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Flowering in Longan (*Dimocarpus longan* L.) Induced by Hormonal Changes Following $KClO_3$ Applications

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Summary

Flower induction in longan (*Dimocarpus longan* L.) following the application of 8 g$KClO_3$ m^{-2} canopy as soil drench at mature leaf stage was associated with
a) increased concentrations of the cytokinins zeatin and zeatinriboside in both the bud meristem and the surrounding leaf primordia around 7 to 10 days before first flowers appeared;
b) reduced leaf stomatal conductance, transpiration and net CO_2 assimilation;
c) a transient increase in leaf ethylene evolution, and
d) lower export rates of gibberellins, auxin, abscisic acid, and zeatin and zeatinriboside from leaves.

The reduced amount of auxin and gibberellins exported from leaves and typically favouring vegetative growth, in combination with increased levels of cytokinins in buds, known to support floral bud development, might play a key role in flower induction not only in longan, but also for other perennial fruit tree crops.

Key words. auxin – ethylene – flower induction – leaf gas exchange – off-season fruit – potassium chlorate

Introduction

Longan (*Dimocarpus longan* L.) is a subtropical fruit crop with a brown-skinned, white flesh fruit of increasing export potential in Southeast Asia (Fig. 1). A major production constraint is the inconsistency of flowering, hence alternate bearing due to an insufficiently met chill requirement.

The discovery of $KClO_3$ as flower inducing agent was a combination of luck and precise horticultural observation, when someone in Northern Thailand linked the sudden flower formation in a longan tree to this substance where a container with this chemical was accidentally rinsed. First experiments confirmed the effectiveness of $KClO_3$ to induce flowers and subsequent research attempted to develop the most suitable application time and procedure (Manochai et al. 2005). Since that time, the use of $KClO_3$ became a common practice amongst growers, which helped considerably to overcome the problem of biennial bearing and to produce economically viable off-season longan fruit. The unique flower inducing effect of $KClO_3$ in longan within 17–28 days of treatment provides an excellent opportunity for scientists to study the physiological mechanisms during the transition process from vegetative to floral bud development. The effectiveness of $KClO_3$ is much reduced when applied to trees with a new flush of growth and this could be related to increased auxin export from young leaves (Hegele et al. 2004a). This study attempts to uncover temporal and spatial hormonal changes in various tissues of longan trees following $KClO_3$ application. This might improve our knowledge of the mode of action of $KClO_3$ in longan and the control of flower induction (FI) in perennial fruit trees in general.

Material and Methods

Plant material and photosynthesis measurement

Thirty-two five-year-old longan trees, cv. 'Daw', grown at an experimental orchard near Majo University, Chiang Mai, in Northern Thailand, were used in this experiment. 'Daw' is the most popular grown variety in Thailand and under local climatic conditions known as a consistent fruit bearing cultivar (Menzel et al. 2002). The trees were arranged in a randomised block design of eight plots with four trees each. Following commercial practice, 8 g of $KClO_3$ (Rengthol Co. Ltd., China) per square meter of leaf canopy were applied to half of the plots. Throughout a 20 days period and at 1–3 day intervals between 8–28 May 2001, 24 terminal and 96 lateral buds for extraction of free endogenous hormones and 48 leaves for measuring hormone export were taken per plot and treatment at each sampling date.

Four leaves per tree were used to record net CO_2 assimilation, transpiration, stomatal conductance and leaf temperature, using a portable gas analyser (CIRAS 1, PP-Systems, UK). Similar data were also obtained from 20 two-year-old potted longan trees treated with 1 g $KClO_3$ per pot applied as a soil drench to half of the plants.

Hormone extracts

Immediately after sampling buds were frozen in liquid nitrogen, transported on dry ice from Thailand to Germany and freeze-dried. Twenty buds per replication were dissected into meristems and surrounding leaf primordia,

