene regulation of flower induction (Wagner et al., 2004). Overall, 61 genes with altered expression were recorded, including *AP1, CAL, SEP1*and *SEP2*. Most of the known floral genes were up-regulated and a few were down-regulated in a LFY-dependent manner. It is a useful system for mapping genetic circuits of floral organ development. A similar system may be developed to study other routes of organogenesis. Some such studies have already been reported, e.g., oligonucleotide array analysis of hormone-induced gene expression during shoot formation in *Arabidopsis* (Che et al., 2002).Such studies will help to identify useful genes for plant improvement.

Additionally, we carried out some studies on *Paulownia* leaf cultures with the aim of isolating MADS box genes involved in regulating shoot regeneration. The role of MADS box regulatory proteins in flower development is well characterized. Some MADS box genes are also expressed in vegetative tissues, e.g., stolons and tuber of potato express *StMADS11* and *StMADS16*. Hence, we reasoned that some of the MADS box genes may be involved in regulating vegetative shoot development.

We screened a cDNA library (constructed from 10-day-old SF leaf cultures of *Paulownia kawakamii* Ito (*Scrophulariaceae*) and isolated a 1.2 kb cDNA (AF060880) clone containing MADS box (Prakash & Kumar, 2002). This clone was designated as *PkMADS1* (for *Paulownia kawakamii MADS*). RNA gel blots showed that the gene is expressed only in the vegetative shoot apex and newly differentiating shoot buds in tissue culture. The gene was not expressed in any of the floral organs. In situ hybridization (with antisense *PkMADS1* riboprobe) supported the above expression pattern. Functional analysis of *PkMADS1* was attempted by plant transformation. We generated sense (overexpression phenotype similar to wild type) and antisense transgenic plants (stunted and had altered phyllotaxy). Shoot regeneration from leaf explants of antisense transgenic plants was reduced 10-fold compared to that ofthe sense transgenic lines or the wild type. Based on these, we concluded that *PkMADS1* is a regulator of shoot morphogenesis

and is involved in shoot apical meristem organization (Prakash & Kumar 2002).

Cytokinins and organogenesis: Activation tagging mutagenesis studies in *Arabidopsis* helped to identify a gene *CytoKinin Independent 1* (*CKI1*), which is a cytokinin receptor and is involved in regulating shoot regeneration (Kakimoto, 1996). Another cytokinin receptor gene that was identified is *Cytokinin REsponse 1* (*CRE1*) (Inoue et al., 2001). These cytokinin receptors are similar to bacterial two-component histidine kinases (*CRE1* is able to complement His kinase mutants). Further, genes encoding proteins similar to bacterial two component elements are found in *Arabidopsis.* These genes are present as small gene families and include His kinases. Some of them are: His-containing phospho-transfer proteins (AHPs) and two classes of Arabidopsis Response Regulators (ARRs). Overexpression of *CRE1*(receptor) induces ARR6 expression (downstream signal intermediate) (Hutchison & Kieber, 2002). Overexpression of *ARR2* favoredshoot regeneration (Hwang & Sheen, 2001). Ectopic expression of *ARR2* alone was sufficient to promote cytokinin responses in transgenic plants. Also, *ARR5* and *CKI1* were upregulated in *Arabidopsis* during shoot development (Che et al., 2002).

We isolated a cDNA encoding for a cytokinin binding protein from petunia cDNA library prepared from shoot-forming leaf cultures. This cDNA has been named *PETCBP* (our unpublished work). *PETCBP*shows high similarity to cytokinin binding proteins from other plants. The putative cytokinin-binding protein has high sequence similarity to S-adenosyl L-homocysteine hydrolase from human beings. Antisense suppression of the gene in petunia resulted in a delay and decrease in adventitious shoot formation, excessive branching and delayed flowering phenotype.

General conclusions: Phytohormone signaling genes are important for organogenesis in tissue culture. Several genes including MADS box genes (e.g., *PkMADS1* of *Paulownia*) are involved in shoot development. A petunia cDNA (*PETCBP*) in the cytokinin signal transduction pathway has been cloned. It is also involved in shoot development. Antisense *PETCBP* petunia exhibited delayed flowering and excessive branching and hence the gene may be useful in improving leafy vegetable species. Studies such as these provide genes for future crop improvement efforts by genetic enhancement of development either by marker assisted plant breeding or by genetic engineering.

