รายงานฉบับสมบูรณ์

โครงการส่งเสริมกลุ่มวิจัยและพัฒนาพันธุ์พืชตระกูลถั่ว

โดย

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และคณะ

ตุลาคม 2543

สัญญาเลขที่ RTA/06/2539

รายงานวิจัยฉบับสมบูรณ์

โครงการส่งเสริมกลุ่มวิจัยและพัฒนาพันธุ์พืชตระกูลถั่ว

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สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

กิตติกรรมประกาศ

โครงการส่งเสริมกลุ่มวิจัยและพัฒนาพันธุ์พืชตระกูลถั่ว ได้รับการสนับสนุนด้านทุนวิจัยจากสำนักงานกองทุนสนับสนุนการวิจัย และได้รับการสนับสนุนด้านสถานที่วิจัย วัสดุ อุปกรณ์ และบุคลากร จากมหาวิทยาลัยเกษตรศาสตร์ และ Asian Regional Center – Asian Vegetable Research and Development Center (ARC-AVRDC)

สรุปย่อสำหรับผู้บริหาร

รหัสโครงการ : RTA/3980006

ชื่อโครงการ : โครงการส่งเสริมกลุ่มวิจัยและพัฒนาพันธุ์พืชตระกูลถั่ว

ระยะเวลาที่ทำการวิจัย : ตุลาคม 2539 – ตุลาคม 2543

วัตถุประสงค์ของโครงการ : เพื่อสร้างนักวิจัยรุ่นใหม่ที่มีความสามารถสูง และสร้างองค์ความรู้เพื่อการวิจัยและพัฒนาพันธุ์พืชตระกูลถั่วผลิตเมล็ด ให้เหมาะสมกับสภาพการเพาะปลูกของประเทศไทย

วิธีการ :

1. การสร้างนักวิจัยรุ่นใหม่ที่มีความสามารถสูง ได้แก่

1.1 การปฏิบัติงานร่วมกันระหว่างเมธีวิจัยอาวุโสกับคณาจารย์รุ่นใหม่ที่เพิ่งจบปริญญาเอก และผู้เชี่ยวชาญจากห้องปฏิบัติการเฉพาะทาง เพื่อทำงานร่วมกันในทุกขั้นตอน ตั้งแต่การตรวจเอกสาร วางแผนงาน ดำเนินการวิจัย วิเคราะห์ข้อมูล สรุปผล ตลอดจนเรียบเรียงเพื่อการตีพิมพ์ร่วมกันในวารสารทางวิชาการระดับนานาชาติ

1.2 การผลิตบัณฑิตระดับปริญญาโทและเอก โดยส่วนใหญ่มีเมธีวิจัยอาวุโสเป็นประธานคณะกรรมการที่ปรึกษา และมีคณาจารย์รุ่นใหม่ตาม 1.1 เป็นกรรมการที่ปรึกษา ร่วมกันฝึกฝนนิสิต-นักศึกษาให้เป็นทั้งคนเก่งและคนดี

2. การสร้างองค์ความรู้เพื่อการวิจัยและพัฒนาพันธุ์พืชตระกูลถั่ว ได้แก่

2.1 การสร้างผลงานให้สามารถตีพิมพ์ในวารสารวิชาการนานาชาติได้

2.2 ผลงานที่ก้าวหน้าพอสมควร แต่ยังไม่ได้นำไปตีพิมพ์ ก็นำเสนอในที่ประชุมวิชาการนานาชาติ หรือในประเทศ

2.3 องค์ความรู้ที่เป็นรูปธรรม ได้แก่ พันธุกรรมใหม่ ๆ ของพืชตระกูลถั่วที่พัฒนาขึ้นมาโดยโครงการฯ สามารถใช้เป็นแหล่งของพันธุกรรมเพื่อการปรับปรุงพันธุ์พืชตระกูลถั่วต่อไปในอนาคต

2.4 การจัดประชุมสัมมนาทางวิชาการและเผยแพร่ผลงานในสื่อต่าง ๆ ที่เหมาะสม

ผลที่ได้รับ :

