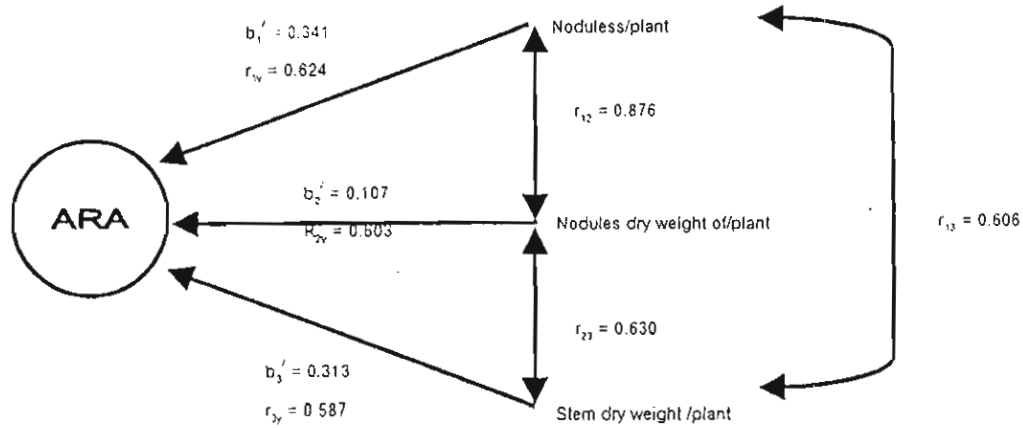
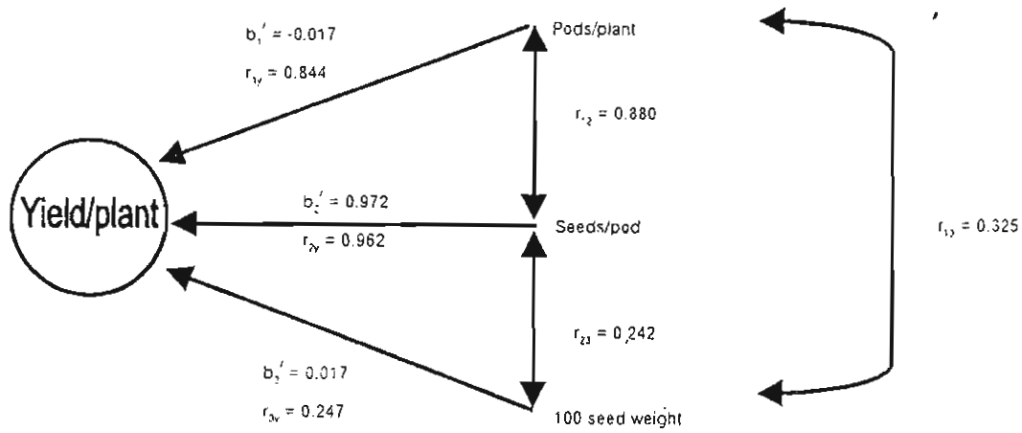
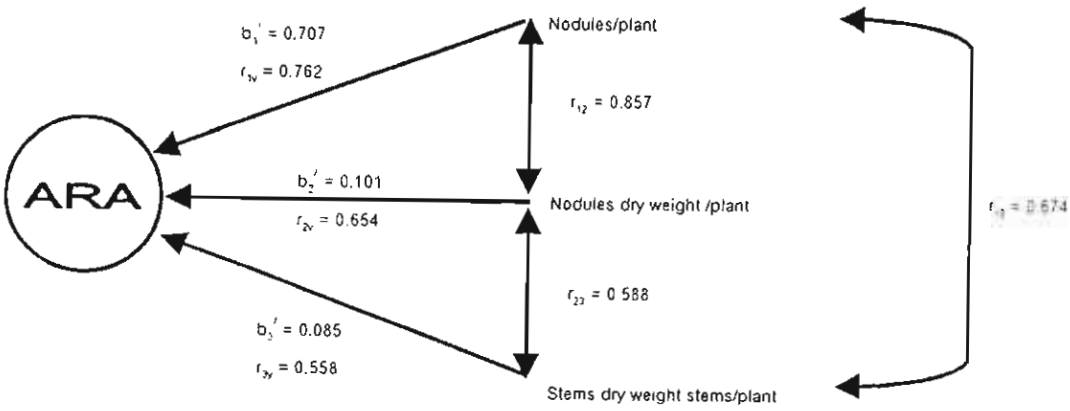


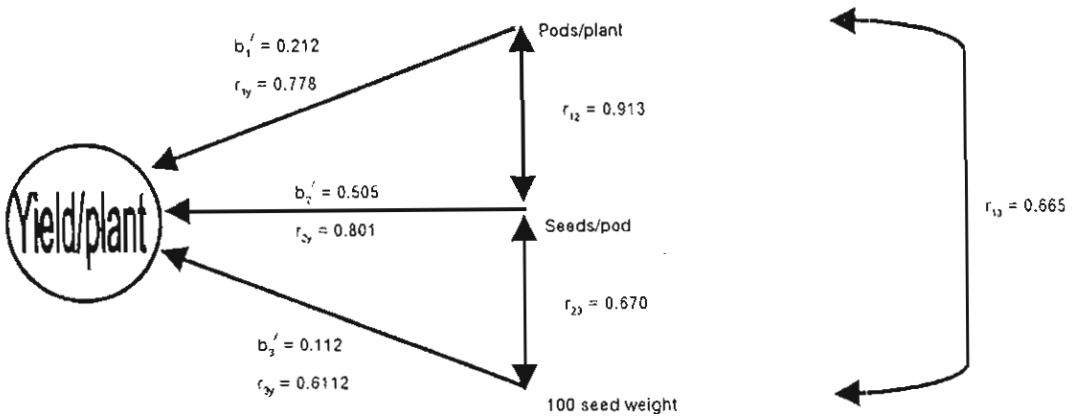
ภาพที่ 1. แสดงค่าสหสัมพันธ์ระหว่างองค์ประกอบการตรึงไนโตรเจน จากการวิเคราะห์แพทโคเอฟฟิเซียน ของผลการทดลองในถังซีเมนต์



ภาพที่ 2. แสดงความสัมพันธ์ระหว่างผลผลิตกับองค์ประกอบผลผลิต จากการวิเคราะห์แพทโคเอฟฟิเซียน ของผลการทดลองในถังซีเมนต์



ภาพที่ 3. แสดงความสัมพันธ์ระหว่างองค์ประกอบการตรึงไนโตรเจนของถั่วเหลืองลูกชั่วที่ 2 โดยการวิเคราะห์แพทโคเอฟฟิเซียน



ภาพที่ 4. แสดงความสัมพันธ์ระหว่างผลผลิตกับองค์ประกอบของผลผลิตของถั่วเหลืองลูกชั่วที่2 โดยการวิเคราะห์แพทโคเอฟฟิเชียน

สัมมนาวิชาการพันธุศาสตร์ ครั้งที่ 13

พันธุศาสตร์กับการพัฒนาที่ยั่งยืน

Genetics and Sustainable Development

5–7 มิถุนายน 2546

การจำแนกพันธุกรรมถั่วเหลืองในภาคเหนือของประเทศไทยโดยใช้เครื่องหมายโมเลกุลแบบ SSR

Identification of Soybean Germplasm in Northern Thailand Using SSR Markers

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Pornpan Pooprompan[1], Jun Abe[2], and Peerasak Srinives[3]

บทคัดย่อ

ในการศึกษาขนาดของอัลลีล Simple Sequence Repeat (SSR) 9 ตำแหน่ง ในถั่วเหลือง 14 ตัวอย่าง (accession) ที่เก็บรวบรวมจากเขตภูมิศาสตร์ที่แตกต่างกันในภาคเหนือของประเทศไทย และสายพันธุ์แนะนำของทางราชการ 7 สายพันธุ์ ร่วมกับการจำแนกลักษณะทางสัณฐานวิทยา พบว่า พันธุกรรมของถั่วเหลืองที่นำมาศึกษาในครั้งนี้มีจำนวนอัลลีลเฉลี่ย 4.7 ค่าเฉลี่ยดัชนีความหลากหลายทางพันธุกรรม 0.67 การจัดกลุ่มด้วยวิธี Unweighted Pair-Group Method of Arithmetic Average (UPGMA) โดยใช้ความถี่ของอัลลีล พบว่า พันธุ์พื้นเมือง (ถั่วเน่า) ที่เก็บมาจากหมู่บ้านชาวเขา และสายพันธุ์แนะนำ พันธุ์สุโขทัย 1 และสุโขทัย 2 สามารถแยกออกจากสายพันธุ์อื่นๆ ได้อย่างชัดเจน พันธุ์สจ.5 และตัวอย่างที่เก็บมาจากจังหวัดแม่ฮ่องสอน ถูกจัดอยู่ในกลุ่มเดียวกัน ซึ่งเกิดจากการที่มีอัลลีลร่วมกัน ส่วนพันธุ์ สจ.4 และเชียงใหม่ 60 มีความใกล้เคียงกันกับตัวอย่างที่เก็บมาจากอำเภอแม่แจ่ม จังหวัดเชียงใหม่ ซึ่งสอดคล้องกับลักษณะทางสัณฐานวิทยาอีกด้วย อาจสรุปได้ว่า ถั่วเหลืองที่เก็บรวบรวมมาส่วนมากมีความใกล้ชิดกับพันธุ์แนะนำ สจ.2 สจ.4 สจ.5 และเชียงใหม่ 60 และยังพบว่า พันธุ์พื้นเมืองมีความแตกต่างจากพันธุกรรมในกลุ่มที่นำมาศึกษาครั้งนี้ การที่ถั่วเหลืองที่เก็บมาส่วนใหญ่มีความคล้ายคลึงกับพันธุ์ สจ.2 สจ.4 และ สจ.5 น่าจะชี้ให้เห็นว่า ตัวอย่างเหล่านี้เป็นพันธุ์ที่แนะนำในแหล่งนั้นๆ หรือเป็นพันธุ์ที่มีการปะปนกันระหว่างพันธุ์แนะนำกับพันธุ์พื้นเมือง ซึ่งเป็นไปได้ว่า ตัวอย่างเหล่านี้พัฒนามาจากการผสมข้ามระหว่างพันธุ์แนะนำกับพันธุ์พื้นเมือง หรือเกิดจากการนำสายพันธุ์ถั่วเหลืองจากแหล่งอื่นๆ มาปลูก

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Abstract

Fourteen accessions of soybean collected from different geographic regions in Northern Thailand and seven released cultivars were analyzed for allele size profiling at 9 simple sequence repeat (SSR) loci. Morphological characters were also characterized in the experimental field. The SSR loci produced an average of 4.7 alleles with mean gene diversity of 0.67. Cluster analysis of allelic frequencies by the UPGMA method clearly separated landraces (Thua Nao) which were collected at a hill tribe village and released cultivars, SK1 and SK2, from the others. The accessions collected from Mae Hong Son Province and released cultivar SJ5 formed a single cluster. This group share a common SSR allele. The released cultivars CM60 and SJ4 were closely related to accessions collected from Mae Chaem District, Chiang Mai Province, and these relationship due mainly to their similarity in morphological traits. The results obtained from SSR analysis and morphological data indicated that most accessions are close to the cultivars released in this area, viz. SJ2, SJ4, SJ5 and CM60. The native accessions are quite different from the remaining accessions and cultivars. The similarity of most accessions to SJ2, SJ4 and SJ5 indicated that the accessions were the cultivars released in this area themselves or the mixtures of the cultivars and landraces. In addition, there is a possibility that these accessions were either the derivatives from natural hybridization between the landraces and the released cultivars, or the advanced breeding lines/cultivars from unknown sources.