Acknowledgements: Most of the cited work from my laboratory was done by my graduate students: Pavan Prakash, Royston Low, Dhinoth Kumar and Godge Mandar Radhakisan. Funding for the research in our laboratory was provided by the National University of Singapore.

References

Che P, Gingerich DJ, Lall S, Howell SH. 2002 Global and hormone-induced gene expression changes during shoot development in *Arabidopsis*. ***Plant Cell*** 14:2771-2785.

Ecker JR. 1995. The ethylene signal transduction pathway in plants. ***Science*** 268:667

Hutchison CE, Kieber JJ.2002. Cytokinin signaling in *Arabidopsis*. ***Plant Cell*** 14: Suppl S47-59.

Hwang I, Sheen J. 2001. Two-component circuitry in *Arabidopsis* cytokinin signal transduction. ***Nature*** 413: 383-389.

Inoue T, Higuchi M, Hashimoto Y, Seki M, Kobayashi M, Kato T, Tabata S, Shinozaki K, Kakimoto T. 2001. Identification of CRE1 as a cytokinin receptor from *Arabidopsis*. ***Nature*** 409:1060-1063.

Kakimoto T. 1996. CKI1, a histidine kinase homolog implicated in cytokinin signal transduction. ***Science*** 274:982-985.

Mizukami Y, Fischer RL. 2000. Plant organ size control: AINTEGUMENTA regulates growth and cell numbers during organogenesis. ***PNAS, USA*** 97:942-947.

Prakash AP, Kumar PP. 2002. *PkMADS1* is a novel MADS-box gene regulating adventitious shoot induction and vegetative shoot development in *Paulownia kawakamii*. ***Plant Journal*** 29:141-151

Wagner D, Wellmer F, Dilks K, William D, Smith MR, Kumar PP, Riechmann JL, Greenland AJ, Meyerowitz EM. 2004. Floral induction in tissue culture: a system for the analysis of LEAFY-dependent gene regulation. ***Plant Journal*** 39:273-282.

Wang Y, Kumar PP. 2004. Heterologous expression of *Arabidopsis ERS1* causes delayed senescence in coriander. ***Plant Cell Reports*** 22:678683.

Yuxin H, Xie Q, Chua NH. 2003. The *Arabidopsis*auxin-inducible gene *ARGOS* controls lateral organ size. ***Plant Cell***15:1951-1961.

PRAKASH P. KUMAR

Present Address : Associate Professor & Deputy Head, Department of Biological Sciences
National University of Singapore, 10 Science Drive 4, Singapore 117543

E-mail : dbskumar@nus.edu.sg

Education

1976-1979, B.Sc. Botany, Zoology & Chemistry, Mysore University, India, First Class.

1979-1981, M.Sc. Botany, St. Joseph's College Trichy, Madras University, India, First Class, First Rank.

1983-1988, Ph.D. University of Calgary, Calgary, Alta., Canada

Professional Experience

July 1999-onwards: Associate Professor, Department of Biological Sciences, National University of Singapore.

October 2000-onwards: Deputy Head, Dept of Biological Sciences, National University of Singapore.

Publications (2004)

1. Wang Y, Kumar PP. Heterologousexpression of *Arabidopsis ERS1* causes delayed senescence in coriander. Plant Cell Rep (2004) 22: 678683.
2. Zhang P, Pwee KH, Tan HT, Kumar PP. Conservation of class C function of floral organ development during 300 million years of evolution from gymnosperms to angiosperms. Plant J (2004) 37:566-577.
3. Yu H, Ito T, Zhao Y, Peng J, Kumar PP, Meyerowitz EM. Floral homeotic genes are targets of gibberellin signaling in flower development. Proc Natl Acad Sci (2004) 101:7827-7832.
4. Wagner D, Wellmer F, Dilks K, William D, Smith MR, Kumar PP, Riechmann JL, Greenland AJ, Meyerowitz EM. Floral induction in tissue culture: a system for the analysis of LEAFY-dependent gene regulation. Plant J (2004) 39:273-282.