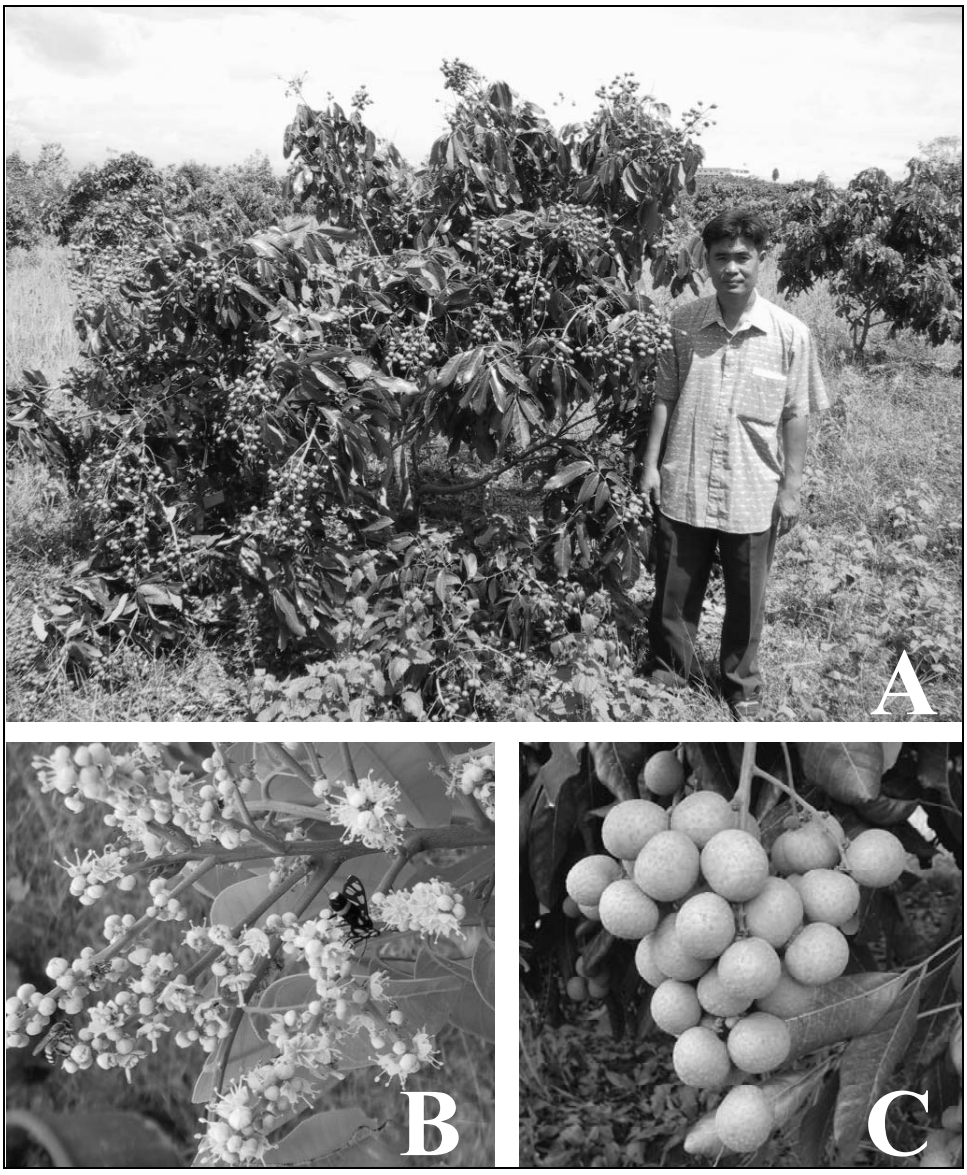


Fig. 1. Off-season longan production by application of $KClO_3$. (A) Fruit bearing longan tree after soil drench with $KClO_3$ next to non-fruiting control tree (in the background to the right side). (B) longan flowers pollinated by insects. (C) Bunch of longan off-season fruit at harvesting time.

then ground separately in liquid nitrogen using mortar and pistil and extracted overnight in 50 mL of 80 % MeOH. Samples were purified using a combination of in-soluble polyvinylpolypyrrolidone (PVP; Sigma, Germany), DEAE Sephadex™ A-25 (Ammersham, Sweden) and SepPak C_{18} (Waters, Germany) columns according to the procedure described by Jimenez et al. (2000).

Diffusible hormones and ethylene

Whole shoots were harvested and transported in moistened plastic bags to the laboratory at Chiang Mai University. Forty-eight freshly cut leaves per treatment, plot and sampling time were then placed into the cavities of two 24 well tissue culture plates. To collect samples for indole-3-acetic acid (IAA)-export, the cavities of one plate were filled with 3 mL 0.1 M phosphate buffer at a pH of 6.2 and solidified with 0.8 % agar. To gain phloem derived hormones such as abscisic acid (ABA), cytokinins (CKs) and gibberellins (GAs), 200 mM EDTA was added to the second plate to prevent the closure of the phloem. The plates were placed in airtight containers, equipped with a septum, to withdraw gas samples and kept for 20 h at 25 °C and 100 % relative humidity in the dark. After 20 h, the atmosphere inside the container was analysed for ethylene concentration by taking gas samples and injecting triplicates of 1 mL into a gas chromatograph (Shimadzu GC 14 B, FID detector, 150 °C injector temperature, Porapak N column, 55 °C column temperature, 70 ml min^{-1} flow rate, N_2 carrier gas).