รายละเอียดดังปรากฏในผลลัพท์ (ouput) ของโครงการฯ

1. ผลงานการสร้างนักวิจัยรุ่นใหม่ที่มีความสามารถสูง

1.1 โครงการฯ มีคณาจารย์รุ่นใหม่ที่เพิ่งจบปริญญาเอกร่วมอยู่ในโครงการ 4 คน และได้ทำงานวิจัยร่วมกันอย่างได้ผลดีมาโดยตลอด

1.2 ตั้งแต่โครงการฯ ได้เริ่มดำเนินการมาในเดือนตุลาคม 2539 มีนักศึกษาเป็นจำนวนมากติดต่อขอทำวิทยานิพนธ์ในโครงการฯ ขณะนี้มีผู้จบการศึกษาแล้ว 5 คน เป็นระดับปริญญาเอก 1 คน ปริญญาโท 4 คน ผู้ที่จบการศึกษา 3 คน (เอก 1 คน โท 2 คน) กลับไปทำงานในหน่วยราชการในสังกัดเดิมและได้รับตำแหน่งสูงขึ้น อีก 2 คนที่ยังไม่มีงานทำก่อนการศึกษาเมื่อจบแล้วคนหนึ่งได้งานที่บริษัทเจียไต๋เมล็ดพันธุ์ จำกัด อีกคนหนึ่งได้งานที่ศูนย์วิจัยและพัฒนาเทคโนโลยีชีวภาพ กรมวิชาการเกษตร

2. ผลงานการสร้างองค์ความรู้เพื่อการวิจัยและพัฒนาพันธุ์พืชตระกูลถั่ว

2.1 โครงการฯ ได้มีผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ 8 เรื่อง ดังแสดงไว้ในภาคผนวก

2.2 โครงการฯ ได้เสนอผลงานในที่ประชุมนานาชาติ 7 เรื่อง เป็นการเสนอภาคบรรยาย 5 เรื่อง และภาคโปสเตอร์ 2 เรื่อง จากการประชุมนานาชาติ 5 ครั้ง ใน 5 ประเทศ

ได้เสนอผลงานในที่ประชุมวิชาการในประเทศทั้งสิ้น 17 รายการ เป็นภาคบรรยาย 9 รายการ ภาคโปสเตอร์ 8 รายการ บางรายการได้นำเสนอผลงานวิจัยมากกว่า 1 เรื่อง

2.3 ได้สร้างพันธุกรรมใหม่ ๆ ของถั่วเขียวและถั่วเหลืองกว่า 10 ลักษณะ โดยมีวัตถุประสงค์เพื่อ (1) ส่งเสริมให้เกษตรกรใช้โดยตรง (2) เพื่อให้นักปรับปรุงพันธุ์พืชนำไปใช้ประโยชน์ต่อไป และ (3) เพื่อใช้เป็นตัวอย่างในการศึกษาด้านพันธุศาสตร์และการปรับปรุงพันธุ์พืชของนิสิต-นักศึกษา ในระดับมหาวิทยาลัยต่อไป

2.4 นอกจากนี้โครงการฯ ยังได้เชิญนักวิชาการผู้มีชื่อเสียงในระดับนานาชาติและระดับประเทศ มาร่วมสัมมนากับนักวิจัยในโครงการฯ นักศึกษา และผู้สนใจรวม 14 ครั้ง เกิดประโยชน์เป็นอย่างมากต่อการวิจัยและพัฒนาพันธุ์พืชของประเทศไทย

โครงการฯ ยังได้นำเสนอผลงานทางวิทยุกระจายเสียงต่าง ๆ เพื่อให้ความรู้แก่ประชาชนในด้านการปรับปรุงพันธุ์และพืชตระกูลถั่ว ปีละประมาณ 2 ครั้ง อีกด้วย

Executive Summary

Project Code : RAT/06/2539

Project Title : Project on Promotion of Legume Cultivar Research and Development Team

Project Duration : October 1996 – October 2000

Project Objectives : To foster and produce high caliber researchers and to produce body of knowledge in legume cultivar research and development appropriate to Thailand

Methodology :

1. To foster and produce high caliber researchers.

1.1 The Senior Research Scholar has worked closely with new Ph.D. graduates and researchers in specific labs in all research aspects, beginning from reviewing of literature, planning and conducting of research, data analyses and interpretation, until writing up for possible publication in international journals.

