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B!OCHEMICAL CHARACTERIZATION OF THE *aba2* AND *aba3* MUTANTS IN *ARABIDOPSIS THALIANA*.

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Intensive screening of *Arabidopsis thaliana* for abscisic acid (ABA) deficient mutants has resulted in the identification of fourteen mutants allelic to *aba1*. However, two new mutants, *aba2* and *aba3*, have recently been identified. Unlike *aba1*, there is no variation in the carotenoid composition of *aba2* and *aba3*. Thus, suggesting both mutants are impaired in the later steps of ABA biosynthesis. Cell-free extracts from the two mutants were assayed for their ability to convert the ABA precursors, xanthoxin and ABA-aldehyde, to ABA. Cell-free extracts of *aba2* and *aba3* were unable to convert xanthoxin to ABA. Extracts from *aba2* were able to oxidize ABA-aldehyde to ABA as efficiently as wild type extracts, indicating that this mutant is blocked in the conversion of xanthoxin to ABA-aldehyde. The *aba3* mutant was unable to oxidize ABA-aldehyde to ABA. Lesions at this step may result from a defect in the aldehyde oxidase apoprotein or a molybdenum cofactor (MoCo), which the aldehyde oxidase requires. The mutant *aba3* also lacked several additional enzyme activities, which require a MoCo, such as xanthine dehydrogenase. This research was supported, in part, by National Science Foundation grant No. IBN 9118377, and U.S. Department of Energy grant DE-FG02-91ER20021

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CAROTENOGENIC AND ABSCISIC ACID BIOSYNTHESIZING ACTIVITY IN A CELL-FREE SYSTEM

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Abscisic acid is considered an apocarotenoid formed by cleavage of a C-40 precursor and subsequent oxidation of xanthoxin and abscisic aldehyde. Confirmation of this reaction sequence is still awaited, and might best be achieved using a cell-free system capable of both carotenoid and abscisic acid biosynthesis. An abscisic acid biosynthesizing cell-free system, prepared from flavedo of mature orange fruits, was used to demonstrate conversion of farnesyl pyrophosphate, geranylgeranyl pyrophosphate and all-*trans*-β-carotene into a range of β,β-xanthophylls, xanthoxin, xanthoxin acid, 1',4'-*trans*-abscisic acid diol and abscisic acid. Identification of product carotenoids was achieved by high performance liquid chromatography and on-line spectral analysis of individual components together with co-chromatography. Putative C-15 intermediates and product abscisic acid were unequivocally identified by combined capillary gas chromatography-mass spectrometry. Kinetic studies revealed that β-carotene, formed from either farnesyl pyrophosphate or geranylgeranyl pyrophosphate, reached a maximum within 30 minutes of initiation of the reaction. Thereafter, β-carotene levels declined exponentially. Catabolism of substrate β-carotene into xanthophylls, putative abscisic acid precursors and product abscisic acid was restricted to the all-*trans*-isomer. However, when a combination of all-*trans*- and 9-*cis*-β-carotene in the ratio 1:1, was used as substrate formation of xanthoxin, xanthoxin acid, 1',4'-*trans*-abscisic acid diol and abscisic acid was enhanced. Biosynthetically prepared [^{14}C]-all-*trans*-violaxanthin and [^{14}C]-9'-*cis*-neoxanthin were used as substrates to confirm the metabolic interrelationship between carotenoids and abscisic acid. ("This research was supported by the Foundation for Research Development, Pretoria, South Africa").