The diffusates were pooled from 4 (IAA) to 24 (other hormones) agarblocks and extracted overnight in 10 mL of 100 % MeOH per block. After evaporation of the solvent, the samples were dissolved in 0.1 M acetic acid and the pH was adjusted to <3. A combination of a plastic syringe with a glass fibre disc (Macherey-Nagel: 85/220) and SepPak C_{18} (Waters) column was used to remove EDTA. The eluate (80 % MeOH / 0,1M acetic acid) was reduced to dryness in a vortex evaporator and dissolved in 0.01 M ammonium acetate, pH 7.4. Further sample purification followed the same procedure mentioned earlier but without the PVP column.

Quantitative hormone analysis

The quantification of the various plant hormones in the purified extracts or diffusates was achieved in triplicates by Radio-Immuno-Assay (RIA) (WEILER 1980), using polyclonal antibodies for Z / ZR, isopentenyladenine (iAde) / isopentenyladenosine (iAdo), indolylaceticacid (IAA), abscisic acid (ABA), and gibberellic acid (GA_3), with cross reaction for GA_1 and GA_{20}.

Results

Physiological leaf parameters

Following application of $KClO_3$, leaf net CO_2 assimilation, transpiration and stomatal conductance significantly declined throughout the observation period which was most pronounced in the young potted longan seedlings under controlled environmental conditions (Fig. 2).

Ethylene evolution and diffusible hormones out of leaves

Six days after application, the $KClO_3$ treatments had a threefold increased leaf ethylene evolution rate compared to the controls (Fig. 3). Nine days following application and toward the end of the observation period when first flowers in treated trees appeared, another significant raise in leaf ethylene production occurred.

The most interesting changes of plant hormones exported from leaves in response to $KClO_3$ application was observed in the amount of IAA, cytokinins and GA_3 equivalents. Only one day after treatment, a significant higher auxin export of more than 120 % over the controls was found. Thereafter, on day 5, the export rate steeply declined by almost 60 % when compared to the controls (Fig. 4). While export of Z / ZR from leaves of treated trees was greatly reduced over most of the observation period (Fig. 5A), diffusion of iAdo / iAde, the precursors of Z / ZR, was initially lower, then higher from day three to day seven and lower again until 17 days after treatment when compared to control leaves (Fig. 5B). Leaves of treated trees exported significantly less GA_3 equivalents than untreated controls, except on day 20, the last sampling day (Fig. 5C). ABA export from leaves did neither show a clear trend over time nor significant differences between treatments (Fig. 5D).

Extractable hormones from buds and leaf primordia

The greatest differences in extractable hormones from terminal (Fig. 6) and lateral buds (Fig. 7) as well as the surrounding leaf primordia (data not shown) were measured for CKs. In buds and the surrounding leaf primordia a considerable increase in the highly biologically active Z / ZR as well as its precursors iAde / iAdo was found. While terminal bud meristems and their surrounding tissue showed two distinct peaks at 5–7 and 17 days after treatment, there was a constant increase from day 5 until day 17 in lateral buds.

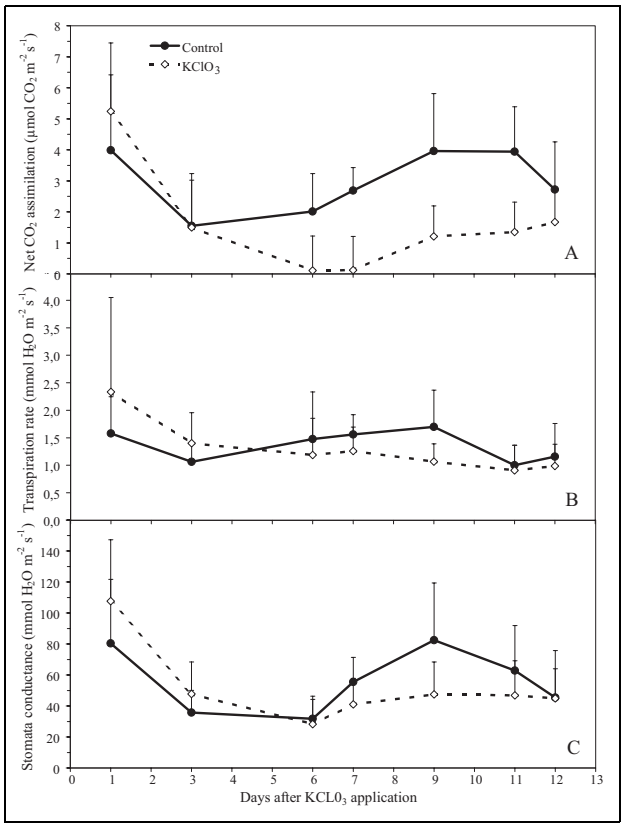


Fig. 2. Effect of $KClO_3$, applied as soil drench, on physiological leaf parameters of young longan seedlings in the 2001 pot experiment: (A) Net CO_2 assimilation, (B) Transpiration, (C) Stomata conductance.