คำนำ

ถั่วเหลืองเป็นพืชที่มีการเพาะปลูกในแถบเอเชียตะวันออกและตะวันออกเฉียงใต้มาเป็นเวลานานแล้ว จึงเป็นแหล่งกำเนิดของพันธุ์พื้นเมืองจำนวนมาก อันเป็นผลมาจากการปรับตัวเข้ากับสภาพแวดล้อมและความหลากหลายของรูปแบบการนำถั่วเหลืองมาประกอบอาหาร ข้อมูลของความหลากหลายและความสัมพันธ์ทางพันธุกรรม สามารถนำไปใช้ในการคัดเลือกพ่อแม่พันธุ์ เพื่อการปรับปรุงพันธุ์อย่างมีประสิทธิภาพมากที่สุด ในการประเมินความหลากหลายทางพันธุกรรม สามารถใช้วิธีการทางด้านเครื่องหมายโมเลกุลร่วมกับลักษณะทางสัณฐานวิทยา เครื่องหมายโมเลกุลชนิด Microsatellite หรือที่เรียกอีกอย่างหนึ่งว่า Simple Sequence Repeat (SSR) ซึ่งเป็นดีเอ็นเอที่มีลำดับเบสซ้ำกันอย่างต่อเนื่อง ได้ถูกนำมาใช้อย่างกว้างขวางในการทำแผนที่ยีน การจำแนกพันธุ์พืช และการศึกษาทางด้านพันธุศาสตร์ประชากร เนื่องจากเป็นเครื่องหมายโมเลกุลนี้มีระดับของความแปรปรวนสูง พบกระจายอยู่ทั่วไปในจีโนมของสิ่งมีชีวิตชั้นสูง สามารถตรวจสอบได้ง่าย ตัวอย่างเช่น Cregan *et al.*, (1999) ได้ทำแผนที่เครื่องหมายโมเลกุลแบบ SSR ซึ่งมีมากกว่า 600 ตำแหน่งบน 20 กลุ่มลิงเกจของจีโนมถั่วเหลือง Tanya *et al.*, (2001) ในการจัดกลุ่มความสัมพันธ์ระหว่างสายพันธุ์ถั่วเหลือง

วัตถุประสงค์ของการวิจัยครั้งนี้เพื่อศึกษาความสัมพันธ์ทางพันธุกรรมระหว่างถั่วเหลืองที่เก็บรวบรวมมาจากแหล่งทางภูมิศาสตร์ที่แตกต่างกันในเขตภาคเหนือ กับถั่วเหลืองสายพันธุ์แนะนำของทางราชการ โดย

จำแนกความแตกต่างของขนาดอัลลีล SSR ที่พบใน 9 ตำแหน่งบนกลุ่มลิงเกจต่างกัน

อุปกรณ์และวิธีการ

ถั่วเหลืองที่ใช้ในการทดลอง

สำรวจและเก็บรวบรวมพันธุ์ถั่วเหลืองจากแหล่งปลูกต่างๆ ในเขตภาคเหนือ ได้แก่ จังหวัดเชียงราย เชียงใหม่ แม่ฮ่องสอน พะเยา และแพร่ จำนวน 17 ตัวอย่าง (accession) และสายพันธุ์แนะนำของทางราชการ จำนวน 7 พันธุ์ ได้แก่ พันธุ์นครสวรรค์ 1 สจ.2 สจ.4 สจ.5 เชียงใหม่ 60 สุโขทัย 1 และสุโขทัย 2 ทำการศึกษาลักษณะทางสัณฐานวิทยาโดยปลูกทดสอบและบันทึกข้อมูลต่างๆ

การวิเคราะห์เครื่องหมายโมเลกุลแบบ SSR

เครื่องหมายโมเลกุลแบบ SSR ที่มี 3 นิวคลีโอไทด์เรียงซ้ำกันอย่างต่อเนื่อง จำนวน 9 ตำแหน่ง บนลิงเกจกลุ่มต่างๆ ของถั่วเหลือง (ตารางที่ 1) นำมาใช้ศึกษาพันธุกรรมของประชากรถั่วเหลืองที่เก็บรวบรวมมาครั้งนี้ โดยติดฉลากปลายด้านหนึ่งของไพรเมอร์ (forward primer) ด้วยสีฟลูโอเรสเซนต์ (fluorescent dye) แต่ละชนิด ดังนี้ 6-FAM (blue), HEX (green) หรือ NED (yellow) สกัดดีเอ็นเอจากใบอ่อนของแต่ละประชากรที่เก็บรวบรวมมาด้วยวิธีของ Doyle and Doyle (1990) ทำปฏิกิริยาพีซีอาร์ปริมาตร 20 μl ซึ่งประกอบไปด้วย 30 ng ของ genomic DNA, 0.25 μM of dNTP, 0.5 units *Taq* polymerase (TaKaRa, Japan), 1 × PCR buffer (10 mM of Tris-HCl pH 8.3, 50 mM of KCl, 1.5 mM $MgCl_2$) ทำปฏิกิริยาพีซีอาร์โดยใช้เครื่อง GeneAmp PCR system 9700 (Perkin Elmer/Applied Biosystems, Foster City, USA) จำนวน 32 รอบ

ตารางที่ 1 ช่วงของขนาดอัลลีล จำนวนของอัลลีล และดัชนีความหลากหลายทางพันธุกรรมของถั่วเหลืองสายพันธุ์แนะนำ และถั่วเหลืองที่เก็บรวบรวมจากภาคเหนือของประเทศไทย

Locus	Linkage group	Allele size range (bp)	No. of alleles	Gene diversity (H)
Satt 600	D1b+W	158-221	4	0.49
Satt 002	D2	111-147	6	0.92
Satt 156	L	190-232	5	0.75
Satt 063	B2	106-163	5	0.66
Satt 180	C1	216-279	6	0.88
Satt 038	G	157-199	5	0.75
Satt 262	O	228-282	3	0.51
Satt 197	B1	135-204	5	0.56
Satt 228	A2	215-254	3	0.50
Mean			4.7	0.67

ใช้อุณหภูมิและเวลาเป็นวินาที ดังนี้ 94°C 30 s, 46°C 30 s, 68°C 30 s นำ PCR products ที่ได้ในแต่ละไพ
เมอร์ที่ติดฉลากด้วยสีฟลูโอเรสเซนต์แต่ละชนิด และมีขนาดของอัลลีลที่แตกต่างกัน มาผสมรวมกัน ดังนี้ 1.5
μl of 6-FAM label, 5.0 μl of HEX label และ 1.5 of NED lebel และน้ำกลั่น 20 μl แล้วแบ่งปริมาตร 1.5
μl มาผสมรวมกับ loading buffer ที่ประกอบด้วย DNA ขนาดมาตรฐาน (internal size standard; GeneScan
500) ที่ติดฉลากด้วย ROX (red) หลังจาก denaturation ที่อุณหภูมิ 94°C เป็นเวลา 5 นาที จึงนำมาทำ
อีเล็คโตรโฟรีซีสด้วยเครื่อง sequencer ABI 377 (Perkin Elmer/Applied Biosystems. Foster City. USA) และ
วิเคราะห์ขนาดของอัลลีลด้วยโปรแกรม GeneScan

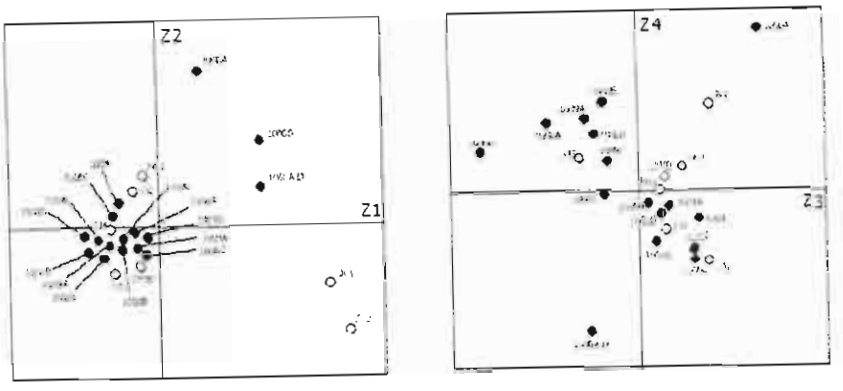
วิเคราะห์ค่าดัชนีความหลากหลายทางพันธุกรรม (genetic diversity index. H) โดยใช้ค่าความถี่
ของแต่ละอัลลีลบน SSR แต่ละตำแหน่ง ด้วยวิธีของ Nei's unbiased statistics (1987) ดังนี้ $H = 1-\sum Pi^2$ โดยที่
Pi คือความถี่ของอัลลีลที่ i วิเคราะห์ จากนั้นจัดกลุ่ม (cluster analysis) ความแตกต่างทางพันธุกรรมด้วยวิธี
Unweighted Pair-Group Method of Arithmetic Average (UPGMA)

ผลและวิจารณ์

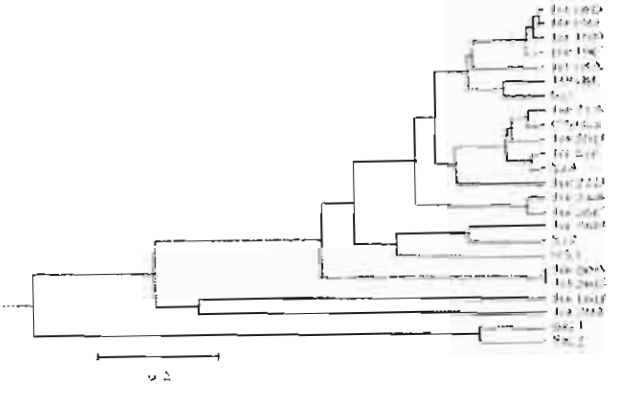
การวิเคราะห์เครื่องหมายโมเลกุลแบบ SSR ใน 9 กลุ่มลิงเกจ ของถั่วเหลืองที่เก็บรวบรวมมา
จำนวน 14 ตัวอย่าง และพันธุ์แนะนำของทางราชการ 7 พันธุ์ พบความหลากหลายของอัลลีลอยู่ในช่วง 3-6
อัลลีล มีค่าเฉลี่ย 4.7 โดยที่ locus Satt 180 มีจำนวนอัลลีลมากที่สุดเท่ากับ 6 อัลลีล locus Satt 262 และ locus
Satt 228 มีจำนวน อัลลีลน้อยที่สุดเท่ากับ 3 อัลลีล ค่าดัชนีความหลากหลายทางพันธุกรรม (H) อยู่ระหว่าง
0.49-0.92 โดยมีค่าเฉลี่ย 0.67

จากการวิเคราะห์ Principle Component Analysis โดยใช้ความถี่ของอัลลีลของ SSR ทั้ง 9 loci เพื่อ
ศึกษารูปแบบความแปรปรวน พบว่า มีการกระจายดังแสดงในภาพที่ 1 ใน component 1. 2. 3 และ 4 มีค่า
เท่ากับ 24.3 %. 14.2 %. 12.4 % และ 9.2 % ตามลำดับ ใน component Z1 และ Z2 แสดงให้เห็นว่าตัวอย่าง
10/20A. 10/16B. 10/26A และ 10/26D กับพันธุ์สุโขทัย และ สุโขทัย 2 แยกออกจากตัวอย่างและพันธุ์
แนะนำของทางราชการอื่นๆ อย่างชัดเจน ส่วนใน component Z3 และ Z4 พันธุ์ สจ.5 และตัวอย่าง 10/18A.
10/18B. 10/19A. 10/19B. 10/19C และ 10/16B ซึ่งเก็บมาจากบริเวณจังหวัดแม่ฮ่องสอน สามารถแยกออก
มาจากพันธุ์อื่น เนื่องมาจากพันธุกรรมในกลุ่มนี้ให้อัลลีลเดียวกันขนาด 205 bp ที่ locus Satt 150 และ 151 bp
ที่ locus Satt 063 ทำให้การจัดกลุ่มของพันธุ์ สจ.5 และตัวอย่างในกลุ่มนี้แยกออกมาเป็นกลุ่มเดี่ยวๆ ดัง
แสดงในภาพที่ 2 โดยเฉพาะอย่างยิ่ง พบ locus Satt 063 ซึ่งเป็นอัลลีลขนาด 151 bp ในทุกตัวอย่างของกลุ่มนี้
แต่ไม่พบในพันธุ์ สจ.5

จากการวิเคราะห์เครื่องหมายโมเลกุลแบบ SSR ชี้ให้เห็นว่าถั่วเหลืองที่เก็บรวบรวมมามีบางตัวอย่าง
ได้แก่ 10/16B, 10/20A. 10/26A และ 10/26D แตกต่างไปจากสายพันธุ์แนะนำและตัวอย่างอื่น ๆ ในขณะที่
ตัวอย่างส่วนมากสามารถจัดอยู่ในกลุ่มพันธุ์แนะนำ พันธุ์ สจ.2 สจ.4 และ สจ.5 จากการวิเคราะห์
เครื่องหมายโมเลกุลร่วมกับลักษณะทางสัณฐานวิทยา ชี้ให้เห็นว่า ตัวอย่างต่างๆ ที่เก็บรวมรวมมา น่าจะเป็น
พันธุ์แนะนำของทางราชการ หรือเป็นพันธุ์ที่ปะปนกันระหว่างพันธุ์พื้นเมืองกับพันธุ์แนะนำ อย่างไรก็ตาม
มีความเป็นไปได้ที่ตัวอย่างเหล่านี้จะพัฒนามาจากการผสมข้ามระหว่างพันธุ์พื้นเมืองกับพันธุ์แนะนำของทาง



ภาพที่ 1 แผนผังการกระจายของถั่วเหลืองพันธุ์แนะนำและถั่วเหลืองที่เก็บรวบรวมจากภาคเหนือของประเทศไทยจากการ Principle Component Analysis โดยใช้ความถี่ของอัลลีล SSR 9 loci



ภาพที่ 2 แผนผังการจัดกลุ่มของถั่วเหลืองสายพันธุ์แนะนำและที่เก็บรวบรวมจากภาคเหนือของประเทศไทยด้วยวิธี UPGMA โดยใช้ความถี่ของอัลลีล SSR 9 loci

ราชการ หรืออาจเป็นสายพันธุ์ของทางราชการที่เกษตรกรนำไปปลูก โดยยังไม่ได้รับรองพันธุ์ก็ได้ สำหรับพันธุ์เชียงใหม่ 60 มีความแตกต่างอย่างชัดเจนกับพันธุ์พื้นเมืองหรือพันธุ์แนะนำดั้งเดิม แต่ถูกจัดอยู่ในกลุ่มเดียวกับพันธุ์ สจ.4 เนื่องจากสจ.4 เป็นสายพันธุ์พ่อของเชียงใหม่ 60 นั่นเอง สรุปได้ว่า ในการจัดกลุ่มของแต่ละตัวอย่าง ให้ผลสอดคล้องกับสภาพทางภูมิศาสตร์ตามแหล่งที่เก็บรวบรวมมา

คำขอบคุณ

งานวิจัยนี้ได้รับทุนสนับสนุนการวิจัยจาก Japan Society for the Promotion of Science (JSPS) ศูนย์เทคโนโลยีชีวภาพเกษตรมหาวิทยาลัยเกษตรศาสตร์ และสำนักงานกองทุนสนับสนุนการวิจัย

เอกสารอ้างอิง

Cregan, P.B., T. Jarvik, A.L. Bush, R.C. Shoemaker, K.G. Lark, A.L. Kahler, N. Kaya, T.T. Vanoai, D.G. Lohnes, J. Chung and J.E. Specht. 1999. An integrated genetic linkage map of soybean genome. Crop Sci. 39: 1464-1490.

