

P6.24**Use of RAPD in assessing genetic variability in *Tilia cordata* to facilitate appropriate reestablishment of native trees**

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Lincolnshire contains some of the most important examples of small-leaved lime woodland in Britain. The trees have been managed since the 11th century by coppicing and many of the trees studied are several hundred years old.

The genetic diversity of small leaved lime (*Tilia cordata*) is being investigated using RAPD markers to estimate the genetic relationships between trees. The RAPD technique provides a convenient method of assessing the differences in the genetic composition of related individuals when there is no DNA sequence information available. Initial results of RAPD analysis indicate that the trees from two separate woodland areas although largely similar show two separate clusters. This suggests that RAPDs will be a suitable tool in this study and will show how the genetic variation of the trees is affected by their location not only in their own wood but also within nearby ancient woodlands.

Tissue culture techniques are also being developed to enable the successful micro-propagation of *T. cordata*. Factors such as sterilization procedures, plant growth regulator concentrations, temperatures and day length are being manipulated to produce optimum conditions for the production of explants. Using the plant growth regulators 6-benzylaminopurine (BAP) and naphthaleneacetic acid (NAA) it has been possible to induce both root and leaf material. Using tissue culture techniques to produce new seedlings would enable the woods to be managed in a way that maintains the inherent genetic integrity of the *Tilia cordata*.

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P6.25**Bioinformatics: A tool to explain gene diversity**

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Sequencing technology has now reached such a level of sophistication that it is quite common for a large stretch of DNA to be sequenced and for that sequence to be manipulated/stored in a computer database. It is possible once a nucleotide/protein sequence has been deduced to search an existing database for a similar, homologous, sequence and for generic gene or protein coding region. It is more relatively straightforward to use sequence analysis software to search a new sequence for identity within a chosen database.

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P6.26**Genetic modification of plant stature by manipulation of gibberellin metabolism: An alternative to chemical growth regulators**

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Gibberellins (GAs) are endogenous plant hormones that control key aspects of growth and development. Chemical growth retardants that inhibit GA biosynthesis are used extensively in horticulture to modify plant stature, increasing production costs, manpower and associated environmental risks. An alternative strategy is genetic manipulation of GA metabolism in plants to induce similar phenotypic changes. Two species, *Solanum nigrum* and *Nicotiana glauca*, have been employed as targets for *Agrobacterium*-mediated gene delivery. The constructs used in this study contained the CaMV 35S promoter driving GA-biosynthetic genes including *MmGA3ox1* and *MmGA3ox2* from *Marah macrocarpus*, *AtGA20ox1* from *Arabidopsis thaliana* and *des* from the fungus *Gibberella fujikuroi*, which may increase bioactive GAs and promote plant growth. The *PcGA2ox1* gene from *Phaseolus coccineus* may decrease the concentrations of bioactive GAs by deactivation, so decreasing stature. Double transformations of both species with 35S:*MmGA3ox1*+35S:*MmGA3ox2*, 35S:*MmGA3ox1*+35S:*AtGA20ox1* and 35S:*PcGA2ox1*+35S:*des* genes are also being carried out in order to evaluate the combined effect of both genes in modifying GA metabolism. Both species have been transformed with *MmGA3ox1*, *MmGA3ox2*, *PcGA2ox1*, *AtGA20ox1* and *MmGA3ox1*+*MmGA3ox2* genes; gene presence and expression are being confirmed by PCR and RT-PCR, respectively. GC-MS analyses are being undertaken on transgenic plant tissues to determine the changes in the contents of precursor, bioactive and deactivated GAs. Increase in stature has been observed in *S. nigrum* transformed with 35S:*MmGA3ox1*, 35S:*MmGA3ox2* and 35S:*MmGA3ox1*+35S:*MmGA3ox2* genes. Modification of plant stature by such a transgenic approach may have application in commercial horticulture.