1.2 The Senior Research Scholar, with the researchers in 1.1, helped guiding M.S. and Ph.D. students during their course of study. The aim is to produce graduates high in both knowledge and moral.

2. To produce body of knowledge in legume cultivar research and development appropriate to Thailand.

2.1 To produce research work of high quality for publication in international standard journals.

2.2 The on-going research which is not in the publication stage, can be presented in either international or domestic meetings.

2.3 To produce novel legume genotypes for future use in cultivar improvement.

Research Output :

Detial of the research output is given in the text.

1. High caliber researchers produced

1.1 Four new Ph.D. holders have worked smoothly in the project and made continual contribution to all research activities.

1.2 Since the inception in October 1996 a number of graduate students have proposed to work with the project. At the moment, the project has produced 5 graduates, 1 Ph.D. and 4 M.S. Three of them (1 Ph.D. and 2 M.S.) went back to their own governmental offices and were promoted. One M.S. graduate found a plant beeding job at Chia Tai Seed Co. Ltd., the other one got a job at Biotechnology Research and Development Center, Department of Agriculture.

2. Body of knowledge produced

2.1 The project has published 8 articles in international journals as shown in the appendix.

2.2 The project has presented 7 articles in 5 international meetings in 5 countries. These included 5 oral presentations and 2 posters.

2.3 Over 10 novel mungbean and soybean genotypes were produced from the project with the following aims : (1) to extend directly to the farmers (2) to be utilized as a germplasm source for legume breeders, and (3) to be utilized as materials for the study in plant genetics and breeding by students.

2.4 The project has invited experienced scientists from overseas and within the country to give altogether 14 seminars to researchers, graduate students in the project, and interested audience. The activity is definitely beneficial to plant cultivar research and development in Thailand.

The project also presented its activity through radio stations about twice a year.

Research Output (จากทุนเมธีวิจัยอาวุโส สกว.)

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ

1.1 Ubonprasert B, Srinives P, Lamseejan S, Prabuddham P, Peyachoknagul S. Effects of gamma radiation of soybean cotyledon explants on embryo induction and regeneration in a somatic embryo cycling system. SABRAO J. Breed & Gen. 1998 ; 30 (1) : 19-24. (เอกสารแนบ 1)

1.2 Ngampongsai S, Srinives P, Lamseejan S, Peyachoknagul S. Interspecific hybridization of mungbean [*Vigna radiata* (L.) Wilczek] and related *Vigna* species using embryo rescue techniques. J. ISSAAS 1998 ; 4(2) 98-104. (เอกสารแนบ 2)

1.3 Yan QS, Tongpamnak P, Srinives P, Opena RT. Identification of RAPD marker associated with downy mildew resistance in soybean using bulked segregant analysis of near-isogenic lines. Thai J. Agric. Sci. 1998 ; 31(4) : 527-532. (เอกสารแนบ 3)

1.4 Kowsurat S, Srinives P, Kasemsap P, Lamseejan S. Effects of the multiple leaflet gene on agronomical and physiological characters of mungbean (*Vigna radiata*). J. Agric. Sci, Cambridge. 1999 ; 133 : 321-324. (เอกสารแนบ 4)

1.5 Sarikarin N, Srinives P, Kaveeta R, Saksoong P. Effect of seed texture layer on bruchid infestation in mungben (*Vigna radiata* (L.) Wilczek). Sci. Asia. 1999 ; 25 : 203-206. (เอกสารแนบ 5)

1.6 Xu PW, Srinives P. Perspective and economic analysis of virus-free garlic in commercial production. Thai J. Agric. Sci. 1999 ; 32 (in press) (เอกสารแนบ 6)

1.7 Xu PW, Srinives P, Yang CL. Rapid multiplication of virus-free garlic by inflorescence meristem culture and induction of multi-bulbils. Thai J. Agric. Sci. 1999 ; 32 (in press) (เอกสารแนบ 7)