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เรื่องย่อการสัมมนาวิชาการประจำปี 2545
โครงการส่งเสริมกลุ่มวิจัยและพัฒนาพันธุ์พืชตระกูลถั่ว
มหาวิทยาลัยเกษตรศาสตร์

ณ Imperial Phukaew Hill Resort อ. เขาค้อ จ. เพชรบูรณ์

วันที่ 28-30 มีนาคม 2545

Glufosinate-Resistant Soybean : Biochemical Basis and Negative Cross-Resistance

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Objective

The specific objectives were to evaluate the difference in glufosinate tolerance of 16 soybean cultivars and breeding lines, to determine ammonia accumulation by genotypes with contrasting response to glufosinate to provide biochemical basis of the difference, and to determine their cross-resistance to other herbicides.

Methods

1. Treating glufosinate herbicide on 16 soybean cultivars and record their growth response at the whole plant and cell levels.
2. Conduct ammonia accumulation test after treating of the herbicide.
3. Determine cross-resistance of the soybean genotypes to the other herbicides.

Results

In the field screening, soybean cultivar "SJ 4" showed tolerant response while GC 87016 breeding line was susceptible. With the introduction of glufosinate, a soybean cell line resistant up to 10^{-6} M glufosinate was obtained through direct selection of diploid cells in the suspension culture. Determination of ammonia accumulation in the herbicide-treated soybean indicated that the normal cells accumulated up to 15-fold more ammonia than the resistant ones. This information suggested that lower ammonia accumulation in the resistant cells can be used as an indicator for selection of glufosinate-resistant soybean. Additionally, there was no cross-resistance between glyphosate, imazethapyr and primisulfuron.

Conclusions

Glufosinate-resistant soybean was determined at the whole plant and cell levels to provided information on the biochemical mechanism and negative cross-resistance to glyphosate, imazethapyr and primisulfuron.

Key words: Glufosinate-resistant soybean, ammonia accumulation, cross-resistance cell suspension culture

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1. Pornprom T, Surawattananon S and Srinives P (2000) Differential tolerance of soybean genotypes to glufosinate. SABRAO J. of Breeding & Genetics 32, 73-80.

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Progress on Biotechnology of Soybean

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Objective

To review the progress on biotechnology of soybean in three different areas

Methods

The three different areas of biotechnology are 1) plant tissue culture and protoplast technology 2) genetic transformation 3) genomic research including gene mapping and molecular marker development

Results

Plant tissue culture and protoplast technology are the basic technique for soybean biotechnology. Plant regeneration from callus, protoplast and somatic embryogenesis are the key point of applications in this area and will be focused.

Genetic transformation is the only technique for making transgenic soybean. The methods of gene transfer and some of transgenic soybeans will be presented.

Genomic research including gene mapping and molecular maker development for certain genes are the one of the most popular research topic at this day. Marker assisted selection is the major application of the study which will be discussed.

Conclusions

Biotechnology of soybean is used as a tool to accelerate the achievement of breeding program. It also makes us more understanding the pattern and the control of gene expression which can be applied for designing the novel soybean cultivars.

Key words : soybean, biotechnology, plant tissue culture, genetic transformation, genomic research

Selected References :

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The Possibility of Grafting Mungbean (*Vigna radiata*) and Blackgram (*Vigna mungo*) on Sweet Potato (*Ipomoea batatas*)

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Objective

To study the possibility of grafting mungbean (*Vigna radiata)* and blackgram (*V. mungo)* on sweet potato (*Ipomoea batatas)* stock.

Methods

In this grafting experiment the scion was the seedlings from mungbean variety "KPS 1" and blackgram "PSL 2", while the stock was sweet potato accession "No. 197". Sweet potato stock was propagated by stem cuttings into 6 inch plastic bags and let establish until 15 days. Three-day-old mungbean and blackgram seedlings were grafted on the stock in the morning and afternoon of each day. In each grafting, make sure that the cambium layers of the stock and scion were in good contact. Plastic thread was used to wrap the grafting point to protect the wound from drying.

Results

The result showed that mungbean and blackgram plants could temporarily grow on sweet potato stem for a period of time before wilting and dying off. Most of the scion died 2 days after grafting, while a few plants survived until flowering and setting pods (Table 1). The seedlings grew very slowly into small plants. Morphologically, the grafted mungbean had narrower leaves than the parental cultivar. The mature seeds will be harvested and sown for further study.

Table 1. Distribution of surviving mungbean and blackgram seedlings at different days after grafting on sweet potato stock.

Days after grafting	2	4	6	8	10	15	20	25	35	Total
Mungbean (KPS 1)	26	1	7	2	5	5	8	3	3	60
Blackgram (PSL 2)	27	5	1	3	10	7	3	1	3	60

Grafting in the morning and afternoon did not yield different number of survival days (Table 2). Seedlings of PSL 2 blackgram survived longer than that of KPS 1 mungbean. If the cambiums at the grafted point were not completely joined together the seedlings would start to wilt and the wound of sweet potato was rotten. The survival rate was much better when the grafted point was so near to soil surface that some of the seedling roots touched the soil. The experiment will be repeated in the coming rainy season.

Table 2. Average number of days from grafting to dying of mungbean and blackgram seedlings grafted on sweet potato stock in the morning and afternoon

Seedling	8:00-9:00 AM	3:00-4:.00 PM	Varietal Average *
Blackgram (PSL 2)	20.5	20.7	20.6
Mungbean (KPS 1)	16.9	16.7	16.8
Time Average ns	18.7	18.7	

CV=17.9% *= significant at 0.5% level, ns = non significant

Conclusion

Mungbean and blackgram seedlings could temporarily lived on sweet potato stock before wilting and dying. Only 3 plants survived until flowering and setting pods. Grafting in the morning and afternoon gave similar survival duration. However, the surviving seedlings must extend some roots into the soil surface. The experiment will be repeated to further refine the technique.

Key words: mungbean, *Vigna radiata*, blackgram, *Vigna mungo*, sweet potato, *Ipomoea batatas*, grafting

Selected References :

1. Meng Zaohuang (2001) Happy looking for mungbean and sweet potato wedding. The distant grafting's major bridge. Henan Science Daily. Translated into English by Mr. Zhou Jihong, China.
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Inheritance of Waxy Leaf Mutant in Mungbean (*Vigna radiata* (L.) Wilczek)

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Objective

To determine the inheritance of waxy leaf mutant in mungbean

Methods

Segregating F_2 progenies were obtained from a cross between waxy leaf and green petiole mutant mungbean line and normal leaf and purple petiole line. These lines were induced from F_2 of the cross between the cultivated mungbean 'Chi Nat 36' (*V. radiata* var. *radiata*) and the wild mungbean 'TC 1966' (*V. radiata* var. *sublobata*), using the gamma doses between 10-70 krad.

Results

The linkage between waxy leaf and petiole color in F_2 plants were tested against the expected Mendelian ratio using Chi-square (χ^2). The results showed that normal leaf is completely dominant to waxy leaf at the χ^2value of 1.176 with the acceptable probability of 0.25-0.50. The purple petiole was completely dominant to the green one at χ^2 value of 0.024 (P = 0.75-0.90). The linkage test between genes controlling both traits revealed that the χ^2 value was low (0.648). Yet, The χ^2 test for dihybrid (testing againt 9:3:3:1 ratio) indicated that gene controlling waxy leaves is independent from that controlling petiole color.

Conclusions

Normal leaf is completely dominant to waxy leaf, while purple petiole is completely dominant to the green one. Linkage test between genes controlling both traits showed that they located on different chromosomes.

Key words : mungbean, waxy leaf, purple petiole, linkage.

Selected References :

1. Shanmugasundaram, S. and D.H. Kim. (1996) Mungbean, pp 137-173. *In* P.N. Bahl and P.M. Salimath (eds) Genetics, Cytogenetics and Breeding of Crop Plants (Vol. 1). Oxford & IBH Pub. Co. Pvt. Ltd., New Delhi, India.
2. Srinives, P., N. Hual-alai, S. Saengchote and S. Ngampongsai. (1999) The use of wild relatives and gamma radiation in mungbean and backgram breeding, pp 205-218. *In* Proc. 7th MAFF, Japan International Workshop on Genetic Resources. NIAR, Tsukuba, Ibaraki, Japan.

Correlation of Some Qualitative Characters with Yield and Yield Components in Soybean [*Glycine max* (L.) Merr.]

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Objective

To study on segregation and correlation between some qualitative characters with yield and yield components in soybean.

Methods

Four crosses were made from 4 soybean cultivars, viz. G 8441 x G 1099, G 8441 x G 8483, G 1093 x G 8483 and G 1093 x G 1099. The parental cultivars were rather diverse in qualitative characters, such as leaflet size, leaflet shape, flower color, seed coat color, and seed size. Segregation and correlation of the qualitative traits with yield and yield components were done in the F_2 generation.

Results

In all 4 crosses, plant height, number of nodes per plant, number of branches per plant, number of pods per plant, number of seeds per plant, and number of seeds per pod showed positive correlation with the qualitative characters and yield. Plants with large leaflet size had higher yield than the smaller ones. Ovate leaflet type had higher yield than the lanceolate one. Purple flower color type had higher yield than the white one. Yellow seeded type was found linking with high yield. Large seed size plants had higher yield than the smaller ones.

Conclusion

The following qualitative characters cannot be used as criteria for selection to improve yield and yield components in soybean under studied.

Keywords : soybean, correlation, qualitative characters, yield, yield components

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2. Dawande, V.B. and D.N. There. 1993. Effect of seed size on yield in soybean[*Glycine max* (L.) Merr.]. Soyabean Abstr. 18(96) : 13, 70-85.
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4. Woodworth, C.M. 1923. Inheritance of growth habit, pod color, and flower color in soybeans. J. Am. Soc. Agron. 15 : 481.

Agrobacterium-mediated transformation system in mungbean (*Vigna radiata* (L.) Wilczek)

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Objective

To study the cointegration and expression of an insecticidal protein gene, cholesterol oxidase (*choA*), in mungbean.

Methods

Constructed plasmid pCAMBIA 1301-choA was transformed into *Agrobacterium rhizogenes* strain K599 and *A. tumefaciens* strain EHA 105 for mungbean transformation. Cotyledons from different ages of mungbean seedlings were inoculated with both bacteria. Selection was done on a medium supplemented with an appropriate antibiotic. Histochemical assay for GUS (β–glucuronidase) was performed to confirm the transformation.

Results

The two-day-old cotyledons that were co-cultured with hairy root bacteria showed higher ability to produce branched roots than did the others. An average of 11 branched roots were formed on both the wounded abaxial site and the hypocotyl cut end. Eleven of 75 individual lines(13.25%) were GUS positive. In addition, cotyledons that were cut and cultured on MB medium supplemented with 2 μg/ml BA for 4 days before co-culture with *A. tumefaciens* using hairy root method revealed high transformation ability (31.25%). Ten out of 32 inoculated cotyledons showed positive GUS activity. One shoot emerged from this GUS-positive cotyledon showed GUS activity on its shoot and leaves. To confirm the permanent cointegration of the gene, the assay of cholesterol oxidase activity and Western blot analysis need to be done and are in progress.

Conclusion

This study of mungbean transformation was not yet successful. It was observed that the cultivars, storage time of mature seeds, co-culture condition and culturing condition were the important factors affecting the transformation of mungbean.

Key words : mungbean, *Vigna radiata*, *Agrobacterium*-mediated transformation, cholesterol oxidase (*choA*), hairy root

Selected References :

1. Cho HJ, Widholm JM, Tanaka N, Nakanishi Y and Murooka Y (1998) *Agrobacterium rhizogenes*-mediated transformation and regeneration of the legume *Astragalus sinicus* (Chinese milk vetch). Plant Science 138:53-65.
2. Jaiwal PK, Kumari R, Ignacimuthu S, Potrykus I and Sautter C (2001) *Agrobacterium tumefaciens*-mediated genetic transformation of mungbean (*Vigna radiata* L. Wilczek)-a recalcitrant grain legume. Plant Science 161:239-247.
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Evaluation of Genetic Relationship among Soybean Genotypes from Diverse Genetic Bases

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Objective

To evaluate genetic relationship among soybean genotypes from diverse genetic bases.

Methods

Eight Thai, 5 Korean and 3 wild soybeans were analyzed for DNA marker relationship based on SSR and SNPs. Then, the relationship was shown through clustering by NTSYS-pc software.

Results

The dendrograms prepared through cluster analysis using SSR and SNPs are shown in Fig 1 and 2, respectively. For SSR marker, the soybean genotypes can be grouped into four clusters, while SNPs separated them into eight clusters.

