FINAL REPORT

GRDC
GRAINS RESEARCH & DEVELOPMENT CORPORATION

GRS36

Control of flowering time and subsequent seed formation by manipulation of AtMYB82

PROJECT DETAILS

PROJECT CODE:	GRS36
PROJECT TITLE:	CONTROL OF FLOWERING TIME AND SUBSEQUENT SEED FORMATION BY MANIPULATION OF ATMYB82
START DATE:	01.02.2001
END DATE:	01.10.2005
SUPERVISOR:	ROGER PARISH
ORGANISATION:	LA TROBE UNIVERSITY, BOTANY DEPT.
CONTACT NAME:	ROGER PARISH

Summary

The aim of the project was to investigate the *Arabidopsis* gene AtMYB82 and its role in influencing flowering time and flower formation. This was achieved using a variety of molecular techniques to attempt to turn off and over-express AtMYB82, ascertain exactly when and where it is expressed and perform comparative analysis of wild-type and mutant plants.

Old or Archival Reports (Projects that concluded in 2007 or earlier)

The information contained in these older reports is now several years old, and may have been wholly or partially superseded or built upon in subsequent work funded by GRDC or others. Readers should be aware that more recent research may be more useful for their needs. Findings related to agricultural chemical use are also potentially out of date and are not to be taken as a recommendation for their use.

Conclusions

AtMYB82 is crucial in directing the formation of functional flowers in *Arabidopsis*. Altering its expression pattern produces highly distorted, non-viable flowers. Evidence also suggests that AtMYB82 can influence leaf development and flowering time. AtMYB82 is expressed in the tissues in which phenotypes have been observed. There are no obvious candidates for functional redundancy of AtMYB82, making it highly unusual among most other MYBs and many other genes involved in flowering and floral development. AtMYB82 appears to have two splicing sites, which means two different transcripts can be made and therefore potentially two different proteins. Hence, AtMYB82 could have more than one role in controlling flower formation and/or flowering time.

Analysis of AtMYB82 mutants has provided the following information:

- Down regulation of AtMYB82 seems to affect flowering time and leaf formation.
- Over-expression of AtMYB82 appears to be lethal, even when induced in adult plants.
- Ectopic AtMYB82 expression in flowers severely alters the structure of all four whorls.

Recommendations

Literary searches have revealed two new leads as to how AtMYB82 may function:

1. A paper was published last year in which two bHLH transcription factors were shown to be capable of interacting with the AtMYB82 protein. Although the exact function of these bHLH proteins is unknown, it has been shown that they are involved in regulating phenylpropanoid biosynthesis, most notably in the seed coat. It is possible that one or more of these proteins may be required to bind to the AtMYB82 protein in order for it to assume a functional conformation. This could be ascertained by targeted T-DNA insertional mutagenesis or RNAi against the relevant bHLH genes, and assessing any resulting phenotypes that bear similarities to those observed in the work presented here.

2. A group of MADS-box genes called the SEPELLATA genes can influence the development of all four floral whorls. Very recently a paper was published in which one of these was ectopically expressed (SEP3), producing a mutant with phenotypic abnormalities very similar to those seen in various AtMYB82 mutants. Knock-out mutants of this, and other SEP genes, produce further, similar mutations. The SEP genes are expressed in the same tissues as AtMYB82 (soon after AtMYB82 expression ceases), can influence the formation of all floral organs, and produce mutants with striking similarities to those observed in AtMYB82 mutants. One or more of the SEP genes are, therefore, strong candidates for potential downstream targets of AtMYB82.

Potential interactions of these genes with AtMYB82 could be investigated in the future without too much difficulty. Doing so would potentially tell us what genes are both upstream and downstream of AtMYB82. This would enable us to ascertain the precise pathway in which AtMYB82 is located, and its position within that pathway.

Outcomes

AtMYB82 has been found to be an important gene in controlling flower formation. By expressing it ectopically, individual

floral organs were transmuted into displaying incorrect organs, e.g. anthers became carpel-like, petals became sepal-like, etc.

The ability to understand just how flowering is regulated is crucial in attempting to manipulate such processes in order to produce crops better suited to particular environments. Flowering is a critical event in plant development, but is also extremely complicated genetically, with many factors, both genetic and environmental, influencing both when and how flowering occurs. Furthermore, being the crucial event in reproductive success, there are many 'back-up systems' to ensure flowering and seed set take place.

AtMYB82 plays a crucial role in the regulation of floral development. Hence, this work adds to our knowledge of how flowering is regulated, knowledge that is applicable to all plants including crops important to the grains industry.

