



**Institute of Crop Science Section of Crop Physiology
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**Physiological and Molecular
mechanisms of flowering in longan
(*Dimocarpus longan* Lour.)**

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Chapter 2

Morphological changes during flower bud formation in longan (*Dimocarpus longan* Lour.): an improved method for resin embedding and the use of nuclear magnetic resonance imaging

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2.1 Summary

To study histological changes during flower bud development in longan, a resin embedding method has been modified. Samples from longan buds were fixed in formalin/acetic acid/alcohol and embedded in glycol methacrylate-methyl methacrylate using a modified dehydration and embedding technique by vacuum infiltration. Sectioning of longan buds 17 days after treatment with the flower inducing chemical $KClO_3$ clearly showed development of floral bud meristem. In contrast only vegetative tissue was observed in non-treated plants. In addition to conventional light microscopy, bud morphology was also investigated by nuclear magnetic resonance imaging technique. The NMR method was applied to non-destructively observe longan bud development based on apparent water content of the bud tissue. Cross and longitudinal bud sections revealed a low water content in vegetative longan buds. However, the technique is not yet suitable for histological studies within the bud meristem of woody perennial trees, largely due to the fact that the low tissue water content combined with the small sized buds resulted in insufficient image resolution.

Key words. Bud meristem, floral development, histology, NMR, potassium chlorate.

Chapter 3

Hormonal changes during flower formation of longan

(Dimocarpus longan Lour.) in response to manipulative measures

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3.1 Abstract

Mature leaves are an essential prerequisite for the successfully induction of flowering in longan (*Dimocarpus longan* Lour.) trees through the application of potassium chlorate ($KClO_3$). However, little is known about the physiological role of leaves during flower induction and in particular in the context with hormonal changes within the tree. Hormone concentrations in terminal and lateral buds, bark and wood during flower bud formation of longan induced either by $KClO_3$ or in combination with girdling and defoliation treatments were analyzed by Radio-Immuno-Assay. Untreated control trees remained vegetative, while trees treated with $KClO_3$ and in combination with apical shoot defoliation flowered within one month of application. The results show that flowering occurred in all treatments except the untreated controls. Trees treated with $KClO_3$ alone and in combination with apical shoot defoliation flowered terminally at the shoot apex, whereas in combination with girdling flowering only occurred on the 1st lateral bud below the girdle. These results suggest that $KClO_3$ effectively induces flowering when mature leaves are present at or in close proximity to the flower bud. The flowering response presumably is brought about by an increase of Z[9R]Z and concomitantly low IAA concentrations in varying plant tissues. It can be assumed that the increased concentrations of endogenous CKs were, depending on the presence of mature leaves, due to de novo biosynthesis or deconjugation of stored CKs in various plant organs which in turn might be regulated by IAA. Buds in the untreated controls remained vegetative which might have been caused by a low CK:IAA ratio. Moreover, girdling will likely disrupt the basipetal IAA transport in the phloem parenchyma cells and the resulting increased auxin concentration above the girdle will inhibit deconjugation of stored CKs and therefore promote vegetative growth.

Chapter 4

Identification and expression analysis of flowering genes in KClO₃ treated longan (*Dimocarpus longan* Lour.)

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4.1 Summary

Longan, *Dimocarpus longan* Lour, a subtropical fruit crop is specifically responsive to the chemical potassium chlorate (KClO_3), inducing off-season flowering. However, the mode of action how this chemical triggers flower induction and which genes are activated in the molecular pathway is not yet elucidated. Two putative floral integrator-like genes, *Dimocarpus longan* FLOWERING LOCUS T1 and 2 (*DIFT1* and *DIFT2*) were isolated from longan and both translated sequences revealed a high homology to *Arabidopsis thaliana* FT (AtFT). Results of a phylogenetic analysis suggest that *DIFT1* and *DIFT2* are members of the FT protein family in plants. We examined the transcription of *DIFT1*, *DIFT2* and the putative meristem identity target gene *APETALA 1*-like of longan (*DIAP1*) to analyze gene activation during flower induction due to KClO_3 application. *DIAP1* was up-regulated three weeks after treatment. The results suggest that KClO_3 induced flowering in longan acts via a pathway regulating *DIAP1*-gene expression. However, the transcription pattern of *DIFT1* and *DIFT2* does not conclusively show that *DIAP1* transcription is controlled by DIFTs.
