endocytosis. There are vitellogenin (Vg), lipophorin (Lp), cathepsin B-like protease (VCB), vitellogenic carboxypeptidase (VCP) as four major yolk proteins. For the research of the characterization of VCP receptor, VCP, 53 kDa, was purified effectively by chromatofocusing and Con A Sepharose affinity chromatography from mosquito ovaries, and had two bands separated by 4-15% gradient native PAGE.

E201

Chemical Induction of Cytochrome p450s in the Liquid Cultured Cells of Jerusalem artichoke (*Helianthus tuberosus* L.)

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Cytochrome p450 inducers phenobarbital and aminopyrine were examined for their effect on liquid cultured cells of Jerusalem artichoke (*Helianthus tuberosus* L.). Cytochrome p450 content and, to a lesser extent, the activity of t-cinnamate 4-hydroxylase were induced by the treatment of 8 mM phenobarbital to 10-day old cultured cells. On contrast aminopyrine, which is well known p450 inducer in intact plant system, did not significantly affected on both cytochrome p450 content and cinnamate 4-hydroxylase activity. Interestingly cultured Jerusalem artichoke cells was found to have a noticeable amount of cytochrome p420, which was not detected in Tulip bulb. And treatment of chemicals markedly increased the content of cytochrome p420 in Jerusalem artichoke comparing to that in control. Apparently this enhancement could arise by a specific manner since the activity of peroxidase, another heam protein, was not affected, or even inhibited, by the treatment of chemicals.

E202

Brassinosteroids in Shoots of Maize Seedlings are Biosynthesized by the Late C6-oxidation Pathway

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GC-MS/SIM analyses of 4-demethylsterols in shoots of maize seedlings revealed that campesterol and campestanol, biosynthetic precursors for the early and late C6-oxidation pathway for brassinosteroids (BRs) biosynthesis, were contained in the shoots. To determine which pathway is operative, endogenous BRs in the shoots were examined. 6-Deoxocastasterone and castasterone, members of the late C6-oxidation pathway, were successfully identified, but any members of the early C6-oxidation pathway were not identified, suggesting that BRs in the shoot were biosynthesized by the late C6-oxidation pathway. In the presentation, confirmatory results established by enzymatic conversions of BRs included in the late C6-oxidation pathway of the maize shoots will be also discussed.

E203

Brassinolide and (26, 28-$^2H_6$)-brassinolide are Differently Demethylated by Loss of C-26 and C-28, Respectively, in *Marchantia polymorpha*

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Metabolism of brassinolide in *Marchantia polymorpha* was investigated by use of in vivo suspension cultured cells. GC-MS analysis of metabolites derived from nonlabelled brassinolide and [26, 28-$^2H_6$]-brassinolide revealed that brassinolide was converted to 26-norbrassinolide while [26, 28-$^2H_6$]-brassinolide to [26,-$^2H_3$]-28-norbrassinolide. It seems that *Marchantia* cells recognized [26, 28-$^2H_6$]-brassinolide as a xenobiotic rather than brassinolide and deteriums attached to C-28 significantly affect demethylation reaction due to isotopic effect. Thus, demethylation of brassinolide in planta seems to proceed by loss of C-26 rather than C-28. The present finding is the first evidence for demethylation metabolism of brassinosteroids. The biological activity of 26-norbrassinolide was 10-fold reduced as examined by the rice lamina inclination test. However, because of its high biological activity, it remains difficult to conclude straightforward whether or not C-26 demethylation serves as an important deactivation process of brassinolide.

E204

Biosynthesis of Brassinosteroids in Primary Roots of Maize

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We have demonstrated that brassinosteroids (BRs) are involved in gravitropic response of primary roots of maize. To examine whether BRs are indeed biosynthesized in the roots, identification of BRs and their biosynthetic precussors and occurence of biosynthetic enzymes in the root were carried out. GC-MS/SIM analyses revealed that 6-deoxocastasterone and castasterone, members of the late C6-oxidation pathway, were contained in the roots. In addition, presence of campesterol and campestanol, biosynthetic precursors of 6-deoxocastasterone and castasterone, were demonstrated in the roots. These suggested that a biosynthetic pathway from campesterol to castasterone via campestanol and 6-deoxocastasterone, namely the late C6-oxidation pathway, is present to produce BRs in the roots. A microsomal fraction obtained from the roots successfully catalyzed conversion of 6-deoxocastasterone to castasterone, which provided the presence of castasterone oxidase in the roots. Taken together, it is clear that BRs are biosynthesized via the late C6-oxidation pathway in primary roots of maize to show gravitropic curvature.

E205

Cytochrome P450 Monooxygenases Catalyze Biosynthesis of Brassinolide from Typhasterol or 6-Deoxocastasterone via Castasterone in *Phaseolus vulgaris* Cells

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A microsomal fraction prepared from cultured cells of *Phaseolus vulgaris* catalyzed conversions of typhasterol to brassinolide intermediated by castasterone. This indicates that typhasterol 2a-hydroxylase and brassinolide synthase catalyzing the conversion of typhasterol to castasterone and castasterone to brassinolide, respectively, are integral proteins in the membrane of the cells. For the activity, both typhasterol 2a-hydroxylase and brassinolide synthase required NADPH and O_2. Furthermore,