

ปริมาณไอโซฟลาโวนอยด์หลักและโปรตีโอมิกสึโนไบและหัวกวาวเครือขาว *Pueraria mirifica*



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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต

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MAJOR ISOFLAVONOID CONTENTS AND PROTEOMICS IN LEAVES AND TUBERS OF
Pueraria mirifica

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A Dissertation Submitted in Partial Fulfillment of the Requirements
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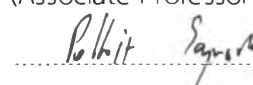
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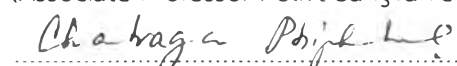
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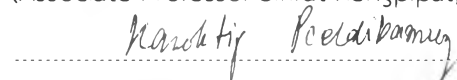
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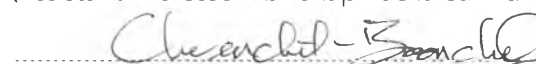
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จุฬามาศ จิ่งสุขเจริญ : ปริมาณไอโซฟลาโวนอยด์หลักและโปรตีโอมิกส์ในใบและหัว
 กวาวเครือขาว *Pueraria mirifica*. (MAJOR ISOFLAVONOID CONTENTS AND
 PROTEOMICS IN LEAVES AND TUBERS OF *Pueraria mirifica*) อ.ที่ปรึกษา
 วิทยานิพนธ์หลัก: รศ. ดร. วิชัย เชิดชูวิศาตร์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. ดร.
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การศึกษาการเปลี่ยนแปลงของสารไอโซฟลาโวนอยด์หลักได้แก่ พิวราริน (puerarin)
 ไดด์ซีน (daidzin) เจนิสทิน (genistin) ไดด์ซีน (daidzein) และเจนิสไตน์ (genistein) ของต้น
 กวาวเครือขาว *Pueraria mirifica* ทั้งสามสายพันธุ์ (PM-III, PM-IV and PM-V) ในแปลงปลูก
 ทดลองที่จังหวัดราชบุรีทุกเดือนเป็นระยะเวลา 1 ปี โดยใช้วิธีวิเคราะห์ลิควิดโครมาโตกราฟีแบบรี
 เวอร์สเฟส (RP-HPLC) พบว่าสัดส่วนปริมาณสาร พิวราริน ไดด์ซีน เจนิสทิน และ ไดด์ซีนในใบ
 กวาวเครือขาวขึ้นกับสายพันธุ์และสัมพันธ์กับอุณหภูมิและปริมาณน้ำฝน การศึกษารูปแบบของ
 โปรตีนที่แสดงออกในหัวและใบกวาวเครือขาวทั้ง 3 สายพันธุ์โดยใช้การศึกษาโปรตีโอมิกส์เพื่อ
 เข้าใจวิถีสังเคราะห์การไอโซฟลาโวนอยด์มากขึ้น วิเคราะห์โปรตีนโดยใช้ฟีนอลเป็นวิธีที่เหมาะสมใน
 การสกัดโปรตีนจากกวาวเครือขาวซึ่งให้ปริมาณโปรตีนและความชัดของแถบโปรตีนมากกว่า
 วิธีการสกัดแบบ ทริส-ไฮโดรคลอริก (Tris-HCl) และไตรคลอโรเอซิติกเอซิด-อะซีโตน (TCA-
 Acetone) โปรตีนที่ได้จะถูกแยกออกจากกันโดยใช้วิธีโพลีอะคริลาไมด์เจลอิเล็กโตรโพลีซิสแบบ 2
 มิติ (2D-PAGE) และวิเคราะห์โปรตีนที่ได้ด้วยเทคนิคแมสสเปคโตรเมตรี การศึกษานี้ได้เปิดเผย
 รูปแบบโปรตีนที่เป็นลักษณะเฉพาะในหัวและใบกวาวเครือขาวซึ่งแบ่งตามหน้าที่ของโปรตีนได้
 ดังนี้ 1. โครงสร้างของเซลล์ 2. การป้องกัน 3. เมตาบอลิซึม 4. การสังเคราะห์โปรตีน 5. โปรตีน
 สะสม 6. การตอบสนองต่อความเครียด 7. การขนส่ง และ 8. โปรตีนไม่ทราบหน้าที่ การเก็บเกี่ยว
 ในแต่ละฤดูมีการแสดงออกของโปรตีนที่แตกต่างกัน ปริมาณโปรตีนในหัวกวาวเครือขาวเพิ่มขึ้นใน
 หน้าหนาวขณะที่ใบกวาวเครือขาวพบโปรตีนเพิ่มขึ้นในหน้าร้อน พบโปรตีนที่เกี่ยวข้องกับการ
 สร้างสารทุติยภูมิเช่น กลูตาไธโอนเอสทรานสเฟอเรส และ กลูตาไธโอนรีดักเทส เป็นต้น นอกจากนี้
 ยังพบโปรตีนที่เกี่ยวข้องกับการสร้างสารไอโซฟลาโวนอยด์ ได้แก่ ซาลิโคไนโอโซเมอเรส (CHI) ไอโซฟ
 ลาโวนซินเธส (IFS) ไฮโดรอะซีลทรานสเฟอเรส (HAT) ยูริดีนไทโรฟอสเฟสกลูโคซิลทรานสเฟอเรส (UGT) และไอ
 โซฟลาโวนรีดักเทส (IFR) โปรตีนเหล่านี้มีความสัมพันธ์กับปริมาณสารไอโซฟลาโวนอยด์อย่างมี
 นัยสำคัญ จากผลการศึกษาทำให้เข้าใจกลไกการผลิตสารทุติยภูมิในหัวและใบกวาวเครือขาว ไม่
 เพียงแต่การแสดงออกของโปรตีนแต่ยังมีความสัมพันธ์กับการสร้างสารทุติยภูมิในฤดูกาลต่างๆ