1.8 Pornprom T, Surawattananon S, Srinives P. Ammonia accumulation as an index of glufusinate-tolerant soybean cell lines. Pestic. Biochem. Physiol. 2000 ; 68 (in press). (เอกสารแนบ 8)

2. **ผลงานตีพิมพ์ในวารสารวิชาการในประเทศไทย**

ไม่มี ถ้านับ Thai J. Agric. Sci. และ Sci. Asia เป็นวารสารวิชาการนานาชาติ

3. **หนังสือ**

ไม่มี

4. **การจดทะเบียนสิทธิบัตร**

ไม่มี

5. **การเสนอผลงานในที่ประชุมวิชาการนานาชาติ**

ได้เสนอผลงานแล้ว 7 เรื่อง ดังนี้

5.1 Srinives P. Collaborative mungbean breeding research between AVRDC and its Southeast Asian partners with emphasis to Thailand. Workshop on International Mungbean Consultation, Indian Agricultural Research Institute, New Delhi, India. 7-11 September 1997. (เอกสารแนบ 9)

5.2 Srinives P, Nopparat S, Kaveeta R, Jintakanon S. An inheritance of mungbean tolerance to microessential element deficiency in Takhli soil series. The VIII General Congress, Society for the Advancement of Breeding Researches in Asia and Oceania (SABRAO), Seoul Olympic Parktel, Seoul, Korea Rep. 24-28 September 1997. (oral) (เอกสารแนบ 10)

5.3 Lamseejan S, Ubonprasert B, Srinives P, Wongpiyasatid A, Jompook P. *In vitro* selection for tolerance to iron deficiency in soybean. The VIII General Congress, Society for the Advancement of Breeding Researches in Asia and Oceania (SABRAO), Seoul Olympic Parktel, Seoul, Korea Rep. 24-28 September 1997. (poster) (เอกสารแนบ 11)

5.4 Srinives P, Sanitchon J, Sriphadej S. Genetic resistance of *Fusarium solani* and *Macrophomina phaseolina* causing soybean sudden death syndrome in Thailand. World Soybean Research Conference VI, Sheraton Chicago Hotel and Towers, Chicago, Illinois, USA. 4-7 August 1999. (oral) (เอกสารแนบ 12)

5.5 Yan QS, Tongpumnuk P, Srinives P, Opena RT. Preliminary identification of RAPD markers linked to downy mildew resistance in soybean by using near isogenic line. World Soybean Research Conference VI, Sheraton Chicago Hotel and Towers, Chicago, Illinois, USA. 4-7 August 1999. (poster) (เอกสารแนบ 13)

5.6 Srinives P, Hual-alai N, Saengchot S, Ngampongsai S. The use of wild relatives and gamma radiation in mungbean and blackgram breeding. The 7th MAFF International Workshop on Genetic Resources. National Institute of Agrobiological Resources, Tsukuba. Japan. 13-15 October 1999. (เอกสารแนบ 14)

5.7 Pornprom T, Surawattananon S, Srinives P. Isolation and characterization of glufosinate tolerance in soybean cell lines. Proceedings I (B) : The 17th Asian-Pacific Weed Science Society Conference. Bangkok, Thailand. 22-27 November 1999. (เอกสารแนบ 15)

6. การเสนอผลงานในที่ประชุมวิชาการในประเทศไทย

ได้เสนอผลงานรวม 17 รายการ ดังนี้

6.1 พีระศักดิ์ ศรีนิเวศน์. งานวิจัยจีโนมพืชตระกูลถั่ว. การประชุมระดมความคิดเรื่องการวิจัยจีโนมพืช. มหาวิทยาลัยเกษตรศาสตร์ กทม. 8 สิงหาคม 2540. (เอกสารแนบ 16)

6.2 นงนุช สาริการินทร์, พีระศักดิ์ ศรีนิเวศน์, วรวิทย์ โสรัจจาภินันท์, สนั่น ริ้วทองซุ่ม, เหี้ยมหาญ เจเถื่อน. การพัฒนาสายพันธุ์ถั่วเขียวคู่แฝดที่ต้านทานด้วงเจาะเมล็ด. น. 50-58. ใน รายงาน การประชุมวิชาการถั่วเขียวแห่งชาติครั้งที่ 7. โรงแรมโกลเดนแกรนด์ จ.พิษณุโลก. 2-4 ธันวาคม 2540. (เอกสารแนบ 17)