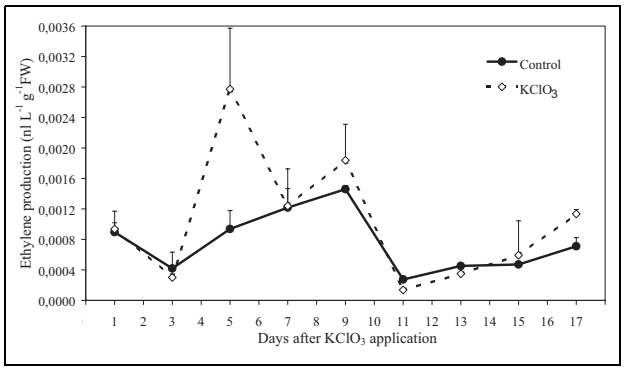


Fig. 3. Ethylene production per g FW of 24 longan leaves within 20 h at various times after treatment of trees with $KClO_3$.

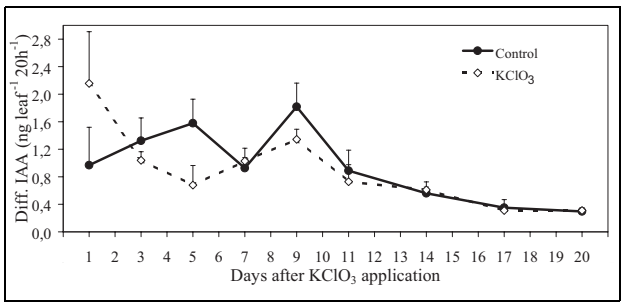


Fig. 4. Diffusible IAA out of longan leaves after application of $KClO_3$ as soil drench.

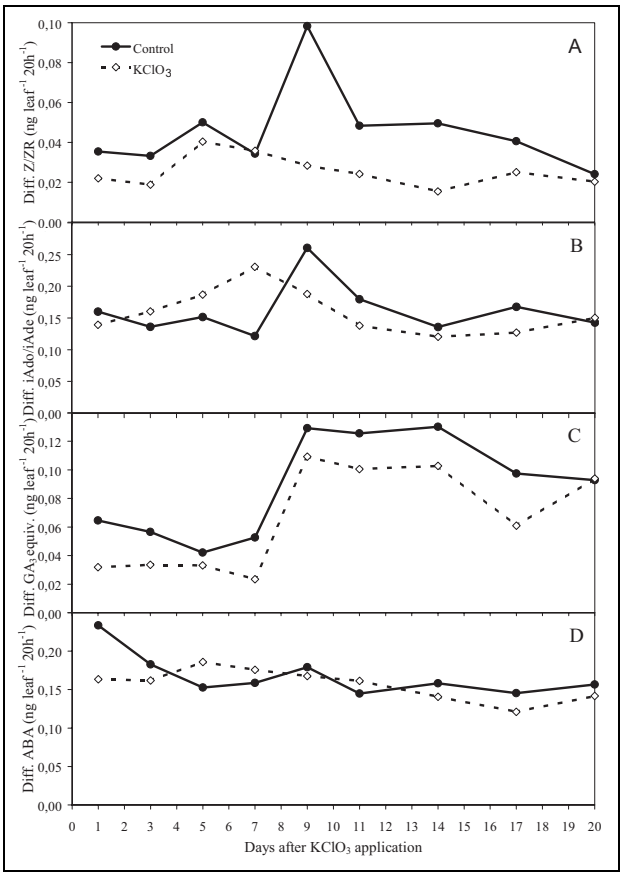


Fig. 5. Diffusible cytokinins (Z/ZR (A), iAdo/iAde (B)), GA_3 equivalents (C) and ABA (D) out of the phloem of longan leaves after application of $KClO_3$ as soil drench.