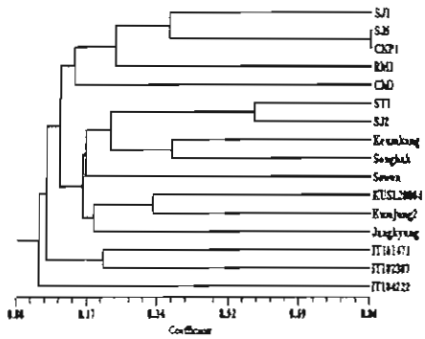

Fig. 1 Clustering by SSR

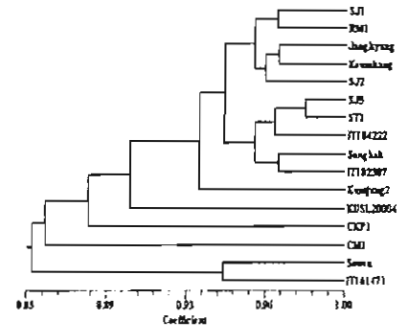

Fig. 2 Clustering by SNPs

Conclusion

The SSR dendrogram could separate the Thai, Korean and wild soybeans more distinctly than the SNPs dendrogram. This is because SSR was surveyed on larger and less specific region.

Keywords : Soybean, Simple Sequence Repeat, SSR, Single Nucleotide Polymorphisms, SNPs.

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2. Diwan N and Cregan PB (1997) Automated sizing of fluorescent-labeled simple sequence repeat (SSR) markers to assay genetic variation in soybean. Theor. Appl. Genet. 95, 723-733.
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Inheritance of seed storability in soybean (*Glycine max* (L.) Merrill)

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Objective

To determine the relative importance of genotype and environment in controlling seed storability in soybean

Methods

The two good storability soybean lines, GC 4670 (black-seeded) and GC 10999 (yellow-seeded) were crossed with three soybean varieties which have poorer storability, viz. 'Nakhon Sawan 1', 'Chiang Mai 60' and 'KUSL 20004'. Three crosses were obtained and derived up to F_3 seeds and established as F_3 lines. F_4 seeds of the lines were investicated in laboratory for electrical conductivity and seed germination.

Results

The histogram of seed germination frequency in all three soybean populations showed the same skewness pattern. The recessive effect of gene conditioning seed germination was detected. The germination percentage was found affected by seed coat color. Green-seeded group showed the highest percent of germination. However, black and brown seeded groups had more hard seed that would be germinated upon scarified.

Conclusions

All three soybean populations showed similar distribution histograms in seed germination among F3 lines with the tendency that seed storability was controlled by recessive gene effect. Seed coat color might be linked with seed germination after storage.

Keywords : soybean, seed storability, germination test, inheritance

Selected References :

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Genetic of the Resistance to *Phytophthora sojae* in Soybean (*Glycine max* (L.) Merrill)

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Objective

To determine the inheritance of resistance to Phytophthora root and stem rot in soybean grown in Thailand.

Methods

Soybean crosses were produced from KUSL 20004 x GC 87018-12-2B-1, GC 89045-13 x GC 87018-12-2B-1and GC 85039-1-2-1-1 x 87018-12-2B-1. GC 87018-12-2B-1 is susceptible to the fungus *Phytophthora sojae* while the rests are resistant. The F_2's were sown in cement blocks filled with infested soil to record for disease reaction. The normal F_2 plants were harvested for F_3 seeds, grown in an infested field, and counted for number of diseased and normal plants in the segregating rows. The number were tested for goodness-of-fit against the 3 to 1 ratio using Chi-square. Quantitative traits were also observed in plant height (cm), number of pods/plant, number of seeds/plant, grain yield (g/plant) and 100 seed weight (g). A group comparison t-test was used to compare between the traits observed on the normal vs infected plants.

Results

The segregation ratio of resistant to susceptible plants was found to be 3:1 in F_2 as well as segregation F_3 families, revealed that resistance to the disease was conditioned by a single dominant gene. Yet the number of segregating and resistant F_{23} families distributed at 2:1 ratio and thus confirmed the monogenic inheritance. The infected plants normally died before harvesting. The surviving diseased plants gave lower yield and yield components than the resistant plants. However, plant height was the same in all 6 soybean families regardless of disease infection.

Conclusion

Resistance to *P. sojae* was conditioned by a single dominant gene. Yield reduction in the diseased plants was due mainly to reduction in seed size. Although the infected plants normally died before harvesting.

Key words : Resistance, *Phytophthora sojae*, Soybean

Selected References :

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Tissue culture for gene transfer in mungbean (*Vigna radiata* (L.) Wilczek)

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Objective

To study tissue culture and gene transfer in mungbean cv. KPS1.

Methods

The cotyledonary nodes of mungbean seedling germinated on MS medium with or without growth regulator for 2, 3, 4 and 5 days were cultured on MB medium containing BA at various concentrations with or without 0.1 mg/l NAA. The multiple shoots were rooted on ½ MB medium with of or without NAA or IBA. In addition, gene transfer was conducted via the plasmid pCAMBIA 1301 from *Agrobacterium tumefaciens* strain EHA 105. Multiple shoots and cotyledons wounded by various techniques were used with this co-cultivation system.

Results

Cotyledons of 5-days-old seedling germinated on MS hormone free medium were cut and cultured on MB medium supplemented with 2 mg/l BA gave the highest number of shoots. Root was induced in ½ MB hormone free medium or ½ MB supplemented with 0.5 mg/l IBA. For transformation experiment, blue spots were observed on leaves and elongated shoots wounded by silicon carbide and sonicator. Some surviving shoots were not elongated and tended to become callus. To determine the stable transformants, molecular approach will be applied.

Conclusions

Tissue culture of mungbean cv. KPS1 was successful. However, transformation of mungbean showed transient and chimera expression of the *gus* gene. Transgenic plant was not obtained due to difficulty of plant regeneration from callus.

Keyword : mungbean, tissue culture, transformation

Selected References :

1. Lamseejan S, Wongpiyasatid A and Smutkupt S (1991) Effect of gamma-rays on *in vitro* culture of mungbean cotyledon. Kasetsart J. (Nat. Sci. Suppl.). 25,15-20.
2. Gulati A and Jaiwal PK (1994) Plant regeneration from cotyledonary node explants of mungbean (*Vigna radiata* (L.) Wilczek). Plant Cell Rep. 13,523-527.
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Genetic diversity in 15 yardlong bean accessions revealed by RAPD analysis

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Objective

To study level of genetic diversity in 15 yardlong bean (*Vigna unguiculata* (L.) Walp. ssp. *sesquipedalis* Verdc.) accessions.

Methods

Three yardlong bean accessions from Thailand and 12 accessions from Bangkladesh, China, Laos, Philippines and Taiwan were analyzed for their genetic diversity using RAPDs and cluster analysis by NTSYS-pc software.

Result

Of 45 random decamers screened, 26 showed 51 polymorphic bands of the size ranging between 600 and 2000 basepairs across the 15 yardlong bean accessions. Cluster analysis using UPGMA based on Nei and Li coefficient of similarity, generated five main clusters (Fig. 1) with high coefficient of similarity.

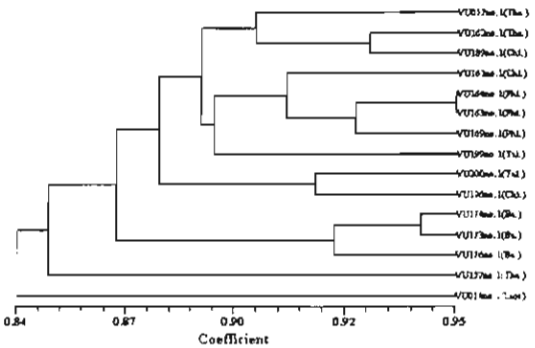

Fig. 1 UPGMA dendrogram of 15 *Vigna unguiculata* (L.) Walp. ssp. *sesquipedalis* Verdc. (yardlong bean) accessions based on Nei and Li coefficient of similarity.

Conclusion

The 15 yardlong bean accessions were devided into five clusters using UPGMA with high coefficient of similarity.

Keywords: yardlong bean, RAPD, genetic diversity, *Vigna unguiculata* (L.) Walp. ssp. *sesquipedalis* Verdc.

Selected References:

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2. Amadou H.I., P.J. Bebeli and P.J. Kaltsikes. 2001. Genetic diversity in Bambara groundnut (*Vigna subterranea* L.) germplasm revealed by RAPD markers. Genome 44: 995-999.

เอกสารแนบ 11

เรื่องย่อการสัมมนาวิชาการประจำปี 2545/46
โครงการส่งเสริมกลุ่มวิจัยและพัฒนาพันธุ์พืชตระกูลถั่ว
มหาวิทยาลัยเกษตรศาสตร์

ณ มหาวิทยาลัยเชียงใหม่ อ. เมือง จ. เชียงใหม่

วันที่ 9-11 พฤษภาคม 2546

Content

Oral Presentation

Poster Session

Microsatellite Markers Linked to Sudden Death Syndrome Resistance in Soybean

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Objectives

To verify QTLs underlying sudden death syndrome (SDS) resistance in soybean and to identify microsatellite markers linked to QTLs controlling the SDS.

Materials and Methods

A mapping population was developed from the cross between the susceptible soybean line GC89045-13-1 with the resistant line GC87018-12-2B-1. Finally, 104 RILs were obtained. A soil infested technique with *Fusarium solani* was employed to determine reaction of each RIL to the pathogen in the greenhouse condition. Disease severity was scored as phenotypic data. DNA from RILs were extracted and used for amplification of 500 microsatellite primers to obtain genotypic data. The MAPMAKER and mQTL softwares were used to construct a linkage map and locate the QTL controlling SDS resistance.

Results

One hundred and six out of 500 primers were found polymorphic. Fifteen linkage groups were constructed from 81 markers while 25 markers were found unlinked. Broad-sense heritability of disease severity was 56% of total variation in the greenhouse experiment. The QTL on linkage group J was identified by SATT183, SATT456, SCT065 and SCT001 as a new QTL conditioning the resistance to SDS.

Conclusion

A new QTL conditioning SDS resistance was found locating on linkage group J. It was identified by markers SATT183, SATT456, SCT065 and SCT001.

Keywords : sudden death syndrome, *Fusarium solani,* microsatellite marker, quantitative trait loci (QTL)

Selected References :

Njiti, V. N., M. A. Shenaut, R. J. Suttner, L. E. Schmidt and P. T. Gibson. 1996. Soybean response to sudden death syndrome : Inheritance influenced by cyst nematode resistance in Pyramid x Douglas progenies. **Crop Sci.** 36 : 1165–1170.

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High Temperature Sensitivity of Chlorophyll Fluorescence in some Pulse Legume Species

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Objectives

To determine the effect of high temperature stress on chlorophyll fluorescence Induction of *Glycine max, Vigna radiata, V. unguiculata, Phaseolus lunatus* and *P. acutifolius*.

Materials and Methods

The temperature dependence of chlorophyll fluorescence induction was studied in pulse legume plants grown under the same temperature and light intensity in a phytotron with day/night temperature of 17/24°C, at the increasing rate of 1°K/min with fluorometer (pulse amplitude module, PAM). The responses of the thylakoid membrane and the efficiency of the excitation of photosystem II at high temperatures of 24°, 32° and 40°C were measured by saturation pulse method.

Results

The breakpoints of all studied species were observed around 43° to 48°C when the minimum fluorescence induction (Fo) was plotted against temperature. The result explains the mechanism underlying the occurrence of the breakpoints. The kinetics of chlorophyll fluorescence quenching parameters induced in photosynthetic system of pulse legume leaves are used to identify their heat tolerance. The possibility of using breakpoint temperatures and the chlorophyll fluorescence quenching parameters to indicate adaptation of the thylakiod membranes organization and functioning under superoptimal growth condition of pulse legumes is discussed.

Key words: pulse legumes, chlorophyll fluorescence induction, minimum fluorescence induction (Fo), breakpoint temperature, heat tolerance

Selected References:

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Measurement of Leaf Response to Calcareous Soil in Mungbean

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Objectives

1) To estimate correlation between 2 leaf response parameters, green index and chlorophyll content.
2) To determine the genetic control of calcareous soil tolerance in mungbean.

Materials and Methods

The F_2 mapping population for gene tagging was obtained from a cross between NM 10 - 12 (tolerant parent) and KPS1 (susceptible parent). The F_2 seeds were sown in a calcareous soil field at Nakhon Sawan Field Crops Research Center to observe on plant reaction. Leaf green index of each F_2 plant was mesured by chlorophyll meter. Subsequently, leaf discs from the same sampled leaves were assessed for chlorophyll content.

Results

1) Correlation coefficient between chlorophyll content and green index was 0.969 (P=0.000) indicated that both parameters were highly correlated.
2) F_2 plant segregation fitted a 3:1 ratio of tolerant green plant to susceptible yellow plant. This indicated that the trait is controlled by one locus of gene which tolerance is dominant over susceptibity.

Conclusion

1) Mesurement of chrolophyll content and/or green index can be used to determine field reaction of mungbean to calcareous soil.
2) Genetic control of tolerance to calcareous soil in mungbean is conditioned by single dominant gene.

Keywords : calcareous soil, mungbean, green index, chlorophyll content

Selected References

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Plantlets Regeneration from *Stylosanthes hamata* Callus

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Objectives

To develop the protocol for plant regeneration from callus of ***Stylosanthes hamata***.