สาขาวิชา เทคโนโลยีชีวภาพ

ปีการศึกษา 2556

ลายมือชื่อนิสิต

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5173807723 : MAJOR BIOTECHNOLOGY

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JUTARMAS JUNGSUKCHAROEN: MAJOR ISOFLAVONOID CONTENTS AND PROTEOMICS IN LEAVES AND TUBERS OF *Pueraria mirifica*. ADVISOR: ASSOC. PROF. WICHAJ CHERDSHEWASART, Ph.D., CO-ADVISOR: ASSOC. PROF. POLKIT SANGVANICH, Ph.D., CHANTRAGAN PHIPHOBMONGKOL, Ph.D., 202 pp.

The monthly variation of major isoflavonoid contents namely puerarin, daidzin, genistin, daidzein and genistein from 3 different cultivars of *Pueraria mirifica* (PM-III, PM-IV and PM-V) in field trial at Ratchaburi province were investigated by the aid of RP-HPLC analysis. The proportions and net levels of puerarin, daidzin, genistin and daidzein in *P. mirifica* leaves were found to depend on the plant cultivar and to correlate with cultivation temperature and rainfall amount. The determination of protein pattern in *P. mirifica* tubers and leaves were performed via proteomics approach to better understanding the isoflavonoid biosynthesis pathway. The phenolic method was the suitable protein extraction due to the high protein yield and clearly separated protein band when comparing with other methods (Tris-HCl and TCA-Acetone extraction). Proteins were separated by using 2D-PAGE and mass spectrometry technique. The novel proteome patterns in tubers and leaves were revealed according to 8 major protein functions; 1) cell structure 2) defense 3) metabolism 4) protein synthesis 5) storage protein 6) stress response 7) transportation and 8) unidentified protein. The seasonal harvesting was expressed in difference protein pattern. Protein expression in tuber was up-regulated in winter whereas, in leaf was up-regulated in summer. Many proteins involved in secondary metabolite also found such as glutathione S-transferase and glutathione reductase. Furthermore, protein involved in pathway of bioactive isoflavonoids biosynthesis were expressed; chalcone isomerase (CHI), isoflavone synthase (IFS), cytochrome p450, UDP-glycosyltransferase (UGT) and isoflavone reductase (IFR). Moreover, these proteins also have significant correlated with isoflavonoid contents. From these results, the more understanding of the plant secondary metabolite production in *P. mirifica* tuber were recorded and not only protein expression but also relation of secondary metabolites production in different seasonal harvesting.

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