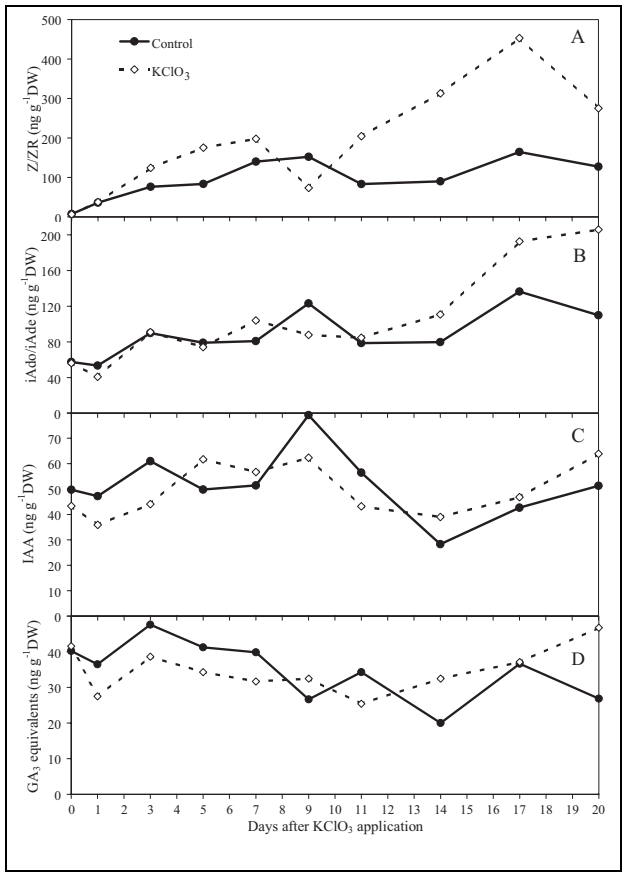


Fig. 6. Extractable free hormones out of terminal longan buds after soil drench application of $KClO_3$: Z/ZR (A), iAdo/iAde (B), IAA (C), GA_3 equivalents (D).

IAA concentration in terminal buds was mostly but not consistently reduced over the first two weeks after $KClO_3$ application, whereas slightly increased in the last week of observation. Apart from insignificantly lowered concentrations during the beginning and end of the observation period, IAA in lateral buds and primordia was mostly higher in the $KClO_3$ treated trees compared to the controls. Concurrently to the distinctive Z/ZR pattern (Fig. 6A), GA_3 equivalents in treated terminal buds were reduced from day 1 to day 7 while higher on day 14 and 20 than those in the controls. In contrast, the later flowering lateral buds had significantly increased GA_3 equivalents one week after $KClO_3$ application, but thereafter they were slightly reduced to the controls. However, in both cases there was a time period when a high CK concentration occurred concurrently with low GA_3 equivalents (day 1–7 for terminal buds and day 11–17 for lateral buds). GA_3 equivalents extracted from leaf primordia showed no significant differences to the controls except a transient peak at day 11 after $KClO_3$ application for those surrounding the terminal meristems. ABA levels in the bud meristem were mostly below those of untreated controls but no differences were found in the leaf primordia.

Discussion

Considering that the reduction of net CO_2 assimilation in longan after $KClO_3$ application was also observed in mango following flower inducing chemical treatments (Naphrom 2004; Hegele et al. 2004a) and litchi as a consequence of "natural" induction by cool temperature (Chattrakul 2005), it might be assumed that the lowered leaf gas exchange is a general physiological response during the initial stage of FI in subtropical tree species. It is difficult to know whether this decline in photosynthetic capacity is causing or characterising the period of transient rest which is thought to be a necessary prerequisite for the switch toward floral bud development (Menzel and Simpson 1987). Slightly reduced amounts of diffusible ABA from leaves of $KClO_3$ treated trees (Fig. 5D) would rule out any participation of ABA in stomatal closure (Willmer and Fricker 1996) and hence reduction of leaf photosynthesis. The exact mode of action by which $KClO_3$ causes photosynthesis to decline remains still unclear; however, the reduction of chlorate by nitrate reductase to toxic chlorite might be involved in this process (Harper et al. 1981).

Five days after treatment a strong reduction in auxin export from leaves (Fig. 4) coincided with a peak in ethylene evolution (Fig. 3). Elevated concentrations of ethylene within the tissue are known to reduce auxin transport (Beyer 1973; Suttle 1988), but high internal concentrations of auxin can stimulate ethylene production (Russel and Thimann 1988). Therefore it cannot be distinguished whether the reduction of diffusible auxin from leaves has been induced by high rates of ethylene or ele-