Materials and Methods

1) Callus induction: Culture *in vitro* explants (cotyledon, leaf and hypocotyl) of *S. hamata* in MS + 5-20 mg/l 2,4-D or NAA.
2) Plantlets regeneration: Transfer callus obtained from MS + 5 mg/l 2,4-D to MS + (10-25 mg/l) BA or (5-20 mg/l) kinetin.
3) Root induction: Culture the regenerated shoots on MS + (0.5-4 mg/l) IBA or NAA. All treatments were incubated at 25 $\pm$ 2 °C with 16 hr light (28 µmol $m^{-2}s^{-1}$).

Results

1) Callus induction: The best green compact callus was obtained from MS + 5 or 10 mg/l 2,4-D. The media supplemented with NAA at all tested concentrations gave light brown friable callus which was not suitable for regeneration.
2) Plantlet regeneration: The suitable media for plantlet regeneration was MS + 15 mg/l BA which 9 shoots per callus clump were obtained. The compact callus from leaf explants tended to yield more shoots than those of cotyledon or hypocotyl derived callus.
3) Root induction: About 40% of the shoots were able to form roots when cultured in MS + 0.5 mg/l IBA or 2 mg/l NAA.

Conclusion

A plantlet regeneration system of *S. hamata* was developed as follows :-

Callus Induction: MS + 5 mg/l 2,4-D
Shoot Induction: MS + 15 mg/l BA
Root Induction: MS + 0.5 mg/l IBA or MS + 2 mg/l NAA

Key words: Caribbean bean, *Stylosanthes hamata*, callus, plant regeneration

Selected References:

Li, D.W., J. Schmid and E.R. Keller. 1989. Application of growth regulators in the induction and maintenance of calluses of soybean. **Hered.** 11 :1-4.

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Molecular Study of Genetic Diversity in Yardlong Bean (*Vigna unguiculata* (L.) Walp. ssp. *sesquipedalis* Verdc.) and Related *Vigna* species

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Objectives

To study level of genetic diversity in yardlong bean accessions and genetic relationship among related *Vigna* species.

Materials and Methods

Fifteen accessions of yardlong bean and six accessions of related *Vigna* species were analysed for their genetic relationship using RAPD and STMS techniques. Cluster analysis was performed using NTSYS-pc software.

Results

RAPD and STMS dendrograms of yardlong bean and related *Vigna* species were exhibited in Fig. 1, 2 and 3, respectively. Cluster analysis using UPGMA based on Nei and Li coefficient of similarity, generated five main clusters, while STMS separated them into three main clusters. Six accessions of related *Vigna* species can be grouped into four main clusters.

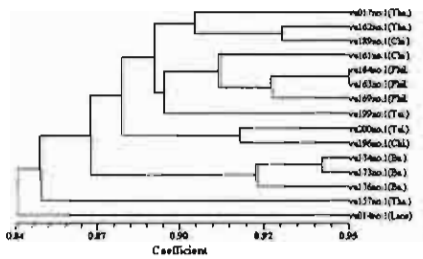

Fig. 1 RAPD dendrogram of yardlong bean

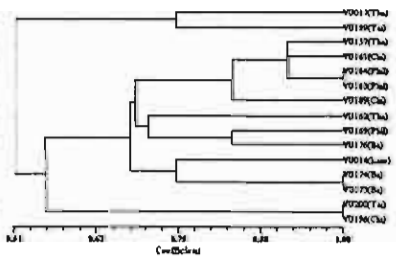

Fig. 2 STMS dendrogram of yardlong bean

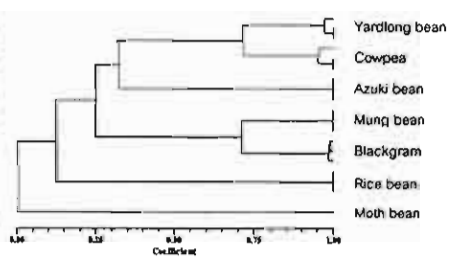

Fig.3 RAPD dendrogram of *Vigna* species.

Conclusions

Both RAPD and STMS indicated narrow genetic base of yardlong bean, but large variation among six *Vigna* species. Moth bean is the most differentiated and cowpea is the closest to yardlong bean.

Key words : yardlong bean, RAPD, STMS, *Vigna* species, genetic diversity

Selected References:

Rolf, F.J. 1998. Numerical taxonomy and multivariate analysis system, **NTSYS-pc Program Manual Version 2.0.** Applied Biostatistics Inc., 3 Heritage Lane, Setauket, NY 11733.

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Inheritance of Epicotyl, Seed Coat and Hilum Colors and Flowering Date in Ricebean (*Vigna umbellata* (Thunb.) Ohwi & Ohashi)

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Objectives

To determine the inheritance of flowering date and the colors of epicotyl, seed coat and hilum in ricebean.

Materials and Methods

Segregating F_2 progenies were obtained from the cross between a Thai cultivated ricebean 'CN800001' having green stem seedling, late flowering, yellow seed coat, and white hilum ring color and a Japanese cultivated ricebean 'Menaga' having red stem seedling, early flowering, red seed coat, and brown hilum ring color. To induce flowering of the progenies, the plants in the planting blocks were covered with a black plastic sheet and subjected to 10 hr photoperiod starting from 20 days after germination until flowering.

Results

The number of plants with different epicotyl color, seed coat color, and hilum ring color in the F_2 plants were counted and tested against the expected 3:1 Mendelian ratio using Chi-square (χ^2) test. The results showed that red epicotyl is completely dominant to green epicotyl at the χ^2 value of 1.12. (P=0.1-0.5). The yellow seed coat is completely dominant to the red one at χ^2 value of 2.79 (P=0.1-0.5). The brown hilum ring is completely dominant to the white one at χ^2 value of 0.21 (P=0.5-0.9). A χ^2 – test of linkage among the three traits revealed that they were inherited independently. The flowering date of the F_2 population showed normal distribution and thus suggested that it is controlled by multigenes.

Conclusion

Red epicotyl, yellow seed coat, and brown hilum ring are dominant to green epicotyl, red seed coat, and white hilum ring, respectively. A linkage test among the three traits showed that they segregated independently. Flowering date is a quantitative trait.

Key word: rice bean, *Vigna umbellata*, seed coat color, epicotyl color, hilum ring color, flowering date

Seleced References:

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Inheritance of Black Seed and White Pod in Mungbean (*Vigna radiata* (L.) Wilczek)

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Objectives

To study the inheritance of seed coat and pod color in mungbean.

Materials and Methods

F_1 seeds of mungbean were obtained by crossing a mutant BS (black seed and black pod) line with other 2 lines, viz. a local cultivar WP (green seed and white pod) and the recommended cultivar KPS2 (green seed and black pod). Their reciprocal crosses were also made. Seed coat color and pod color from each plant in the F_2 generation were recorded. Data were analyzed by Chi – square (χ^2) test against the monogenic 3:1 Mendelian ratio.

Results

The results showed that the F_2 populations from both direct and reciprocal crosses showed 3:1 segregation in both seed coat color and pod color. The black seed coat color is dominant over green seed with the χ^2 value of 0.1-3.12 ($P_{0.05}$ = 3.84). Similarly in pod color, black pod is dominant to white pod color with the χ^2 value of 0.6-1.5.

Conclusion

Seed and pod colors of mungbean are each controlled by a single locus of gene. Black seed and black pod colors are dominant to green seed and white pod color, respectively.

Key word : mungbean, *Vigna radiata*, seed coat color, pod color

Selected References:

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Interspecific Hybridization among Three Asian *Vigna* species

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Objectives

To study crossability and genetic barriers among mungbean, black gram, and rice bean.

Materials and Methods

Mungbean (*Vigna radiata*) 'Kamphaeng Saen 1', black gram (*V. mungo*) 'VM2164', and rice bean (*V. umbellata*) 'Miyazaki', were used as parents. All possible cross combinations were made among these three species.

Results

The crosses mungbean x black gram and mungbean x rice bean were successful only when mungbean was the female parent. Some pods of both crosses grew normally and reached full maturity. Most of the hybrid seeds were normal, while some were crinkled or split. Black gram x rice bean was successful only when black gram was the female parent. Some hybrid pods of this cross developed normally and contained full-size wrinkled seeds. Hybrids from mungbean x black gram were mostly fertile and produced flowers and pods in abundance, but all pods dropped off within a week after flowering. Two hybrid plants which resembled the mungbean parent flowered and set pods profusely. All pods of both plants reached maturity, but contained all crinkled and empty seeds. Mungbean x rice bean produced hybrid pods which reached normal maturity and yielded both normal and abnormal (small, wrinkled or split) seeds. Hybrid plants from this cross flowered plentifully and set a lot of pods. However, the pods shed within a week. Hybrid plants from black gram x rice bean were mostly fertile, grew vigorously, and yielded a lot of normal pods and seeds.

Conclusion

Cross-compatibility among mungbean, black gram, and rice bean was not strong. Mungbean is the most effective female parent. Crossing of mungbean x rice bean and black gram x rice bean is likely to be successful.

Key words: *Vigna* spp., interspecific hybridization, crossability

Selected references :

Ahn, C.S. and R.W. Hartmann. 1978. Interspecific hybridization among four species of the genus *Vigna* Savi. pp. 240-246. *In* R. Cowell, ed. **Proceedings of the First Int. Mungbean Symp.** AVRDC, Shanhua, Tainan.

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Hybridization Techniques in Bambara Groundnut and the Phenotypes of F_1 and F_2

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Objectives

To establish an appropriate technique for hybridization in bambara groundnut (*Vigna subterranea*), and study on phenotype of the resulting F_1 and F_2 profenies.

Materials and Methods

Bambara groundnut varieties TVSu 11, TVSu 870, TVSu 1061, and a Southern Thailand local variety were grown in a field at Songkhla Field Crops Research Center until flowering. Hybridization was done every 30 minutes from 10:00 PM to 9:00 AM to compare 2 emasculation methods, cut and un-cut of petals. The F_1 seeds were germinated and advanced to obtain F_2 seeds. The F_2 segregating population were sown and observed for some major traits.

Results

The total of 23 F_1 seeds were obtained from crossing time between 2:30 to 3:30 AM. Cutting the petals did not harm bambara groundnut flowers. Complete and incomplete dominance of some traits, i.e. leaf shape, canopy type, and pod color could be seen in the F_1 plants. The F_2 phenotypes are under investigation.

Conclusion

The suitable hybridization time for bambara groundnut is between 2:30 to 3:30 AM. Emasculation from the flowers with cut petals is easier and faster to make cross in very early morning.

Key words : *bambara groundnut, Vigna subterranea, hybridization technique,* hybrid traits

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Use of Molecular Markers for Gene Mapping with Application to Nodulation Gene in Soybean

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Objectives

To identify nodulation gene in soybean.

Materials and Methods

Thai and Korean soybean varieties were screened against *Bradyrhizobium japonicum* in Leonard jar. The cultivar SJ2 and Suwon157 were chosen as parents to form a population.

Results

Number of nodules per plant, nodule fresh weight, nodule dry weight, acetylene reduction assay (ARA), and plant dry weight were employed in parental survey. The population has been advanced to obtain F_6 recombinant inbred lines. The phenotypic data will be combined with genotypic data from the SSR and AFLP and construct maps linking to nodulating traits.

Characters	SJ2	Suwon157
No. nodules/plant	18.0	39.67
Nodule fresh weight (g)	0.21	0.89
Nodule dry weight (g)	0.05	0.18
ARA (mmole C_2H_4/plant/hr)	1.47	4.73
Plant dry weight (g)	0.81	2.21

Conclusion

SJ2 and Suwon 157 were chosen as parents for constructing a mapping population of nodulation gene.

Keywords : soybean, nodulation, gene mapping

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Determination of *In Vitro* Antibiotic Resistance for Gene Transformation in *Stylosanthes hamata*

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Objectives

To determine an optimal concentration of antibiotics commonly used in gene transformation protocol in *Stylosanthes hamata.*

Materials and Methods

1) Determination of optimal hygromycin and kanamycin concentrations.

S. hamata calluses (5x5 mm) were cultured on MS medium + 5mg/l 2,4-D + 0-80 mg/l hygromycin. Both liquid and solid of media were used. In the case of kanamycin, the calluses were cultured on MS medium + 5mg/l 2,4-D + 0-60 mg/l kanamycin. The minimum concentration and the duration that all cells were executed were recorded.

2) Determination of optimal cefotaxime concentration.

The calluses of *S. hamata* (1x1 cm) were cultured on MS (solid medium) + 0-300 mg/l cefotaxime for 40 days. After that the calluses were cultured on MS (solid medium) + 15 mg/l BA for 90 days. In case of solid medium, the calluses of *S. hamata* (5x5 cm) were cultured on MS + 5 mg/l 2,4-D + 0-600 mg/l cefotaxime for 4 weeks.

All cultures mentioned above were incubated at 16 hr light (28 $\mu mol/m^2/s$) and 25±2°C.