6.3 สุมณฑา นพรัตน์, พีระศักดิ์ ศรีนิเวศน์, รังสฤษดิ์ กาวีต๊ะ, สุรเดช จินตกานนท์. การถ่ายทอดทางพันธุกรรมของลักษณะความทนทานต่อดินด่างชุดตาคลีในถั่วเขียว. น. 78-82. ใน รายงาน การประชุมวิชาการถั่วเขียวแห่งชาติครั้งที่ 7. โรงแรมโกลเดนแกรนด์ จ.พิษณุโลก. 2-4 ธันวาคม 2540. (เอกสารแนบ 18)

6.4 สมใจ โควสุรัตน์, พีระศักดิ์ ศรีนิเวศน์, พูนพิภพ เกษมทรัพย์, Doo Hwan Kim. ผลของยีนควบคุมลักษณะหลายใบย่อยต่อลักษณะทางสรีรวิทยาและทางพืชไร่ของถั่วเขียว. น. 88-99 ใน รายงาน การประชุมวิชาการถั่วเขียวแห่งชาติครั้งที่ 7. โรงแรมโกลเดนแกรนด์ จ.พิษณุโลก. 2-4 ธันวาคม 2540. (เอกสารแนบ 19)

6.5 วารุณี โสมนัส, พีระศักดิ์ ศรีนิเวศน์, อภิชาติ วรรณวิจิตร, สมวงษ์ ตระกูลรุ่ง, จุลภาค คุ้นวงศ์. การใช้เทคนิค RFLP เพื่อคัดเลือกสายพันธุ์ถั่วเขียวต้านทานด้วงเจาะเมล็ด. น. 220-224 *ใน* รายงาน การประชุมวิชาการถั่วเขียวแห่งชาติครั้งที่ 7. โรงแรมโกลเดนแกรนด์ จ.พิษณุโลก. 2-4 ธันวาคม 2540. (ภาคโปสเตอร์) (เอกสารแนบ 20)

6.6 สุมนา งามผ่องใส, สมยศ พิชิตพร, สมทรง โชติชื่น, วิไลวรรณ พรหมคำ, พีระศักดิ์ ศรีนิเวศน์. การผสมข้ามชนิดระหว่างถั่วเขียวกับถั่วในสกุล *Vigna* ร่วมกับการใช้เทคนิคการเพาะเลี้ยงเอมบริโอ. น. 225-239. *ใน* รายงาน การประชุมวิชาการถั่วเขียวแห่งชาติครั้งที่ 7. โรงแรมโกลเดนแกรนด์ จ.พิษณุโลก. 2-4 ธันวาคม 2540. (ภาคโปสเตอร์) (เอกสารแนบ 21)

6.7 จานุลักษณ์ ขนบดี, พีระศักดิ์ ศรีนิเวศน์, รังสฤษดิ์ กาวีต๊ะ, อนันตชัย เชื่อนธรรม. การคัดเลือกสายพันธุ์ถั่วเหลืองให้มีโปรตีนในเมล็ดสูง. น. 1-8 *ใน* รายงาน การประชุมทางวิชาการของมหาวิทยาลัยเกษตรศาสตร์ครั้งที่ 36. มหาวิทยาลัยเกษตรศาสตร์ กทม. 3-5 กุมภาพันธ์ 2541. (เอกสารแนบ 22)

6.8 ร่วมแสดงนิทรรศการ Cyber-TechnoMart กับสำนักงานพัฒนาวิทยาศาสตร์และเทคโนโลยีแห่งชาติ เมื่อเดือนสิงหาคม 2541 จำนวน 2 เรื่อง คือ

- การพัฒนาสายพันธุ์ถั่วเขียวต้านทานด้วงเจาะเมล็ด
- การพัฒนาสายพันธุ์ถั่วเหลืองต้านทานโรคที่สำคัญ

