Biosynthesis and Biodegradation of Brassinosteroids, New Steroidal Plant Hormones, in Cultured Cells of *Phaseolus vulgaris*

Kim, Seong-Ki

Department of Life Science, Chung-Ang University, Seoul 156-756, Korea

Identification of endogenous brassinosteroids (BRs) and their biosynthetic precursors in *Phaseolus vulgaris* indicated that brassinolide, castasterone, typhasterol, teasterone, 6-deoxocastasterone, campestanol and campesterol are contained in the plant. These are members of the early- and/or late-C6 oxidation pathway for biosynthesis of brassinolide, suggesting that both pathways are included in the plant. To better understand the pathways, *in vitro* enzymatic conversions of BRs were investigated by cell-free systems prepared from suspension cultured cells of *P. vulgaris*.

The crude cytosolic enzyme solution prepared by centrifugal separation (supernatant at 120,000 x g) successfully catalyzed conversions of teasterone to typhasterol *via* 3-dehydroteasterone in the early C6-oxidation pathway and of 6-deoxoteasterone to 6-deoxotyphasterol *via* 6-deoxo-3-dehydroteasterone in the late C6-oxidation pathway. This indicated that enzymes responsible the reactions, namely teasterone dehydrogenase, 3-dehydroteasterone reductase, 6-deoxoteasterone dehydrogenase and 6-deoxo-3-dehydroteasterone reductase, respectively, are soluble enzymes in cytosol of the cells.

The microsomal enzyme solution (pellet at 120,000 x g) catalyzed the conversions of campestanol to 6-oxocampestanol *via* 6α-hydroxycampestanol in the early C6-oxidation pathway and to 6-deoxocathasterone in the late C6-oxidation pathway, indicating that campestanol oxidase and campesterol 22*R*-hydroxylase, respectively, are integral proteins in the membrane, most likely membrane of E.R. The microsomal solution also mediated conversions of typhasterol to brassinolide *via* castasterone in the early C6-oxidation pathway and of 6-deoxocastasterone to castasterone in the late C6-oxidation pathway, providing that enzymes catalyzing the reactions are bound to the E.R. membrane. Furthermore, activities of these enzymes were strongly inhibited by CO, N_2 (anaerobic condition), several cytochrome P450 inhibitors and removal of NADPH from the reaction solution. Therefore, three enzymes, namely typhasterol

2α-hydroxylase, brassinolide synthase and 6-deoxocastasterone oxidase were thought to be NADPH dependent cytochrome P450 monooxygenases.

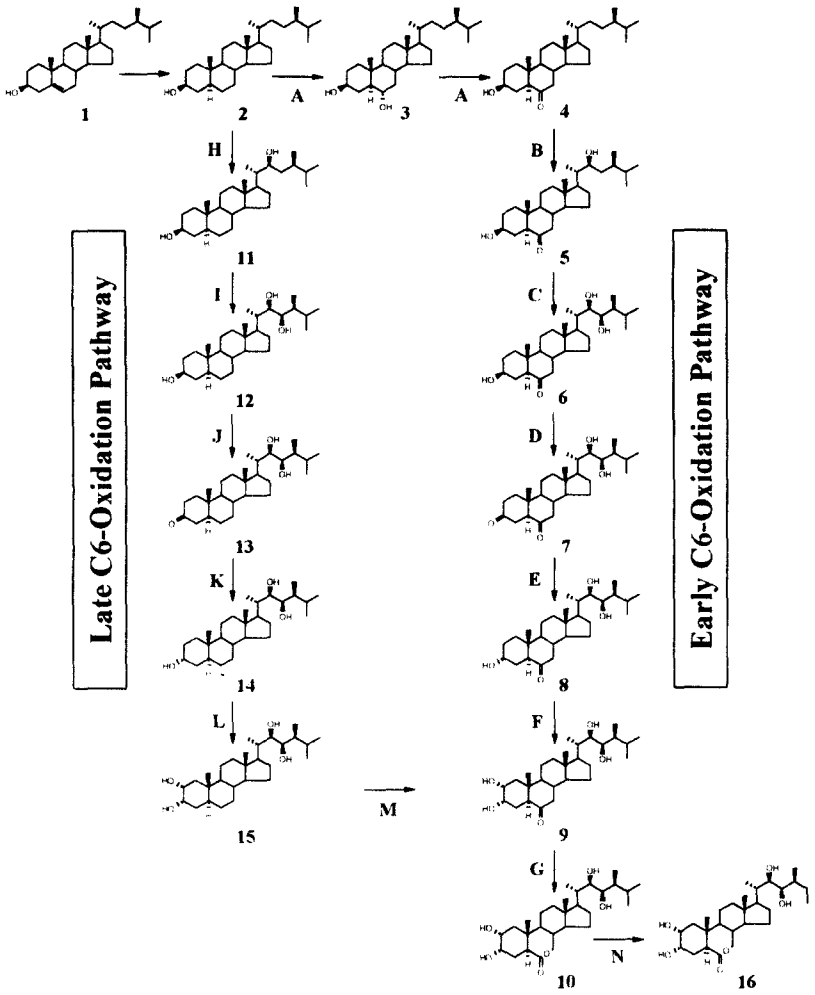

Figure : Pathways for brassinosteroids biosynthesis and biodegradation in *Phaseolus* cells. 1: campesterol, 2: campestanol, 3: 6α-hydroxycampestanol, 4: 6-oxocampestanol, 5: cathasterone, 6: teasterone, 7: 3-dehydroteasterone, 8: typhasterol, 9: castasterone, 10: brassinolide, 11: 6-deoxocathasterone, 12: 6-deoxoteasterone, 13: 6-deoxo-3-dehydroteasterone, 14: 6-deoxotyphasterol, 15: 6-deoxocastasterone, 16: 26-norbrassinolide, A: campestanol oxidase, B: 6-oxocampestanol 22*R*-hydroxylase, C: cathasterone 23*R*-hydroxylase, D: teasterone dehydrogenase, E: 3-dehydroteasterone reductase, F: typhasterol 2α-hydroxylase, G: brassinolide synthase, H: campestanol 22*R*-hydroxylase, I: cathasterone 23*R*-hydroxylase, J: 6-deoxoteasterone dehydrogenase, K: 6-deoxo-3-dehydroteasterone reductase, L: 6-deoxotyphasterol 2α-hydroxylase, M: 6-deoxocastasterone oxidase.

In addition to the biosynthesis of BRs, biodegradation of brassinolide, the end product of the both biosynthetic pathways, was examined. *In vivo* feeding experiment and *in vitro* enzymatic conversion revealed that brassinolide was converted into a biologically less active catabolite, 26-demethylbrassinolide, which was catalyzed by brassinolide 26-demethylase in the cytosol of *Phaseolus* cells.

Based on the results, intracellular movement of BRs during biosynthesis and biodegradation in *Phaseolus* cells and usefulness of these cell-free systems to develop commercially valuable compounds will be discussed in the presentation.