Achievements/Benefits

The precise expression pattern of AtMYB82 was demonstrated, both spatially and temporally, in developing seedlings, developing flowers and floral organs.

Seedlings - AtMYB82 is expressed in seedlings from as early as three days post germination and remains at a constant level until at least 15 days post germination (approx. 10-12 leaf stage). Expression in seedlings is isolated almost exclusively to primordial and meristematic tissue, with some weak expression also present in the petioles of some very young leaves.

Flowers - expression of AtMYB82 is strongest in very early floral buds that are pre-mitotic divisions. Expression then quite rapidly decreases such that by the time flowers begin to open, AtMYB82 expression has all but ceased.

Floral organs - in early flower buds, AtMYB82 expression is strongest in the outer cell layers of the pedicel and developing sepals, with weak expression in the petals and sepals. This expression progressively declines as the flower matures.

An insertion mutant with an altered floral phenotype, (alpha KO mutant) was extensively studied. In this mutant, petals, and occasionally the gynoecium, all appear sepalloid, producing leaf-like petals and unfused carpels. The mutant also produces anthers that fail to dehisce (but often contain viable pollen) and have gynoecium characteristics such as sticky apex filaments (for pollen capture) and adventitious ovules. These anthers are also often shortened in stature and can be extremely misshapen in appearance, sometimes containing no locules (and therefore no pollen) at all. This mutation is caused by dramatically altered AtMYB82 expression in flowers, whereby expression occurs much later than in wild-type. Despite the dramatic flower phenotype, any change in expression of four floral meristem identity genes, four MADS-box ABC regulating genes, two flowering time genes and a jasmonic acid regulated MYB controlling anther dehiscence were not detected. It was shown that the AtMYB82 transcript is not only altered in its expression pattern in this mutant, but the transcript itself is also modified, producing an mRNA partially coded for by the T-DNA insertion.

Many constructs are complete, all of which have been transformed into *Arabidopsis* and analysed to varying degrees:

- Two reporter gene constructs (promoter::GUS and promoter::GUS::3'UTR) demonstrated to great detail the expression pattern of AtMYB82 both in space and time. Extensive staining and sectioning of these plants revealed individual tissues and cell layers in which AtMYB82 is expressed, and at what stage of development.
- Over-expression construct appeared to predominantly produce a lethal phenotype. Three independent transformations with this construct, along the screening of many thousands of seeds, resulted in a solitary transgenic plant. This plant had altered rosette morphology (curled leaves), delayed flowering and severely disrupted flowers that were completely sterile. Those flowers that were produced displayed fused and indistinct whorls.
- Inducible over-expression construct again appeared to be somewhat lethal. Only two transgenic plants were isolated, both of which rapidly died after expression was induced post-flowering.
- Two inducible RNAi constructs consistently produced a phenotype dubbed 'the cabbage' which showed a number of similarities to the phenotype seen in the solitary over-expression plant. It showed traits such as delayed flowering and curled rosette leaves, as well as reduced fertility. However, this phenotype only appeared when plants were grown in sterile tissue culture media and never in soil. Cabbage plants transferred to soil all died. Many of the cabbage plants grown on media also often died prematurely for no apparent reason. Again, this indicated that expression of AtMYB82 is crucial to plant development.
- Constitutively expressed RNAi construct - only two transgenic plants were able to be isolated containing this construct. Only limited analysis was able to be performed on them as they were produced very near the end of the project. They appeared to show no phenotype at all, but expression levels of AtMYB82 in these lines were not determined.

- Endogenous promoter and 3' non-coding region, fused to AtMYB82, were created originally in an attempt to rescue the alpha KO mutant. Later crossing and expression experiments revealed that rescue by complementation in this manner would not be successful. However, a number of transgenic plants containing this construct were produced, all of which appeared indistinguishable from wild-type.

The PhD thesis, in which all of the above data are presented and discussed in detail, is complete.

Other research

Given the unique and highly intriguing nature of the AtMYB82 mutant, I have had several dealings with scientists from other laboratories who are extremely knowledgeable about flowering and floral development. Some of these scientists, particularly Prof. David Smyth from Monash University, may be interested in collaborative work in further ascertaining the precise role of AtMYB82. Prof. Smyth works largely on MADS-box homeogenes (such as SEPELLATA) that direct floral organ identity, but many of the upstream regulators of such genes are unknown. AtMYB82 may well fit the role of just such a gene.

Dr. John Bowman, who is about to take up a federal fellowship position, also at Monash University, is another potential collaborator who has worked extensively on the MADS-box genes in *Arabidopsis*.