6.9 ร่วมแสดงผลงานในฐานะเมธีวิจัยอาวุโส สกว. ในงานนิทรรศการสัปดาห์วิทยาศาสตร์แห่งชาติ ประจำปี 2541 ระหว่างวันที่ 18-22 สิงหาคม 2541 ณ ศูนย์ประชุมแห่งชาติสิริกิติ์ โดยนำเสนอโปสเตอร์ 6 เรื่องคือ

- การใช้เทคนิค RFLP เพื่อคัดเลือกสายพันธุ์ถั่วเขียวต้านทานด้วงเจาะเมล็ด
- การพัฒนาพันธุกรรมถั่วเขียวของไทยโดยใช้วิธีผสมข้ามพันธุ์ป่ากับการฉายรังสี
- การชักนำให้เกิดยอดหลายยอดในถั่วเขียว
- พันธุกรรมที่ต้านทานต่อเชื้อ *Fusarium solani* และ *Macrophomina phaseolina* ในถั่วเหลือง
- การคัดเลือกสายพันธุ์ถั่วเหลืองที่เมล็ดพันธุ์มีอายุการเก็บรักษาได้ยาวนาน
- การพัฒนาสายพันธุ์ถั่วเหลืองโปรตีนสูง

6.10 เสาวณีย์ เหลืองทองอร่าม, ถวัลย์ศักดิ์ เผ่าสังข์, รังสฤษดิ์ กาวีต๊ะ, ประดิษฐ์ พงศ์ทองคำ, พีระศักดิ์ ศรีนิเวศน์. การเกิดคัพภะจากเนื้อเยื่อใบเลี้ยงอ่อนของถั่วเหลือง. การประชุมวิชาการถั่วเหลืองแห่งชาติครั้งที่ 7. มหาวิทยาลัยสุโขทัยธรรมาธิราช อ.ปากเกร็ด จ.นนทบุรี. 25-27 สิงหาคม 2541. (เอกสารแนบ 23)

6.11 สุขุมาภรณ์ ศรีเผด็จ, พีระศักดิ์ ศรีนิเวศน์, รังสฤษดิ์ กาวีต๊ะ, ศรีสุข พูนผลกุล. การศึกษาพันธุกรรมถั่วเหลืองที่ต้านทานต่อเชื้อ *Fusarium solani* และ *Macrophomina phaseolina* ในถั่วเหลือง. การประชุมวิชาการถั่วเหลืองแห่งชาติครั้งที่ 7. มหาวิทยาลัยสุโขทัยธรรมาธิราช อ.ปากเกร็ด จ.นนทบุรี. 25-27 สิงหาคม 2541. (ภาคโปสเตอร์) (เอกสารแนบ 24)

6.12 สุริยน สุภาพ, พีระศักดิ์ ศรีนิเวศน์, ภาณี ทองพำนัก, วรวิทย์ โสรัจจาภินันท์, สนั่น ริ้วทองซุ่ม, เหี้ยมหาญ เจเถื่อน, วันเฉลิม ฐีตานนท์. การคัดเลือกสายพันธุ์ถั่วเหลืองที่เมล็ดพันธุ์มีอายุการเก็บรักษาได้ยาวนาน. การประชุมวิชาการถั่วเหลืองแห่งชาติครั้งที่ 7. มหาวิทยาลัยสุโขทัยธรรมาธิราช อ.ปากเกร็ด จ.นนทบุรี. 25-27 สิงหาคม 2541. (ภาคโปสเตอร์) (เอกสารแนบ 25)

6.13 สมจิตร์ ใจวังเย็น, พีระศักดิ์ ศรีนิเวศน์, รังสฤษดิ์ กาวีต๊ะ, อนันต์ชัย เชื่อนธรรม, จานุลักษณ์ ขนบดี. การพัฒนาสายพันธุ์ถั่วเหลืองโปรตีนสูง. การประชุมวิชาการถั่วเหลืองแห่งชาติครั้งที่ 7 ม.สุโขทัยธรรมาธิราช อ.ปากเกร็ด จ.นนทบุรี. 25-27 สิงหาคม 2541. (ภาคโปสเตอร์) (เอกสารแนบ 26)