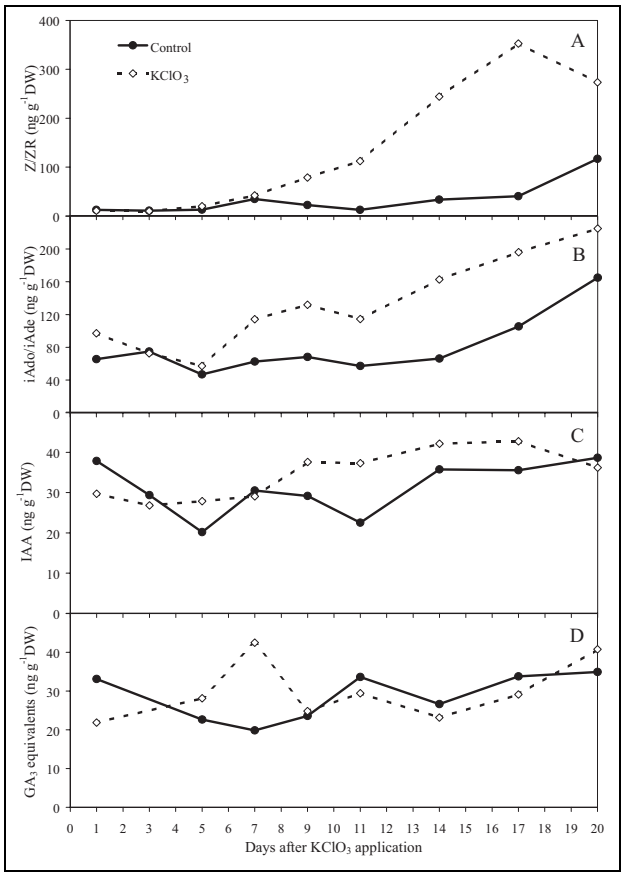


Fig. 7. Extractable free hormones out of lateral longan buds after soil drench application of $KClO_3$: Z/ZR (A), iAdo/iAde (B), IAA (C), GA_3 equivalents (D).

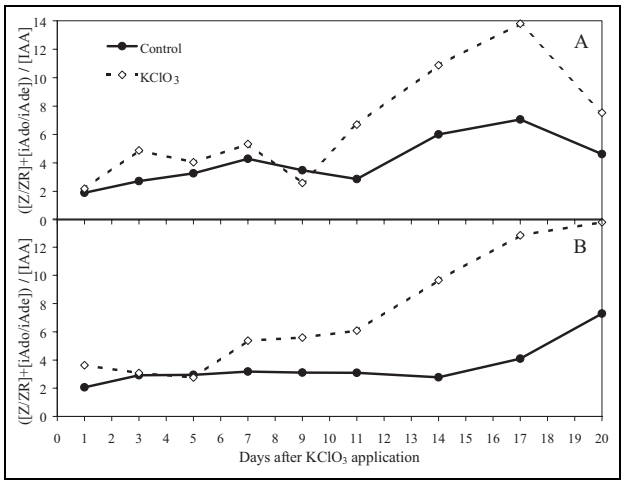


Fig. 8. Relation of extractable free CKs (Z/ZR+iAdo/iAde) to auxin (IAA) in terminal (A) and lateral (B) longan buds after soil drench application of $KClO_3$.

vated ethylene rates were caused by inhibited auxin transport and subsequent internal auxin accumulation. Possible auxin transport inhibiting factors could be proton leakage due to membrane damage as observed in bacteria cell walls due to chlorite exposure (Warf and Kemp 2002) or an increased level of phytotropins. These phenolic plant substances are linked to a specific binding site and control the activity of the IAA export carrier (Rubery and Jacobs 1988), as shown previously by Hegele (2001) in phytoplasma infected plants. Additional measurements of endogenous IAA concentration in leaves and of changes in the transport capacity for ^{3}H-IAA in petioles after $KClO_3$ treatment will provide an improved understanding of the underlying mechanisms. The significance of the higher IAA exports from leaves one day after $KClO_3$ application (Fig. 4) remains unclear but might be related to the onset of FI. Similar results were found in longan after shoot girdling and in mango trees following cold temperature exposure (Hegele et al. 2004b). A potential role of auxin signalling in the process of conjugation / deconjugation of CKs (Parker et al. 1986; Vuylsteker et al. 1998), which are thought to be of major relevance in the transition from vegetative to floral bud development (Hoad 1984; Bernier et al. 1993), has to be clarified by future studies. The generally reduced Z / ZR export from leaves after $KClO_3$ application might be due to a lower conversion rate of iAde / iAdo in leaves, which might also explain the higher amounts of these precursors between day 3 and 7 after the treatment (Fig. 5). Conversely, it could be hypothesised that fewer amounts of Z / ZR are synthesised in roots and/or transported to the leaves following $KClO_3$ application. Apparent "losses" along the transport pathway or in the target tissue (leaf, bud) could be due to higher rates of conjugation. It is therefore unclear, whether the increase in iAde / iAdo export from leaves between days 3 to 7 contributed to the higher CK concentrations in terminal and lateral buds and their surrounding leaf primordia (Fig. 6A, 6B, 7A, 7B).

