

commercial Cyt P450 inhibitors (Cyt c, SKF 525A, aminobenzotriazole and kentoconazole) and CO strongly inhibited both enzyme activities, and the inhibited activity by CO was clearly recovered by illumination of blue light in the presence of O<sub>2</sub>, providing that both enzymes are Cyt P450 monooxygenases. Activity of 6-deoxocastasterone oxidase was also shown in the microsomal fraction in the presence of NADPH as a cofactor. The activity was strongly inhibited by additions of Cyt P450 inhibitors (Cyt c and SKF 525A) in the assay mixture, confirming that 6-deoxocastasterone oxidase in *Phaseolus* cells is also a Cyt P450 monooxygenase. Therefore, it is suggested that typhasterol 2a-hydroxylase, 6-deoxocastasterone oxidase and brassinolide synthase catalyzing the last two steps of the early- and late-C6-oxidation pathway to produce brassinolide are Cyt P450 monooxygenases in *Phaseolus* cells.

#### E206

### Brassinosteroids in *Phaseolus vulgaris* are Predominantly Biosynthesized by the Early C6-Oxidation Pathway.

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Identification of endogenous brassinosteroids (BRs) in *Phaseolus vulgaris* suggested that both the early and late C6-oxidation pathway for biosynthesis of brassinolide are contained in the plant. To determine which pathway is predominantly operative, activities of campestanol oxidase and campestanol 22R-hydroxylase catalyzing the first step in the early and late C6-oxidation pathway, respectively, were examined. The activity of campestanol oxidase was ca 3 times higher than that of campestanol 22R-hydroxylase, indicating

that campestanol is predominantly catalyzed by campestanol oxidase rather than campestanol 22R-hydroxylase. Next, activity of typhasterol 2a-hydroxylase and 6-deoxocastasterone oxidase responsible for the last step to produce castasterone, a direct precursor of brassinolide in the early and late C6-oxidation pathway, respectively, were investigated. Typhasterol 2a-hydroxylase also showed 3 times higher activity than that of 6-deoxocastasterone oxidase, indicating that more castasterone is produced by typhasterol 2a-hydroxylase than 6-deoxocastasterone oxidase. Therefore, it is suggested that castasterone and brassinolide in the *Phaseolus* cells were predominantly biosynthesized by the early C6-oxidation pathway rather than the late C6-oxidation pathway.

#### E207

### Regulation of Gravicurvature by Malformin A1 in the Primary Root of Maize

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Malformin, a small family of cyclic pentapeptides, is an active plant growth regulator isolated from the fungus *Aspergillus niger*. It has been known that malformin induces root curvature and ethylene mediated responses. We studied the action of the purified malformin A1 in the gravitropic response, and examined the possibility that this response might be related to the ethylene. Primary roots pretreated with malformin A1 vertically were placed in a humidified box in the horizontal position, and the curvature was measured using a CCD camera and a time-lapse video cassette recorder. The gravistimulated curvature was

delayed in the  $10^{-6}$  M malformin A1 pretreated roots compared to the control. To explain the role of ethylene in the gravicurvature, we measured the curvature in the presence of ethylene production regulators such as IAA, ACC or cobalt ions. And these results suggested that the inhibition of curvature by malformin A1 might be mediated with ethylene production and the curvature was an inverse proportion to the ethylene production. These results suggested that the gravicurvature might be required the proper internal ethylene level in the primary root of maize.

**E208**

### Effect of Brassinosteroid on the Ethylene Production in the Primary Root of Maize

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Brassinosteroid (BR), isolated from the rape pollen, has been known to regulate the growth and development of plants in the lower concentrations like plant hormones. To elucidate the action of BR, we studied the effect of ethylene production in the primary root of maize in the presence of BR. The ethylene production was increased by the treatment of BR, and this stimulation was proportional to the concentrations of BR. And the stimulated ethylene production was inhibited by the treatment of cobalt ions. Further, the activity of ACC oxidase was stimulated by BR applications. These data suggested that BR stimulated the ethylene production in the conversion step of ACC to ethylene that is regulated by ACC oxidase in the primary roots of maize.

**E209**

### Protective Roles of Exogenous Polyamines against Paraquat Toxicity in Radish Cotyledons

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The effect of exogenously applied polyamines (putrescine, spermidine, and spermine) in reducing the paraquat toxicity on radish (*Raphanus sativus* L. cv. Taewang) cotyledons was investigated to elucidate the physiological role of polyamines in plant oxidative stress resistance. In radish cotyledons, the superoxide-generating paraquat treatment (50 mM) caused a significant oxidative damage accompanying the losses of chlorophyll, carotenoid, and soluble protein. Also, the fresh weight of cotyledons was conspicuously decreased. However, polyamine pretreatments protected the radish cotyledons from paraquat-induced damages. Moreover, different polyamines led to different levels of protection against paraquat toxicity with spermidine (1 mM) being the most effective. The analysis of antioxidant enzymes in response to polyamine treatments showed that whereas putrescine and spermine treatments did not cause any increase in catalase, ascorbate peroxidase and guaiacol peroxidase activities, there were significant increases in catalase and ascorbate peroxidase activities after 1 day of spermidine treatment. It is suggested that the pretreatment of radish cotyledons with 1 mM spermidine among polyamines may induce antioxidant enzymes which lead to increased paraquat tolerance.

**E210**

### Genomic Structure of Canaline-dependent Ornithine Carbamoyltransferase Gene from