Results

Supplementing hygromycin at 50 mg/l to solid media, or 40 mg/l to liquid media were the minimum concentrations that all normal cells were executed within 30 days. For kanamycin, the concentration of 30 mg/l for 30 days can eliminate the normal cells. Cefotaxime at 300 mg/l applied to *S. hamata* callus culture and incubated for 40 days can allow callus to survive and regenerate. These results revealed that the tested concentrations can be used to get rid of *Agrobacterium sp.* in the process of *Agrobacterium*-mediated gene transformation without affecting plant regeneration in *S. hamata.*

Conclusions

The concentration of hygromycin at 50 and 40 mg/l in solid and liquid media, and that of kanamycin at 30 mg/l in solid medium could execute all normal *S. hamata* cells. Thus, they can be used as selective agents in transformation procedures. In order to get rid of *Agrobacterium* sp. from co-culture with *S. hamata* explants, 300 mg/l of cefotaxime was recommended.

Key words: Caribbean bean, *Stylosanthes hamata*, callus, antibiotic resistance

Selected References:

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A Study on Traits Related to Seed Quality with the Application to Selection for Seed Storability in Soybean.

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Objectives

To study on traits related to seed quality and the possibility of using them to improve seed storability in soybean.

Materials and Methods

Thirty soybean lines showing large and vigorous seed were sown in the field with three standard varieties, viz. NS1, CM60, and KUSL20004. They were tested on seed quality, i.e. standard germination test, accelerated aging test, controlled deterioration test, electrical conductivity test, amount of K^+ and Mg^{2+} and soil germination test. Two lines, GC4670 with black seed coat and GC10999 with yellow seed coat, were finally chosen. They were used as female parents to cross with the standard varieties. Three crosses, viz. GC4670 x NS1, GC10999 x CM60 and GC10999 x KUSL20004 were obtained. The hybrid seeds were sown and advanced until F_3 plants were individually harvested. The resulting F_4 seeds were evaluated for the percentage of germination, hard seed and seed viability in different crosses. Seed quality was determined from standard germination test, electrical conductivity test, sand germination test, and oil percentage in seed.

Results

The histograms from both germination percentage and hard seed percentage skewed to the right, revealing that higher germination and hard seed were controlled by recessive genes. In addition, the cross GC4670 x NS1 produced the progenies that have different seed coat colors, viz. yellow, black, brown, green, and yellowish green. The green seed coat lines gave the highest germination percentage of 59.03%, followed by yellowish green, yellow, brown, and black with 41.0, 38.26, 37.08 and 31.78% germination, respectively. For hard seed percentage, the black seed coat line was highest at 29.83%, whereas the brown, yellowish green, yellow, and green seed gave 16.95, 9.23, 3.60 and 1.56%, respectively. Black seed coat group also gave the highest seed viability percentage up to 61.61%, while the green, brown, yellowish, and yellow groups gave 60.58, 54.03 and 50.96%, respectively.

Conclusion

All three soybean populations showed similar distribution histrograms in seed germination among F_4 seed lines with the tendency that seed storability was controlled by recessive gene effect. Seed coat color might link with seed germination and affect seed longevity under storage.

Keywords : soybean, *Glycine max*, seed storability, seed quality, germination test, gene effect

Selected References:

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The Efficacy of Artificial Substrates on Oviposition of Two Bruchid Species (Coleoptera:Bruchidae)

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Objectives

To determine the effect of substrates in artificial seeds on oviposition of *Callosobruchus chinensis* and *C. maculatus*

Materials and Methods

Feeding tests were examined by using artificial seeds prepared from flour of the susceptible mungbean variety 'KPS1' supplemented with beet armyworm artificial diet, flour of sweet potato, tapioca, corn, sorghum, wheat, polished-rice, non-polished rice, soybean, and cowpea. The number of eggs, emerged larvae, emerged adults, non emerged larvae, non emerged adults, and the period from egg to adult were used as parameters in this study.

Results

The highest number of emerged adults was obtained from flour of 50% cowpea + 50% KPS1, followed by 50% wheat + 50% KPS1, and 50% non-polished rice + 50% KPS1 for *C. maculatus*. The maximum number of emerged adults of *C. chinensis* was obtained on 50% non-polished rice + 50% KPS1 treatment.

Conclusion

It appeared that the most favorable supplement of KPS1 flour for both bruchids was non-polished rice, followed by cowpea. Further studies are required to investigate the effect of combined non-polished rice and cowpea seed.

Key words: artificial seeds, *Callosobruchus chinensis*, *C. maculatus*

Selected references:

Kasiwaba K., N. Tomooka, A. Kaga, O.K. Han and D.A. Vaughan. 2003. Characterization of resistance to the three Bruchid species (*Callosobruchus* spp., Coleoptera, Bruchidae) in cultivated rice bean (*Vigna umbellata*). **J. Econ. Entomol.** 96(1): 207-213.

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Effect of Gamma Radiation on Mutation of Parents, F_1 and F_2 Mungbean

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Objectives

1) To study the effect of gamma rays on mutation of different mungbean generations, viz. F_1, F_2 and their parental lines.
2) To identify the mutant characters that may be used as new genetic resources in mungbean breeding programs.

Materials and Methods

Seeds of four mungbean populations were prepared from KPS2, VC6468-11-1B, their F_1 and F_2 by weight of 156, 187, 159 and 212 g, respectively. They were treated with CS-137 gamma rays at 500 Gy. The M_1 seeds were sown in the field. The M_2 seeds were bulk- harvested in each population and finally 7.76 kg of KPS2, 5.12 kg of VC6468-11-1B, 11.02 kg of F_1 and 8.72 kg of F_2 seeds were sown in the field to observe on number and characters of mutant plants.

Results

Several mutants were identified in M_2 generation such as chlorophyll mutation, including albino, light green leaf, variegated leaf, white streak leaf, and xantha. Leaflet shape mutation was identified to include lanceolate leaflet, multiple leaflet, narrow leaflet, round cuneate leaflet, unifoliate leaf, waxy leaf, and wrinkled leaf. Some sterile mutants were also detected.

F_1 irradiated population gave the highest number of mutant of 214 plants (0.1590%), followed by KPS2 (0.1361%), F_2 (0.1584%) and VC6468-11-1B (0.14197%) at 174, 142, and 116 plants, respectively. The heterozygous populations tended to be more sensitive to gamma rays than their parents. All mutant lines will be purified and used for genetic study.

Conclusion

CS-137 gamma rays at 500 Gy affected all four mungbean populations and gave some mutants which never existed in the collected mungbean germplasm. M_3 seeds will be sown to extract for more mutants.

Keywords: mungbean, *Vigna radiata*, mutant, gamma rays

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Hual-Alai, N. 2001. **Effect of Gamma Radiation on Variation of Progenies from Crosses between Cultivated and Wild Mungbeans.** MS Thesis. Kasetsart Univ. Bangkok.

Table 1. Mutant characters found in M_2 of the four mungbean generations irradiated with 500 Gy gamma rays.

Mutant characters	Generations				
	KPS2	VC6468-11-1B	F_1	F_2	Total
Chlorophyll mutation					
abino	113	45	164	105	427
light green leaf	2	2	3	0	7
variegated leaf	2	3	4	3	12
white streak leaf	1	2	2	3	8
xantha	19	13	2	2	36
Leaflet mutation					
lanceolate leaflet	2	2	1	0	5
multiple leaflet	29	37	29	27	122
narrow leafet	2	1	0	0	3
round cuneate leaflet	0	0	0	1	1
unifoliate leaf	2	0	0	1	3
waxy leaf	2	6	5	0	13
wrinkled leaf	0	5	4	0	9
No. mutant plants	174	116	214	142	646
Total seedlings	127,880	81,708	134,607	89,647	433,842
Percentage of mutant	0.1361	0.14197	0.1590	0.1584	0.14890

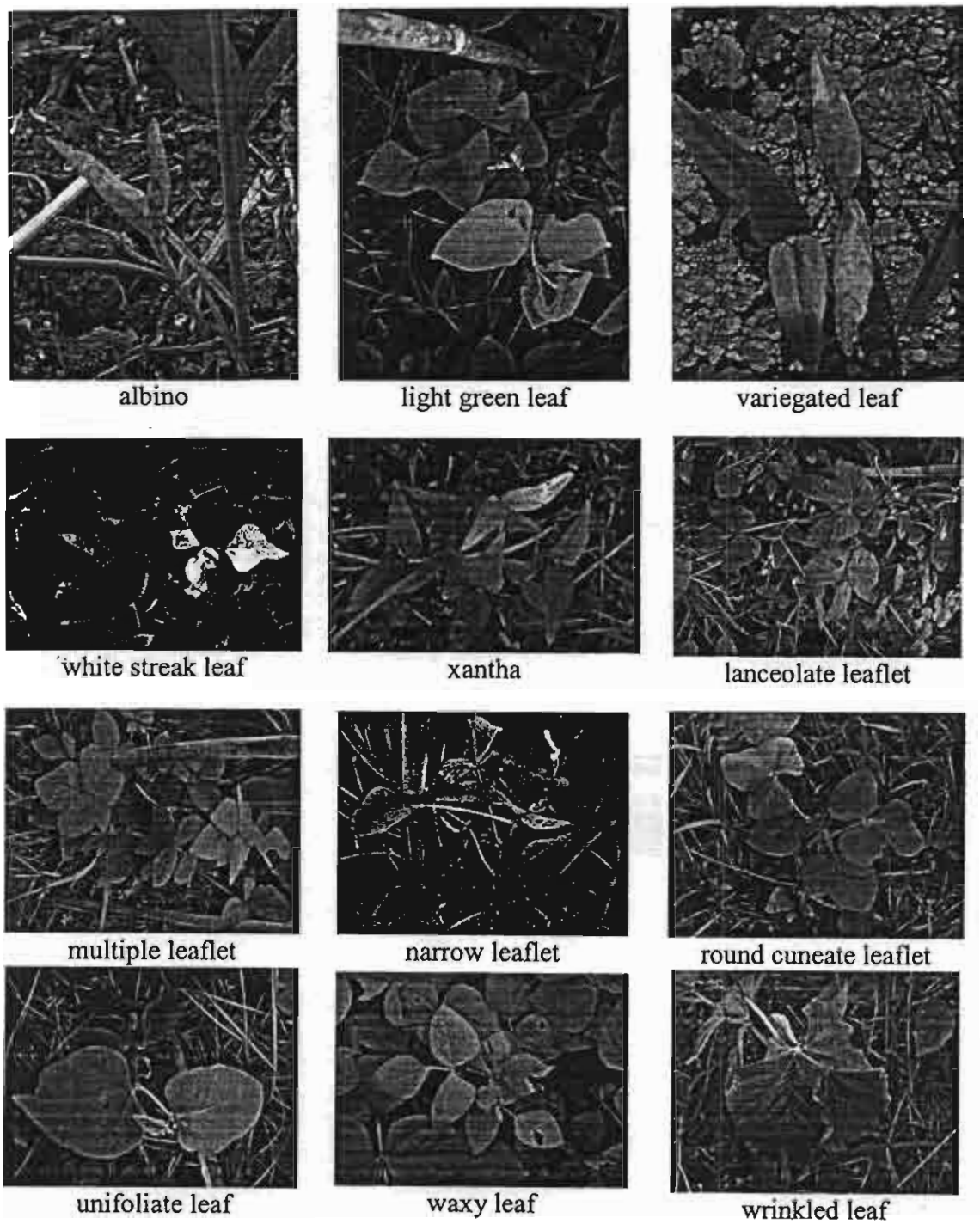

Figure 1. Mutant characters found in M_2 mungbean.

Grafting Bambara Groundnut, Blackgram, Mungbean, Rice Bean and Soybean Scions on Blackgram and Rice Bean Root Stocks.

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Objectives

To study the survival of bambara groundnut, blackgram, mungbean, rice bean and soybean scions on blackgram and rice bean rootstocks. The successful grafting may help inducing mutants as well as facilitating crossings among certain genotypes.

Materials and Methods

Rootstock plants were prepared by sowing blackgram (*Vigna mungo* 'Uthong 2') and ricebean (*V. umbellata* 'Menaga') until they were flowering. The flowering dates for blackgram was 47 days and ricebean was 40 days after sowing. The scions were prepared from 3-10 day-old seedlings of bambara groundnut (*V. subterranea* 'Songkhla 1'), mungbean (*V. radiata* 'KPS2'), soybean (*Glycine max* 'KUSL20004'), Uthong 2 blackgram, and Menaga ricebean. Grafting was done by cutting roots and making a V-shaped stem on scion. The shoot of the rootstocks were cut off about 1-1.5 cm to insert the scions on and tied the joint with plastic film to protect from water. Data were recorded on the number of survival plants at 20 days after grafting.

Results

Mungbean scions survived most (72.73%) on blackgram rootstocks, followed by bambara groundnut and rice bean at 60 and 58.8 %, respectively. None of them survived on soybean scions. On rice bean rootstocks, most surviving scions were bambara groundnut (80 %), followed by mungbean and blackgram at 50 and 32%, respectively. Only one soybean scion was found surviving on rice bean rootstock. It is noted that the survival rates were higher when grafting the *Vigna* spp. on the *Vigna* stock as compared to using soybean as the scion.

The grafted scions grew normally and set pods. Although bambara groundnut is easier to cross after grafting as flowering occurred high above the stock plants, the fertilized flowers required soil to support pod pegging and development.

Conclusion

Grafting among the same genus, such as between *Vigna* and *Vigna,* gave higher success rate than among different genera, such as *Vigna* and *Glycine*. The short plant type species like bambara groundnut can be grafted into taller plant stocks to help facilitating emasculation and pollination.