The significantly reduced export of GA_3 equivalents from leaves until day 17 after $KClO_3$ application (Fig. 5C) could explain the lower GA levels in terminal buds (Fig. 6D), especially during the assumed time of FI at 10 days before first flowers become visible (Sarathum, personal communications). GAs are known to inhibit FI in mango (Tomer 1984; Oosthuyse 1995; Turnbull et al. 1996; Nunez-Elisea and Davenport 1998), citrus (Goldschmidt et al. 1997) and apple (Prang et al. 1997), while GA inhibitors promote flowering (Kulkarni 1988; Salomon and Reuveni 1994; Jose and Reboucas 2000). Consequently low GA concentrations in longan may favour a shift of the bud meristem toward floral development.

Flowering can be promoted by feeding CKs into the xylem of apple (Skogerbø 1992), by cool growing temperatures in litchi and mango (Chen 1990; Naphrom et al. 2004) or by long days in *Arabidopsis thaliana* (Corbesier et al. 2003) and *Sinapis alba* (Lejeune et al. 1994). In all these cases CKs seem to be the major group of hormones involved in FI. $KClO_3$ applications induce longan floral development presumably through an increase in free CKs. The two CK peaks as described for terminal buds and their surrounding leaf primordia coincided with the assumed time of flower induction and flower differentiation, respectively (Fig. 6, 7). The delayed flowering of lateral vs. terminal buds was reflected in a delayed increase of CKs.

In summary it is tempting to assume that higher CK levels and lower concentrations of endogenous GAs are essential for the transition process from vegetative to flo-

ral bud development. The role of auxin remains still unclear; however, it might be that at least during the initial phase of the induction process, lower IAA concentrations are favourable. The relation of CKs to auxin (Fig. 8) might be of greater importance rather than individual hormone concentrations. For example terminal buds of control trees had considerably higher CK and IAA concentrations on day 9 (Fig. 6) than those of treated trees which led subsequently to only vegetative leaf flushing a few days later.

Acknowledgements

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References

Bernier, G., A. Havelange, C. Houssa, A. Petitjean and P. Lejeune 1993: Physiological signals that induce flowering. The Plant Cell **5**, 1147–1155.

Beyer, E. M. 1973: Abscission-support for a role of ethylene modification of auxin transport. Plant Physiology **52**, 1–5.

Chattrakul, A. 2005: Mechanism of physiological responses of litchi when flowering under low temperature condition. Dissertation Chiang Mai University, Thailand, ISBN 974-9888--98-5, 97–101.

Chen, W. S. 1990: Endogenous growth substances in xylem and shoot tip diffusate of lychee in relation to flowering. Horticultural Science **25**, 314–315.

Corbesier, L., E. Prinsen, A. Jacqmard, P. Lejeune, H. Van Onckelen, C. Perilleux and G. Bernier 2003: Cytokinin levels in leaves, leaf exsudate and shoot apical meristem of *Arabidopsis thailiana* during floral transition. J. Exp. Bot. **54**, 2511–2517.

Goldschmidt, E., M. Tamim and R. Goren 1997: Gibberellins and flowering in citrus and other fruit trees: a critical analysis. Acta Hort., **463**, 201–208.

Harper, J. E. 1981: Effect of chlorate, nitrogen source, and light on chlorate toxicity and nitrate reductase activity in soybean leaves. Physiol. Plant. **53**, 505–510.

Hegele, M. 2001: Untersuchungen zu den hormonellen Ursachen der Krankheitsinduktion durch Phytoplasmen. Dissertation Hohenheim Germany, Grauer Verlag Stuttgart Germany, ISBN 3-86186-383-9, 175–181.

Hegele, M., D. Naphrom, P. Manochai, A. Chattrakul, P. Sruamsiri and F. Bangerth 2004a: Effect of leaf age on the response of flower induction and related hormonal changes in longan trees after $KClO_3$ treatment. Acta Hort. **653**, 41–49.

Hegele, M., N. Boonblod, F. Bangerth, D. Naphrom, A. Chattrakul, P. Sruamsiri and P. Manochai 2004b: Changes in photosynthesis, IAA export from leaves and cytokinins in the xylem sap after girdling of young mango trees in combination with different growth regulators and their possible significance for flower induction. Acta Hort. **645**, 417–424.

Hoad, G. V. 1984: Hormonal regulation of fruit bud formation in fruit trees. Acta Hort. **149**, 13–20.

Jimenez, V.M., E. Guevara, J. Herrera and F. Bangerth 2001: Endogenous hormone levels in habituated nucellar *Citrus* callus during the initial stages of regeneration. Plant Cell Reports **10**, 92–100.

Jose, A.R.S. and T.N.H. Reboucas 2000: Use of paclobutrazol in mango orchards in southwest Brazil. Acta Hort. **509**, 713–718.

