V. SUMMARY AND CONCLUSIONS

Program that specifies flower development is complex since it integrates determination of position, number and identity of several distinct organ types from cells that begin as morphologically a identical group called a meristem Hence this pathway provides an excellent model system to study complex genetic and cellular interactions that govern pattern formation in plants Molecular and genetic studies of flower induction, initiation and floral organ development in the model plants *Arabidopsis thaliana* and *Antirrhinum majus* suggested the operation of a cascade of regulatory mechanisms controlling flower genes that culminate in the formation of flowers stereotypic to the species of study Of these, the best characterized genes are those that confer floral meristem identity to cells on the flanks of the shoot apex and the genes that specify the development of floral organs on this floral meristem Cloning and expression pattern analysis of homologs of these genes from various dicotyledonous species suggest the operation of a conserved pathway through evolution of flowering plants (Coen and Meyerowitz, 1991, Weigel, 1995) To assess if similar mechanisms operate in monocotyledon species with different floral architecture, especially in instances where flowers are borne on highly branched inflorescence we have begun to identify and characterize floral regulatory genes from the crop plant rice where reduced flowers are borne on a branched panicle The genes of interest in our investigation are those involved in specifying floral meristem identity and floral organ identity

A spectrum of expressed sequences during early stages of panicle and flower development in rice

To understand gene expression patterns during early stages of panicle development and floral organ specifications in rice we have taken up characterization of random cloned cDNAs from two developmental stage specific libraries Two different panicle specific cDNA libraries were constructed either from panicles at the stage of branching and flower primordia specifications or from panicles where floral organ differentiation is occurring We have carried out partial sequence of ~100 cDNAs and compared these sequence data with known DNA sequences in the data base Approximately sixty-five percent of these cDNAs showed similarity to known genes with recognizable protein motifs, while thirty-five percent represented potentially unknown cDNAs The group of cDNAs identified

through this analysis include potential homologs that are structural genes and they perhaps represent housekeeping genes A number of other genes with homologies to plant protein kinases and leucine rich repeat (LRR) domain containing proteins with diverse functions in signal transduction pathways were found, as were factors that are potential regulatory genes or putative transcription factors In few cases the identified homologs are known to play a specific role in floral development Yet other clones exhibited high similarity to genes whose expression is situation specific, such as salt stress induced proteins or abiotic stresses induced proteins A selected group of cDNAs were studied further for their genomic organization and RNA expression profiles in developing plants We find, not unexpectedly, that these cDNAs represent both single copy and multi copy genes, and genes that express either constitutively or differentially This array of gene expression is expected to reflect the properties of their promoters and provide ways for future manipulation of developmentally regulated expression of the foreign genes in rice In addition, the novel molecules identified are potential candidates that could identify newer classes of protein families

Isolation of a homolog for the *Arabidopsis* floral meristem identity gene *LEAFY* from rice

LEAFY (*LFY*) of *Arabidopsis* is unique to plant kingdom and defines a key transcription regulator with specific role in defining floral meristem (Weigel *et al*, 1992) *LFY* is known to be evolutionarily conserved and *LFY* homologs have been recently isolated from a number of flowering plants A common pattern of RNA expression for these *LFY* homologs is that observed in the incipient and developing floral meristem. However variations in the vegetative expression pattern for some *LFY* homologs are observed To investigate the role of *LFY* homolog in the development of highly branched inflorescence commonly found in the grass family, like those of panicles, we have cloned and characterized its homolog from the rice genome, a representative in this family Using degenerate primers designed based on sequence of *LFY* and *FLO* from *Arabidopsis* and *Antirrhinum* respectively, we initially amplified by RT-PCR, two adjacent conserved domains of the *LFY* homolog from rice These cDNA fragments were used to screen rice genomic libraries in cosmid as well as bacterial artificial chromosome (BAC) vectors Four cosmid clones and a BAC clone were identified as containing genomic fragments

hybridizing to the cDNA Further analysis suggested that these clones are highly related and possibly represent overlapping segments of the same genomic locus The fragments in one of the cosmid OSL4 that hybridizes to the cDNA (two *EcoR*I fragments of 5 0kb and 2 5kb in length) were subcloned ~3 5kb sequence of genomic sequence was obtained from these subclones first with vector based primers and then with internal primers to deduce the genomic sequence of *OSL* (submitted to GenBank, Accession No AF065992) The *OSL* gene (*Oryza sativa LEAFY*) is organized in three exons separated by two introns as deduced by PCR and sequence analysis The position of the introns in the coding sequence are generally conserved when compared to that in other species and size of the introns vary The first intron in the rice gene is smaller of size 208bp and the second intron, larger in size of 1291bp and thus is more similar to those in primitive angiosperms like *Gnetum* (Frohlich and Meyerowitz, 1997) Exon3 contains the most conserved segment in this gene with amino acid identity and similarity running upto 65% Deduced amino acid sequence of *OSL* on alignment with *RFL*, a rice *LFY* cloned independently from Kyozuka *et al* , (1997) showed that except for the two conserved amino acid changes the two cDNAs share identical amino acids We find that *OSL* hybridizes to sequences in rice genome that are other than that from *OSL* as determined by Southern analysis These sequences possibly represent other *LFY* like genes or pseudo-genes