Key word: grafting, ricebean, *Vigna umbellata,* blackgram, *Vigna mungo*, mungbean, *Vigna radiata*, Bambara groundnut, *Vigna subterranea*, soybean, *Glycine max*

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Table 1. Number and percentage of grafted and survived scions when blackgram was used as rootstocks.

Mungbean (KPS2)			Rice bean (Menaga)			Bambara groundnut (Songkhla 1)			Soybean (KUSL20004)		
Grafted	Survived	(%)	Grafted	Survived	(%)	Grafted	Survived	(%)	Grafted	Survived	(%)
11	8	72.73	17	10	58.82	10	6	60	29	0	0

Table 2. Number and percentage of grafted and survived scions when rice bean was used as rootstocks.

Mungbean (KPS2)			Blackgram (UT2)			Bambara groundnut (Songkhla 1)			Soybean (KUSL20004)		
Grafted	Survived	(%)	Grafted	Survived	(%)	Grafted	Survived	(%)	Grafted	Survived	(%)
20	10	50	25	8	32	15	12	80	14	1	7.14

유전자 재조합 작물의 생태적 및 식품 안전성과 농업적 이용

Ecological and Food Safety of Genetically Modified Crops and Their Utilization

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韓國作物學會

목 차

<특강>

Field Crop Research and Development in Thailand

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Thailand has the total area of 513,115 km2. In 1995, 41% of the area are farm holding land, 26% are forest land, and 33% are others. Among the farm holding land (total area of ~210,377 km2 or 21,037,700 ha), roughly 11 million ha are paddy land while 4 million ha are field crop area. In crop year 1998/99, ten major field crops of Thailand ranking in terms of farm value are sugarcane, corn, cassava, soybean, mungbean, peanut, sesame, cotton, sorghum and kenaf (Table 1). Almost 1 million Thai families raise field crops for their major income while the yield and farm gate price are usually low. Thus the field crop growers are among the poorest group of people in the Kingdom. Crops like sugarcane, cassava and mungbean are produced mainly for export in the forms of sugar, cassava products and mungbean products, while soybean and cotton are imported in various forms. Thailand is more or less self sufficient in the other crops.

Research and development (R & D) on field crops, except corn and sorghum, are handled by the government sector. The major organization conducting research in field crops are the Department of Agriculture (DOA), Ministry of Agriculture and Cooperatives, and major public universities, especially Kasetsart University and some other regional universities. Broadly, field crop research and development in Thailand can be divided into crop production research and varietal improvement. The crop production part includes crop physiology, soil fertility, disease and insect pest management, farming systems, organic farming, and farm mechanization. The varietal improvement part includes genetic study, conventional breeding, mutation breeding, and biotechnology. Only a few crop production recommendations are practiced by the farmers due to the lack of cost effectiveness. In contrast, the farmers almost always accept new cultivars once they are available. Public cultivars are disseminated through either DOA or the Department of Agricultural Extension (DOAE) of the same ministry. If an open pollinated public cultivar or a pure line is really good, it will be increased and distributed

C2. Identification of AFLP Marker Linked to Genes Controlling Iron Deficiency Tolerance in Mungbean by Bulked Segregant Analysis.

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Kamphaeng Saen 1 (KPS1), a Thai mungbean cultivar susceptible to iron deficiency, was crossed with NM 10-12 , a tolerant line from Pakistan, to dissect iron deficiency tolerance in mungbean. Single seed descent was practiced to develop 199 recombinant inbred lines (RILs) used in this study. The RILs were sown in a field having calcareous soil and iron deficiency. Chlorosis symptoms were examined between 14 to 24 days after planting. No symptom was found in 146 RILs while 53 of them were affected. The ratio of tolerant to susceptible lines in this population was 3:1. Thus, the RIL population could have 4 genotypes such as *AAII, aaII, AAii* and *aaii,* of which only *AAii* showed the symptom of iron deficiency (Srinives *et al.,* 1997). Seventy-two RILs were randomed and test-crossed using KPS1 as the tester. The test cross progenies from 32 lines were tolerant to iron deficiency while those from 40 lines were susceptible. The ratio of tolerant to susceptible lines was, theoretically, 1:1.

Bulked segregant analysis technique was employed to identify DNA markers linked to iron deficiency. Three DNA pools were generated from the RIL population. Each pool contained 15 individual plants and pooling was based on genotypes of the RILs as identified by test-crossing. DNA pool 1 comprised individuals of genotypes *AAII* and *aaII*. The two genotypes could not be separated since both were tolerant to iron deficiency, and yet their test cross progenies were also tolerant. The genotype of DNA pool 2 was *aaii* conditioning the tolerance but its test cross progenies were all susceptible. The genotype *AAii* contained in DNA pool 3 showed iron deficiency in the RILs as well as their test cross progenies. The DNA pools were analyzed using amplified fragment length polymorphism (AFLP) to identify polymorphic loci. Eight polymorphic bands were found in which DNA pool 1 and 2 followed the banding pattern of the tolerance parent while DNA pool 3 followed that of the susceptible one. All loci were found locating on the same linkage group. One locus of the gene controlling iron deficiency tolerance (most likely the A locus) was found between CGT/CTG and CAG/TAC4 markers flanking at a distance of 2.9 and 3.0 cM from the gene, respectively. While the other locus could not be identified in this study.

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APWSS

The 18th Asian-Pacific Weed Science Society Conference

OFFICIAL PROGRAMME

Beijing, P.R. China, 28 May - 2 June 2001

Saturday 2 June 2001

Concurrent Session	**Resistant Weeds and Crops (2)**
Room:	**Conference Room A**
Chairman:	**Prof. S. Powles and Dr. Y. S. Chen**
08.30	Chairman's Introduction
08.35	Transformation of Spinach (*Spinacia oleracea* L.) for Glyphosate Resistance. *Bevitori, R.,N. R. Burgos, R. E. Talbert, S. Rajguru, and T. E. Morelock*
08.55	Resistance of *Amaranthus palmeri* to ALS-inhibitor herbicides. *Burgos, N. R., Y. I. Kuk, and R. E. Talbert*
09.15	Resistance to Acetolactate Synthase (ALS) Inhibitors in a Biotype of *Monochoria vaginalis* Discovered in Korea. *Hwang, I. T., K.H. Lee, S.H. Park, B.H. Lee, K.S. Hong, S.S. Han, and K.Y. Cho*
09.35	Socio-economic audit of zero tillage in herbicide resistance affected area in India. *Malik, R. K. et. al.*
09.55	Glufosinate-resistant soybean:biological basis and negative cross-resistance. *Pornprom,T. et al*
10.15	Tea/Coffee

ABSTRACT FORM

18th Asian-Pacific Weed Science Society Conference (APWSSC)

May 28 - June 2, 2001 Beijing, China

Glufosinate-Resistant Soybean : Biochemical Basis and Negative Cross-Resistance

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Resistance to glufosinate of 16 soybean cultivars and breeding lines was evaluated at the whole plant and the cell levels. In a field screening, the cultivar "SJ 4" showed resistant response while GC 87016 breeding line was susceptible. The whole plant response indicated that the I_{50} and GR_{50} estimates of growth from the susceptible and resistant cultivars were at 0.250 and 0.500 kg ai/ha glufosinate. With the introduction of glufosinate-resistant soybean cell lines, cell suspension cultures were established on MS basic medium supplemented with B5 vitamins, 0.3% sucrose, and 10 mg/L NAA. Using a stepwise selection with increasing concentration of herbicide, a soybean line resistant to 10^{-6} M glufosinate was obtained through direct selection of diploid cells in the suspension culture. It is referred to as 10^{-6} M glufosinate-resistant soybean cell line. Determination of ammonia concentration in the herbicide-treated cells indicated that the normal cells accumulated up to 15-fold more ammonia than the resistant ones. This information suggested that lower ammonia accumulation in the resistant cells can be used as an indicator to select for glufosinate-resistant soybean cell lines. Additionally, there was no cross-tolerance between glyphosate, imazethapyr, and primisulfuron.

Oral Presentation Oral Poster - Title _____

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中国绿豆产业发展与科技应用

INDUSTRIAL DEVELOPMENT AND TECHNOLOGY UTILIZATION OF MUNGBEAN IN CHINA

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Mungbean Breeding Research at ARC-AVRDC/KU

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Abstract Although the area planted to mungbean in Thailand has declined from over 0.5 million ha to 0.3 million ha during the past decade, the crop still occupied the largest area planted to legumes. All six recommended cultivars grown in the kingdom to date were essentially derived from AVRDC improved lines introduced during the decade of 1976-85. Transferring of mungbean breeding activities to the Asian Regional Center (ARC) located in Kamphaeng Saen Campus of Kasetsart University (KU) in 1993 allowed the scientists from both institutes to practically unite their research projects. Beside developing elite lines to supply to the International Mungbean Nursery, other breeding research activities have also been done. The more interesting topics include breeding for bruchid resistance, breeding for tolerance to iron deficiency, wide crossing and mutant induction by gamma-rays, and investigation on effect of multiple leaflet on major agronomic characters. Funding of the research projects came from Kasetsart Universty, ARC-AVRDC and the Thailand Research Fund.

1 The significance of AVRDC improved mungbean lines to Thailand

Mungbean acreage in Thailand has decreased gradually during to past 10 years. The planted area was well over 0.5 million ha in crop year 1989/1990, dropped down to 0.3 million ha in crop year 1998/99, and expected to become stable ever since (Center for Agricultural Information, 2000). The caused Thailand to lose his position as the world's number one exporter of mungbean and products during the recent years. However, mungbean still occupied the largest planted area in the Kingdom as compared to the area grown to the other legumes. The main reson for the decline in mungbean growing area is competition with the other upland crops, especially sugarcane, corn, cotton and soybean. New improved cultivars of the competitive crops have been released periodically while the most population mungbean cultivars are still 'Kamphaeng Saen1' derived from VC1973A (for dry season planting) and 'Kamphaeng Saen 2'' derived from VC2778A (for rainy season planting). Although being released 15 years ago (in 1986), these two cultivars were

still preferred by Tai farmers due to their high yielding stability.

By the end of 1999, up to 46 breeding lines from the Asian Vegetable Research and Development Center incomes and/or improved nutrition have been made, especially in Thailand, China, Pakistan, Vietnam and Myanmar (Tsou, 2000). In Thailand alone, all 6 major mungbean cultivars were essentially derived from AVRDC improved lines as shown in Table 1(Srinives and Yang, 1978; Srinives, 1990; 1996; 1998; Watanasit et al., 2000).

2 Collaboration between ARC-AVRDC and Thailand

Srinives (1998) emphasized the importance of mungbean in Asia and Australia, with breeding Line VC 1973A was once the most widely grown line in the world. It was released as Kamphaeng 1(KPS 1) in Thailand; as 'Zhoug lu No. 1' and 'Xu Yin No. 1' in China; and 'Seonhwa-nogdu' in the Republic of Korea (Shanmugasundaram, 1998). Dissemination of VC 1973A into the region has been made effectively through either the country collaborators participated in testing of the International Mungbean Nursery (IMN), or through the participants of the Asian Regional Center (ARC) Regional Training Course in Vegetable Production and Research. This demonstrated the importance of collaboration between ARC-AVRDC and Thailand, especially with Kasetsart University (KU), where the ARC is located.

ARC-AVRDC has three major collaborators in Thailand, viz. KU, the Depqrtment of Agriculture (DOA) and Department of Agricultural Extension (DAE). Both departments are attached to Ministry of Agriculture and Cooperatives. Srinives(1998) summarized the joint benefits of this collaboration into 4 items.

1. Utilization of AVRDC mungbean germplasm for trials and release as new cultivars (Table), or as parents in breeding programs.

2. Collaboration with Thailand's mungbean programs, mainly with the DOA's Chai Nat Field Crops Research Center (CNFCRC) and KU.

3. Manpower development through trainings at ARC as well as the AVRDC headquarters.

4. Extension of the elite materials through participants of the Regional Training Course as mentioned above.

Agreement on the collaboration between ARC-AVRDC and KU was spelled out by mungbean breeder of both institutes to jointly the following areas:

1. Resistance to major diseases, especially Cercospora leaf spot and powdery mildew.

2. Resistance to major insect, especially bruchids and pod borers.

3. Genotypes with high yield potential, improved plant type, uniform maturity, and high nutritional seed content.

4. Tolerance to alkaline soils that are deficient in micronutrients.

3 Recent achievement of mungbean breeding at ARC-AVRDC and KU

Collaborative mungbean breeding research between AVRDC and KU began as early as in 1970s, and thus the combined research results are voluminous. The achievement after the establishment of ARC in Thailand in 1992 will be emphasized here. The mungbean research, particularly breeding and plant pathology, was transferred to ARC in 1993. From the founding of AVRDC in 1971 up to 1993, over 6000 *Vigna* crosses (VC) had been developed at AVRDC headquarters. Dr. D. Kim from Korea, the first AVRDC mungbean breeder assigned to Thailand, led the ARC staff in developing more crosses from superior genotypes (Jene-Kritiya et al., 1996). Dr. I. A Malik from Pakistan, who succeeded him, continued to work along the same lines. By the year 2001, ARC have produced over 300 more VCs. Superior derived from the recent crosses were included in the IMNs.

Beside development of mungbean lines, the following breeding research had been achieved during 1993 till to date. The projects were mainly funded by Kasetsart University, ARC-AVRDC and the Thailand Research Fund. Several graduate students and trainees were benefit from these projects.