6.14 นภาพร หวลอาลัย, พีระศักดิ์ ศรีนิเวศน์, วรวิทย์ โสสัจจาภินันท์, สนั่น ริ้วทองซุ่ม. ผลของรังสีแกมมาต่อความแปรปรวนของสายพันธุ์ถั่วเขียวที่เกิดจากการผสมระหว่างถั่วเขียวพันธุ์ปลูกกับพันธุ์ป่า. การประชุมวิชาการถั่วเขียวแห่งชาติ ครั้งที่ 8 มหาวิทยาลัยเกษตรศาสตร์ อ.กำแพงแสน จ.นครปฐม. 18-20 มกราคม 2543. (เอกสารแนบ 27)

6.15 วารุณี โสมนัส, พีระศักดิ์ ศรีนิเวศน์, อภิชาติ วรรณวิจิตร, สมวงษ์ ตระกูลรุ่ง, จุลภาค คุ้นวงศ์. พันธุกรรมที่ควบคุมลักษณะทนทานต่อการขาดธาตุเหล็กในกลุ่มสายพันธุ์ถั่วเขียว. การประชุมวิชาการถั่วเขียวแห่งชาติ ครั้งที่ 8 มหาวิทยาลัย อ.กำแพงแสน จ.นครปฐม. 18-20 มกราคม 2543. (เอกสารแนบ 28)

6.16 ฉัตรนภา ข่มอาวุธ, สนธิชัย จันทร์เปรม, พีระศักดิ์ ศรีนิเวศน์. การเพาะเลี้ยงเนื้อเยื่อถั่วเขียวเพื่อการถ่ายยีน. การประชุมวิชาการถั่วเขียวแห่งชาติ ครั้งที่ 8 มหาวิทยาลัยเกษตรศาสตร์ อ.กำแพงแสน จ.นครปฐม. 18-20 มกราคม 2543. (เอกสารแนบ 29)

16.7 สุภาวิณี แสงโชติ, พีระศักดิ์ ศรีนิเวศน์, รังสฤษดิ์ กาวีต๊ะ, สิรนุช ยามศรีจันทร์. การศึกษาวิธีเพิ่มการติดเมล็ดในลูกผสมข้ามชนิดระหว่าง *Vigna* spp. การประชุมวิชาการถั่วเขียวแห่งชาติ ครั้งที่ 8 มหาวิทยาลัยเกษตรศาสตร์ อ.กำแพงแสน จ.นครปฐม. 18-20 มกราคม 2543. (ภาคโปสเตอร์) (เอกสารแนบ 30)

7. ผลงานอื่น ๆ

7.1 ผลงานการสร้างพันธุกรรม

ได้สร้างพันธุกรรมของถั่วเขียวและถั่วเหลือง (1) เพื่อแก้ปัญหาให้แก่เกษตรกรโดยตรง (2) เพื่อให้นักปรับปรุงพันธุ์พืชนำไปใช้เป็นแหล่งพันธุกรรมเพื่อถ่ายทอดให้กับพันธุ์อื่น ๆ ในโครงการปรับปรุงพันธุ์ และ (3) เพื่อใช้เป็นตัวอย่างในการศึกษาทางพันธุศาสตร์เกษตรและการปรับปรุงพันธุ์พืชในระดับมหาวิทยาลัย