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P6.27**Sugar beet plant–water uptake and plant–water relationships under saline growth conditions**

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Growth and water uptake both decreases when sugar beet plants are irrigated with saline water. To determine the relative condition of physiological traits to these decreases plant fresh and dry weight, plant leaf area, number of leaf per plant, leaf

water (ψ_w) and osmotic (ψ_p) potentials, gas exchange parameters, leaf chlorophyll and Na^+ content were investigated in the sugar beet (*Beta vulgaris* L.) plants, cvs Madison and 7233-P29. Plants were grown in greenhouse condition, in sand culture, and irrigated with a complete nutrient solution supplied with 0 (control), 50, 150, 250 and 350 mM $\text{NaCl} + \text{CaCl}_2$ (in 5:1 ratio), over a period of 2 months. Salinity reduced plant dry weight, height and (reduction of plant leaf area and stomatal density) and physiological changes [reduction of stomatal conductance, transpiration and net CO_2 assimilation (A_{CO_2})]. Leaves appeared healthy and chlorophyll content per unit leaf area increased with increasing salinity. Although reduction of net A_{CO_2} at low levels of salinity can be attributed to stomatal conductance and stomatal density, at high levels of salinity non-stomatal factors cause reduction in net A_{CO_2} . Photosynthetic ability was inversely related to the concentration of either Na^+ or Cl^- in the leaf laminae sampled at the end of experimental period.

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P6.28

Optimisation of micropropagation media for Malaysian banana (*Musa* spp.)

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The current study evaluates the in vitro responses of an economically important Malaysian banana cv. Pisang Nangka AAA to different combinations and concentrations of 6-benzylaminopurine (BAP) and indole-3-acetic acid (IAA) in Murashige and Skoog (MS)-based media. Currently, there are no reports on the successful micropropagation of this cv. and which focus specifically on the synergistic effects of plant growth regulators (PGRs). Data on shoot-tip explants survival, and shoots, leaves and roots formation were taken at 28 days after culture (DAC). Mean survival rates for cultured explants of $87.5 \pm 12.5\%$ to 100% were observed for all treatments. The highest mean number of shoots generated per explant was 5.46 ± 0.22 (20 mg l^{-1} BAP and 0.175 mg l^{-1} IAA). The highest mean number of leaves and roots per explant was 5.15 ± 0.16 and 12.40 ± 0.64 , respectively in control treatment (medium without PGRs). The shoots, leaves and roots size decreased with increasing concentration of BAP and with a fixed concentration number of leaves. Leaf ψ_w and ψ_p decreased with 0.175 mg l^{-1} IAA. The roots formation was totally salinity but leaf turgor pressures were significantly higher in salinised than control plants which suggests that bulk tissue turgor did not limit growth under salinity. Increasing salinity in the irrigation solution led to both morphological changes inhibited at $10\text{--}30 \text{ mg l}^{-1}$ BAP and 0.175 mg l^{-1} IAA. MS medium with 20 mg l^{-1} BAP and 0.175 mg l^{-1} IAA will be used for shoot multiplication of this banana cv. Rooting of shoots (100%) was induced on a PGRs-free medium at 28 DAC. This micro-

propagation protocol will be a foundation for future studies on cryopreservation of banana germplasms.

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P6.29

Galactose from the legume root cap: Structure, signal, toxin, trigger?

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An acidic beta-galactosidase activity was found to be localized in border cells of pea, bean, alfalfa, barrel medic, sorghum, and maize, and to be secreted into the extracellular environment. No activity was detected in radish and *Arabidopsis*, which do not produce viable border cells. A heterologous probe from a tomato galactosidase was used to identify a full length cDNA clone (BRDgal) from a pea root cap library. Whole mount in situ localization (WISH) was used to document that BRDgal mRNA expression is localized in peripheral cells of the root cap as they undergo border cell separation, and in detached border cells; no expression occurs within the body of the root. Multiple efforts to develop viable hairy root clones expressing BRDgal antisense mRNA were unsuccessful, suggesting that inhibiting expression of this enzyme is lethal to development. Galactose, which comprises up to 40% of the root cap mucilage where BRDgal is found and can act as a species-specific chemoattractant and nutrient for microorganisms, was reported by Knudson (1919) to be toxic to plant roots. We confirmed that galactose inhibits root growth, and its effects on cell viability, development, and growth were systematically compared with those of other primary and secondary metabolites encountered by root tips during penetration of the soil environment. Galactose is among the most potent metabolites in its effects, and may constitute a key signal for root cap control of growth and development. Future studies to establish whether cell death occurs by toxicity or by the induction of programmed cell death are warranted.

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P6.30

Production, purification and characterization of 10 *Arabidopsis* xyloglucan endotransglycosylases/hydrolases (XTHs)

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Xyloglucan endotransglucosylase/hydrolases (XTHs) are a class of enzymes that have the ability to cleave and rejoin xyloglucan chains and are considered to play a key role in both the construction and disassembly of the cell wall architecture.