By RNA-RNA *in situ* hybridization we show that RNA expression of *OSL* is co-incident with panicle initiation However, no expression is detected in the vegetative shoot apical meristem We observe continued expression of *OSL* during the elaboration of the branched inflorescence and limited and slightly lower level of RNA expression in the flower primordia In developing flower primordia the RNA is mostly contained in region that contribute to lodicules and stamen organs of the flower By determining the levels of *OSL* expression and that of a second gene *OsMADS1* (which from earlier studies known to be one of the earliest marker for flower meristem in rice) in the same panicle we infer that the floral primordia express low levels of *OSL*, at about the time that *OsMADS1* is first expressed The expression during branching of panicle is suggestive of a role for this transcription factor *OSL*, in maintaining these cells in a transient state of indeterminacy In trying to understand the role of *OSL* expression in the specification of the rice flower we have analysed *OSL* expression in a loss-of-function mutation in rice, *fm29*, whose phenotypes resemble the mutant alleles of *leafy* In this mutation, no floral primordia are

specified and repetitive formation of branch primordia and bract is observed The expression of *OSL* in these repetitive structures are similar to that observed in wild-type plants and thus, the early and high level expression pattern of *OSL* are unaffected by this mutation Further *OsMADS1* transcripts are not detected in *fm29* branching panicles and there we infer a complete transformation of the floral meristem into inflorescence meristem in this mutant Therefore the *fm29* mutant possibly defines a regulator of *OSL* expression that is specific to the floral meristem or this gene acts in a different pathway contributing to floral development Future studies using inter-specific transgenic plants containing *Arabidopsis LFY* or rice *OSL* genes will elucidate the conserved roles of this gene on plant development

Tests for functional conservation of *Arabidopsis* floral regulatory gene products in rice

Molecular genetic analysis of regulators of floral organ identity in diverse species have lead to the proposal of a conserved theme for specifying organ types and suggest that the individual and combined action of A , B and C classes of genes specify four organ types in the developing flower Evidence for functional conservation of these genes across species have come from studies employing constitutive expression of these genes both in homologous and in heterologous systems (Mizukami and Ma, 1992, Mandel *et al* , 1992a) We have chosen to test the effects of ectopic expression of *Arabidopsis* floral organ identity genes specifically those that regulate the development of stamens and carpels, because these are structures that are almost invariantly positioned in Angiosperm flowers We have generated several independent transgenic lines that bear the *Arabidopsis AGAMOUS (AG),* a C function gene or, *APETALA3 (AP3)* or *PISTILLATA (PI)* both being B function genes under the control of constitutive monocot promoters - the rice actin gene promoter or the maize ubiquitin gene promoter These transgenic rice plants were obtained by particle bombardment of embryogenic rice calli with these different cDNA constructs together with a second plasmid bearing the Hygromycin resistance gene and the GUS gene as dominant selectable and scorable markers

We have identified a number of transgenic lines for each of the construct transformed By southern blot analysis of genomic DNA digested with restriction sites predicted to cut once in the transformed construct, we have identified lines that contain

stable integration of transgene with varying copy number On an average 5-10 copies or greater of the transgene were integrated in these lines In some instances several copies of the transgene were integrated at a single locus Plants with stable and some what lower copy number integrations taken for analysis of expression pattern of the transgene by RNA dot blot analysis revealed differing levels of the transgene transcript However surprisingly northern gel blot analysis of the RNA samples did not detect a full length transcripts for any of these transgenes These data suggest very low levels of the stable full length transcript coupled with instability of the transgenic RNA This was reflected in the lack of phenotypic effects in the panicles from these transgenic lines bearing either *AG*, *AP3* or *PI* genes Surprisingly, few of the primary transgenic plants with pACT-AP3 transgene, showed a malformation of lemma and also lodicule The observed pleiotropic effect of conversion of lodicules to glumes is suggestive of suppression of a *AP3* like endogenous rice gene Our failure to see the phenotypic conversions could be due to the multiple insertions of the transgene Another plausible reason could be requirement for simultaneous expression of both *PI* and *AP3* for obtaining phenotypic effects (Krizek and Meyerowitz, 1996a) Thus generation of transgenic plants that ectopically express both the B class genes together with generation of rice transgenic plants with reduced number of transgene insertions would give clues on the possible evolutionarily conserved function of these different *Arabidopsis* organ identity genes in rice