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QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS OF SEED DORMANCY-BREAKING CHEMICALS

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The relative activity of dormancy-breaking chemicals of red rice (*Oryza sativa* L.) has been correlated with octanol/water partition coefficients (Log Ko/w). However, this model was chosen empirically. In this study, quantitative structure *vs.* biological activity relationships (QSAR) were analyzed more rigorously using Molecular Analysis Pro, a PC-based computational chemistry program. The entire set of alcohols, carboxylic acids, esters, aldehydes and ketones (n=42) could still be modeled to Log Ko/w similar to previously published results (n=25). A stable, two-variable model adding density as a bulk steric factor better described the data. While either monocarboxylic acids or primary alcohols also could be described by Log Ko/w, addition/substitution of descriptors for size, shape, density, or dipole moment provided improved QSAR models. Calculations of free energy changes per methylene group for homologous series of linear alcohols or monocarboxylic acids were 639 cal/mole and 236 cal/mole, respectively, for dormancy-breaking activity. The energy barrier for the dormancy-breaking activity of acids was substantially lower for acids, and the mechanisms of action between the two classes of compounds should be different. Preliminary models of lipophilicity-independent functional group effects will also be presented.

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PURIFICATION AND CHARACTERIZATION OF ALLENE OXIDE CYCLASE FROM CORN KERNELS

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Jasmonic acid and its methyl ester are known as signalling molecules in plants. The biosynthesis of jasmonic acid ensues by the oxylipin pathway starting with the lipoxygenase catalyzed peroxidation of linolenic acid to 13-hydroperoxy-linolenic acid which is further converted to 12,13-epoxy-octadecadienoic acid by allene oxide synthase. Subsequent cyclization of the epoxy fatty acid by allene oxide cyclase yields 12-oxo-phytodienoic acid and by reduction of the ring double bond and three rounds of β-oxidation jasmonic acid is formed. In order to investigate the regulation of jasmonic acid biosynthesis we enriched and characterized allene oxide cyclase. This enzyme has been purified to homogeneity by chromatography on hydrophopic interaction, hydroxyapatite and anion exchange columns. Comparison of the native molecular weight determined by gel filtration with the one on SDS-PAGE indicates a dimeric structure for this enzyme. Allene oxide cyclase has a broad pH-optimum of from 6-8 and a pI of 5.5 as estimated by chromatofocusing. The K_M value is 160 μM and V_{max} 40nmol and the enzyme is weakly stimulated by Ca^{2+} but strongly inhibited by the substrate analogue 12,13-epoxy-octadecenoic acid. Compounds known to prevent stress-induced jasmonic acid accumulation such as tetcyclacis or aspirin do not influence enzyme activity in vitro. This research was supported by Deutsche Forschungsgemeinschaft SFB 363 B-4.

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IN VITRO PROPAGATION OF STRELITZIA IMMATURE EMBRYOS

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Strelitzia propagation is a problem for many ornamental plant producers due to its slow development and flower production. It has been established that the use of embryo culture techniques may reduce the time of plant development. We have tested different media in order to optimize shoot and callus formation from strelitzia immature embryos. Our results indicate that the use of cysteine decreases media oxidative browning and that 0.3% sucrose is the best concentration for shoot development. Whereas, the use of GA_3 has no effect on shoot formation, 2mg/L 2,4D can induce callus development. We have also determined the best time to collect immature embryos based on polinization dates and seed morphological chracteristics. (This research was supported, in part, by CAPES, Brazil)

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EFFECT OF ABA AND GA_3 DURING GERMINATION OF *ANADENANTHERA PEREGRINA* (L.) SPEG. EMBRYOS

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We investigated the effects of ABA and GA_3 on the protein profile of embryos and the proteolytic activity in cotyledons during germination of *Anadenanthera peregrina*, a brazilian leguminous wood tree which has non-dormant seeds. Mature cotyledons and embryos were excised and incubated on a sucrose solution supplemented with ABA and/or GA_3. Our results indicated degradation of an embryo polypeptide with approximately molecular weight of 17 kD at 6 hours incubation. Degradation of this polypeptide seems to be blocked by ABA and/or GA_3 and this effect gradually decreases up to 30 hours incubation. Our data suggest that ABA and/or GA_3 blocks but does not induce the synthesis of this polypeptide and that this blockage may be caused by ABA inhibition of the activity of one or more proteases. In cotyledons, we observed a correlation between protein mobilization and enzyme activity (This research was supported, in part, by CNPq and EMBRAPA/CNPMS, Brazil)