Kulkarni, V. J. 1988: Chemical control of tree vigor and the promotion of flowering and fruiting in mango (*Mangifera indica* L.) using paclobutrazol. J. Hort. Sci. **63**, 557–566.

Lejeune, P., G. Bernier, M.C. Requier and J.M. Kinet 1994: Cytokinins in phloem and xylem sap of *Sinapis alba* during floral induction. Physiol. Plant. **90**, 522–528.

Manochai, P., P. Sruamsiri, W. Wiriya-alongkone, D. Naphrom, M. Hegele and F. Bangerth 2005: Year around off season flower induction in longan (*Dimocarpus longan*, Lour) trees by $KClO_3$ applications: potentials and problems. Scientia Hort. **104**, 379–390.

Menzel, C.M. and D.R. Simpson 1987: Effect of cincturing on growth and flowering of lychee over several seasons in subtropical Queensland. Australian Journal of Experimental Agriculture **27**, 733–738.

Menzel, C. M., B.J. Watson and D.R. Simpson 2002: Longan. In: Bose, T.K., S.K. Mitra and D. Sanyal (eds.): Fruits: Tropical and Subtropical, Volume 2. Naya Udyog, Calcutta, India, 259–292.

Naphrom, D., P. Sruamsiri, M. Hegele, N. Boonplod, F. Bangerth and P. Manochai 2004: Hormonal changes in various tissues of mango trees during flower induction following cold temperature. Acta Hort. **645**, 453–457.

Nunez-Elisea, R. and T.L. Davenport 1998: Gibberellin and temperature effects on dormancy release and shoot morphogenesis of mango (*Magnifera indica* L.). Scientia Hort. **77**, 11–21.

Oosthuyse, S.A. 1995: Effect of aqueous application of GA_3 on flowering of mango trees: why in certain instances flowering is prevented, and in others flowering is only delayed? Yearbook of the South African Mango Growers Association **15**, 21–25.

Parker, C.W, B. Entsch and D.S. Letham 1986: Inhibition of cytokinin metabolism. Part 1. Inhibitors of two enzymes which metabolize cytokinins. Phytochemistry **25**, 303–310.

Prang, L., M. Stephan, G. Schneider and F. Bangerth 1997: Gibberellin signals originating from apple fruit and their possible involvement in flower induction. Acta Hort. **463**, 235–241.

Rubery, P.H. and M. Jacobs 1988: Auxin transport and its regulation by flavonoids. In: Pharis, R.P. and S.B. Rood (eds.): Plant Growth substances. Springer Verlag, Berlin, Germany, 419–427.

Russel, W. and K.V. Thimann 1988: Auxin transport and its regulation by flavonoids. In: Pharis, R.P. and S.B. Rood (eds.): Plant Growth substances. Springer Verlag, Berlin, Germany, 428–440.

Salomon, E. and O. Reuveni 1994: Effect of paclobutrazol treatment on the growth and first flowering of intact and autografted seedlings if mango. Sciencia Hort: **60**, 81–87.

Skogerbø, G. 1992: Effects of xylem cytokinin application on flower bud development in apple (*Malus* x *domestica* Borkh.). Norwegian Journal of. Agricultural Science **6**, 473–483.

Suttle, J.C. 1988: Effect of ethylene treatment on polar IAA transport, net IAA uptake and specific binding of N-1-naphthylphthalamic acid in tissues and microsomes from etiolated pea epicotyls. Plant Physiol. **88**, 795–799.

Tomer, E. 1984: Inhibition of flowering in mango by gibberellic acid. Scientia Hort. **24**, 299–303.

Turnbull, C.G.N., K.L. Anderson and E.C. Winston 1996: Influence of gibberellin treatment on flowering and fruiting patterns in mango. Austr. J. Exp. Agr. **36**, 603–611.

Warf, C. C. and G. K. Kemp 2002: Acidified sodium chlorite (ASC): Chemistry and mode of action. In: Abstracts of Papers, 223rd ACS National Meeting, Orlando, FL, United States, April 7–11, 2002, American Chemical Society, Washington, D. C., ENVR-056. CODEN: 69CKQP AN 2002:188741.

Weiler, E. W. 1980: Radioimmunoasssay for differential and direct analysis of free and conjugated abscisic acid in plant extracts. Planta **148**, 262–272.

Willmer C. and M. Fricker 1996: The influence of hormones and other naturally occurring compounds on stomatal behaviour. In: Stomata 2nd edition. Chapman & Hall, London, 193–207.

Vuylsteker, C., E. Prinsen, J.-P. Boutin, H.A. Van Onckelen and S. Rambour 1998: Evidence for nitrate reductase expression during initiation of lateral roots by NAA in chicory. J. Exp. Bot. **49**, 937–944.

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