(1)Breeding for bruchid resistance

There are 2 sources of genes controlling resistance to bruchids (*Callosobruchus maculates* and *C. chinensis*). The first source obtained from a dominant gene available in the wild mungbean *Vigna radiata* var. *aublobata* (TC 1966) conferred qualitative resistance (Fujii et al., 1989). Sarikarin et al. (1999) successfully developed near-isogenic lines (BC9) of KPS 1 and Chai Nat 60 resistance to the bruchids, but could not put them into recommendation for the resistant lines might give adverse effect to consumers. They also found that when texture layer around the seed coat was removed by 15% NaOH, the bruchids laid significantly more eggs than when the layer was kept intact. Although this character has nothing to do with the antibiosis resistance, rough-coated seeds have been proven to harbor less bruchid eggs.

Another resistant source was quantitatively inherited from the accession V 2709 (Talekar, 1989). Duangsong et al. (1993) used Hayman's approach of generation mean analysis to conclude that additive genetic was the most important in conditioning the resistance. However, the lines were not employed in the succeeding breeding programs due to less resistance as compare to the programs of TC 1966.

(2)Breeding for tolerance to iron deficiency

Takhli soil series prevailing the lower northern and the upper central parts of

Thailand is high in pH and resulted in iron deficiency. Srinives et al. (1997) reported that 2 mungbean lines, via. NM 6-97 and NM 10-12 developed by Nuclear Institute for Agriculture and Biology in Pakistan were tolerance to this condition. They found that the F_2-plants from crosses between the tolerance lines and the susceptible cultivars, KPS 1 and KPS 2, segregated at the ratio of 13 tolerance to 3 susceptibility. They concluded that tolerance to iron deficiency in mungbean was controlled by 2 loci of gene with inhibition action. Somanus et al. (2000) have developed a PCR assay for iron deficiency which allowed Srinives and Somanus (2001) to allocate the AFLP markers linked to genes controlling the trait. One locus of gene was found locating between CGT/CTG and CAG/TAC4, franking at the distance of 2.9 and 3.0 cm away.

(3)Wide crossing and mutant induction by gamma-rays

Interspecific hybridization was performed between *Vigna radiata* and related *Vigna* spp. Pod setting percentage was high when using *V. radiata* as female parent. The cross *V. radiata*×*V. radiata* (wild) gave the highest pod setting of 26.1%. Interspecific crosses were successful in *V. radiata*×*V. mungo*, *V. radiata*×*V. mungo* (wild), *V. radiata*×*V. umbellata* (wild), having F_1 seed germination rate of 94.1, 40.3, 6.4, 17.5 and 2.0%, respectively (Ngampongsai et al., 1998). Later, Srinives et al. (1999) took F_2 seeds from the crosses *V. radiata*×*V. radiata* (wild), *V. radiata* (wild)×*V. radiata*, *V. mungo*×*V. mungo* (wild) and *V. mungo* (wild)×*V. mungo* and irradiated with various does of gamma-rays. Mutation was detected in both quantitative and qualitative traits. Among the quantitative traits, early maturity and large-seeded lines were extracted. For qualitative traits, the mutants in cluded leaf mutants (dark green, waxy, multiple, and lpbed), pod mutants (large and top podding), and semi-dwarf plants.

(4)Effect of multiple leaflet on agronomic characters

Near-isogenic lines of mungbean were produced from backcrossing two Thai recommended cultivars, KPS 1 and CN 36, with the multiple leaflet line, V 5926, until BC9. The isogenic lines were evaluated against both recipients to detect of the gene controlling the multiple leaflet on physiological and agronomical characters. It was found that seed yield, plant height and number of pods per plant were greater in the trifoliate leaflet cultivars than the multiple leaflet near-isogenic lines, whereas the numbers of seeds per pod and 1000-seed weights were not significantly different (Kowsurat et al., 1999).

4 Conclusion

All 6 recommend mungbean cultivars grown in Thailand nowadays were essentially derived from AVRDC improved lines. The lines were all developed at AVRDC

headquarters in Taiwan. The mungbean breeding research at ARC-AVRDC and KU were practically united after the establishment of ARC in 1992 followed by transferring of mungbean breeding activities to ARC in 1993. Beside crossing and selecting of elite lines for International Mungbean Nursery, the breeding research activities included basic genetic study, mutation breeding, and exploration of novel traits. The activities that were worth mentioning included breeding for bruchid resistance, breeding for tolerance to iron efficiency, wide crossing and mutant induction by gamma-rays and xploration on effect of multiple leaflet on certain agronomic characters.

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ARC-AVRDC/KU 绿豆育种研究

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摘要 在过去十年,尽管泰国绿豆种植面积由 50×10^4 hm^2 下降到 30×10^4 hm^2,但它仍然是泰国种植面积最大的豆类作物。到目前为止,在泰国推广的 6 个绿豆品种都是 1976—1985 年期间从亚蔬绿豆改良品系中选育的。1993 年,亚蔬中心(AVRDC)将绿豆育种工作从中国台湾转移到位于泰国农业大学(KU)的亚洲区域中心(ARC),从此开始了由 2 个单位科学家联合执行的研究项目。一方面以发展优良品系进行国际绿豆圃(IMN)试验,另外其它育种活动也开始进行。比较感兴趣的课题有抗豆象育种、耐缺铁育种、远缘杂交和 γ-射线诱变效应,以及多叶型绿豆的主要农艺性状调查。研究经费主要来自泰国农业大学、亚洲蔬菜研究与发展中心亚洲区域中心和泰国研究基金。

一、亚蔬绿豆改良品系对泰国绿豆生产发展的重要性

在过去 10 年间泰国绿豆种植面积逐渐减少。据 2000 年泰国农业信息中心报道,1998—1999 年泰国绿豆种植面积从 1989—1990 年的 50×10^4 hm^2,减少到 30×10^4 hm^2。泰国失去了世界第一绿豆及其产品出口国的地位。然而,与其他豆类相比,绿豆仍然是泰国种植面积最大的豆类作物。引起绿豆种植面积减少的主要原因是其它耐旱作物的竞争,尤其是甘蔗、玉米、棉花和大豆。尽管新的绿豆改良品种不断地出现,但种植面积最大的仍然是 Kamphaeng Saen 1(旱季种植)和 Kamphaeng Saen 2(雨季种植)。虽然它们在 15 年前(1986 年)就开始推广,但目前仍是泰国农民首选的高产稳产绿豆品种。

到 1999 年底,已有 46 个亚蔬绿豆育种品系在 22 个国家普及推广,总面积达到 120×10^4 hm^2。有效地提高了农民收入和食物营养水平,尤其是在泰国、中国、巴基斯坦、越南和缅甸(Tsou,2000)。在泰国,6 个推广品种都是从亚蔬绿豆材料中选育的,见表 1(Srinives and Yang,1978;Srinives,1990;1996;1998;Watanasit et al.,2000)。

二、ARC-AVRDC 与泰国之间的合作

Srinives(1998)强调了绿豆在亚洲和澳洲的重要性,VC 1973A 是世界上种植最广的一个绿豆品系。它作为 KPS 1、中绿 1 号和徐引 1 号、Seonhwa-nogdu 分别在泰国、中国、南朝

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鲜推广。通过承担国际绿豆圃试验的合作者和参加 ARC 蔬菜生产和研究培训班的学员，VC 1973A 在各个国家的不同地区进行有效地传播。这证明了 ARC-AVRDC 和泰国尤其是与 ARC 的所在地泰国农业大学合作的重要性。

在泰国，ARC-AVRDC 有 3 个主要合作伙伴。即泰国农业大学(KU)、泰国农业合作部(MAC)的农业局(DOA)和农业推广局(DAE)。Srinives(1998)概述了有关合作在 4 个方面的利益共享：①AVRDC 绿豆种质资源利用，如试验、作为新品种推广或作为亲本材料用于泰国绿豆育种项目(见表 1)；②与泰国农业大学(KU)和 Chai Nat 田间作物研究中心(CNFCRC)合作进行绿豆育种研究；③在亚洲区域中心(ARC)和亚蔬中心(AVRDC)总部进行人才培养；④通过地区培训项目进行优异种质普及推广。

经协商，ARC-AVRDC 和泰国农业大学绿豆育种家在下面几个领域开展合作研究。①抗主要病害，尤其是叶斑病和白粉病；②抗主要害虫，尤其是豆象和豆荚螟；③高产潜力遗传，株型改良，成熟一致和高营养含量；④耐缺少微量元素的碱性土壤。

三、ARC-AVRDC 和泰国农业大学(KU)的最新研究成果

ARC-AVRDC 与泰国农业大学在绿豆育种方面的合作研究，是从 20 世纪 70 年代开始的，并获得了显著成效。这里着重介绍 1992 年 ARC 在泰国成立以后取得的成就。1993 年，AVRDC 的绿豆研究，特别是育种和病理学研究从台湾转移到 ARC(泰国)。1971—1993 年将 6000 多份豇豆属杂交育种材料从 AVRDC 总部(台湾)运到 ARC(Jene-Kritiya et al.，1996)。来自韩国的 D. Kim 博士是 AVRDC 派到泰国的第一个绿豆育种家，他带领 ARC 的工作人员选用优良遗传材料配置更多的杂交组合。之后，来自巴基斯坦的 I. A Malik 博士继续进行这项工作。到 2001 年底，ARC 已有 300 多个新的 VC 系列优良杂交品系进入国际绿豆圃试验(IMN)。

根据绿豆品系的发展，1993 年至今以下几方面的研究是成功的。这个项目主要是由泰国农业大学、ARC-AVRDC 和泰国研究基金资助的。一些研究生和培训人员也受益于这个项目。

(一)抗豆象育种

有两个抗豆象(*Callosobruchus maculates* and *C. chinensis*，四纹豆象和绿豆象)资源，一个来自由显性基因控制的野生绿豆(TC 1966)。1999 年 Sarikarin 等成功地培育出 KPS1 和 Chai Nat 60(BC9)抗豆象近等基因系，但是还未用于生产，因为担心它们对消费者有副作用。他们还发现当种皮周围结构层被 15%氢氧化钠处理后，豆象产卵率明显多于结构层保持完整的种子。尽管这一性状未说明有抗生素的存在，但证明粗糙种皮减少了豆象产卵的场所。

另一个抗源是数量遗传，来自 V 2709(Talekar，1989)。Duangsong 等(1993)利用 Hayman's approach of generation mean analysis 分析方法得出结论，认为 V2709 对豆象的抗性是累加遗传效应。然而这个材料未能成功的用于绿豆抗豆象育种项目，因为它的抗性没有 TC 1966 强。

(二)耐缺铁育种

泰国北部和中上部低洼地区，土壤pH值偏高，铁含量不足。Srinives等(1997)报道巴基斯坦农业生物核原子能研究所(NIABP)培育的NM 6-97 and NM 10-12能忍耐这种缺铁环境。他们发现用耐缺铁品系与KPS1和KPS2杂交，F2群体按13∶3的比率分离，即13个耐、3个感。他们认为绿豆的耐缺铁性由2个遗传基因控制。Somanus等(2000)对绿豆的耐缺铁遗传特性进行了PCR试验，Srinives和Somanus(2001)又进行了AFLP分析，发现一个位点在CGT/CTG和CAG/TAC4之间，距离大约2.9和3.0 cM。

(三)远缘杂交和γ-射线诱变效应

绿豆(*Vigna radiata*)和近缘种*Vigna* spp.杂交，当绿豆作母本时成荚率高。绿豆与野生绿豆杂交时成荚率最高为26.1%。绿豆×黑吉豆、绿豆×野生黑吉豆、绿豆×野生饭豆杂交，F_1种子萌发率分别为94.1、40.9、6.4、17.5和2%(Ngampongsai et al.，1998)。后来，Srinives等(1999)将绿豆×野生绿豆、野生绿豆×绿豆，黑吉豆×野生黑吉豆、野生黑吉豆×黑吉豆杂交F_2种子用不同剂量的γ-射线照射。发现在数量和质量性状上都有变异。在数量性状方面，获得早熟和大粒品系。作为质量性状，诱变体包括叶突变体(浓绿、光滑、多叶、裂叶)，荚突变体(荚大、顶部结荚)和半矮生突变植株。

(四)多叶类型的农艺学效应

用泰国的2个推广品种KPS1和CN36与多叶型种质V5926回交，从BC9中获得绿豆近等位基因系。通过正反交试验，评价控制多叶性状的生理和农艺学特性。发现具三出复叶的栽培品种与多叶近等位基因系在种子产量、植株高度和单株荚数上有很大不同，而单荚粒数和千粒重方面没有显著差异(Kowsurat et al.，1999)。

四、结论

如今在泰国推广的6个绿豆栽培品种实际上都是从AVRDC绿豆改良品系中选育的。这些品系是在台湾AVRDC总部育成的。实际上ARC-AVRDC与泰国农业大学(KU)在绿豆育种方面的合作研究，是1992年ARC成立以后，从1993年开始的。其研究工作主要是通过有性杂交育种手段，选择一些优良品系参加国际绿豆圃试验。另外，其他一些研究活动也在进行，如基础遗传、诱变育种，及一些新性状探索。值得提及的活动有抗豆象育种、耐缺铁育种、远源杂交和诱变育种，以及多叶性农艺性状探讨。