พันธุกรรมดังกล่าวมีดังนี้

พันธุกรรมใหม่ของถั่วเขียวที่พัฒนาโดยโครงการฯ

- ได้ถ่ายทอดยีนควบคุมความต้านทานต่อด้วงเจาะเมล็ด จากพันธุ์ป่า TC1966. ให้แก่ถั่วเขียวพันธุ์ดีของไทย 2 พันธุ์ คือ กำแพงแสน 1 และซัยนาท 60 ได้สายพันธุ์คู่แฝด (near-isogenic line) ซึ่งมีความต้านทานสูงต่อด้วงเจาะเมล็ด แต่ยังไม่สามารถแนะนำเป็นอาหารมนุษย์ได้ เนื่องจากยังไม่ได้ผ่านการทดสอบความปลอดภัยของสารที่ให้ความต้านทานต่อด้วง (Vignatic acids)
- ได้ถ่ายทอดยีนควบคุมความทนทานต่อการปลูกในสภาพดินด่างชุดตาคลี จากพันธุ์ NM6-97 และ NM 10-12 ของปากีสถาน ให้แก่พันธุ์กำแพงแสน 1 และกำแพงแสน 2 ซึ่งจะทำให้พันธุ์ทั้งสองสามารถขึ้นได้เป็นปกติในสภาพดินด่าง
- การพัฒนาถั่วเขียวให้มีใบย่อยหลาย ๆ ใบ เพื่อให้ได้พื้นที่ใบในการสังเคราะห์แสงมากขึ้น และอาจใช้ประโยชน์จากใบเพื่อใช้เลี้ยงสัตว์อีกด้วย
- การนำถั่วเขียวพันธุ์ป่ามาผสมพันธุ์กับพันธุ์ปลูก ทั้งถั่วเขียวธรรมดาและถั่วเขียวผิวดำร่วมกับการฉายรังสี ทำให้ได้พันธุกรรมใหม่ ๆ เกิดขึ้นกว่า 10 ลักษณะ ขณะนี้อยู่ในระหว่างการคัดสายพันธุ์ให้บริสุทธิ์ เพื่อนำมาศึกษาโดยละเอียดต่อไป

พันธุกรรมใหม่ของถั่วเหลืองที่พัฒนาโดยโครงการฯ

- ได้พัฒนาสายพันธุ์ถั่วเหลือง Kasetsart University Soybean Line (KUSL) 20004 จากคู่ผสม Clark 63 x Orba ซึ่งเมื่อตรวจสอบแล้ว พบว่ามีลักษณะดีเด่นหลายประการ เช่น ต้านทานโรคราน้ำค้าง โรค bacterial pustule และโรค sudden death syndrome ในระดับที่น่าพอใจ อีกทั้งยังเกิดปมได้ดีกับไรโซเบียมชนิดต่าง ๆ อีกด้วย นับเป็นแหล่งพันธุกรรมที่มีค่ายิ่งสำหรับใช้ในการปรับปรุงพันธุ์ถั่วเหลืองของไทยในอนาคต

- ได้ถ่ายทอดยีนต้านทานโรคราน้ำค้างจากสายพันธุ์ AGS129 ให้แก่พันธุ์นครสวรรค์ 1 ซึ่งอ่อนแอต่อโรคนี้ ทำให้ได้สายพันธุ์ต้านทาน ซึ่งสามารถใช้ปลูกเป็นการค้าต่อไป และใช้ในการศึกษาด้านพันธุวิศวกรรมของความต้านทานได้อีกด้วย

- ได้พัฒนาสายพันธุ์ถั่วเหลืองที่มีโปรตีนสูงกว่า 45% เพื่อเป็นแหล่งพันธุกรรมในการถ่ายทอดยีนโปรตีนสูง ให้แก่ถั่วเหลืองพันธุ์ไทยต่อไป ถั่วเหลืองโปรตีนสูงมีประโยชน์ทั้งใช้เป็นอาหารมนุษย์และอาหารสัตว์

- อื่น ๆ ได้แก่ การพัฒนาสายพันธุ์ถั่วเหลืองที่เมล็ดรักษาความงอกได้นาน มีกลิ่นเหม็นเขียวน้อยลง ย่อยง่ายขึ้น เกิดปมกับไรโซเบียมได้ดี ต้านทานต่อโรค sudden death syndrome สูงขึ้น เป็นต้น

7.2 การจัดสัมมนาพิเศษ

โครงการฯ ได้เชิญนักวิชาการในประเทศและนานาชาติ มาร่วมสัมมนากับนักวิจัยในโครงการและผู้สนใจ เป็นระยะ ๆ รวม 14 ครั้ง ดังนี้

- 20 สิงหาคม 2540 ; Dr. Christopher J. Lambrides, Researcher จาก CSIRO, Cunningham Laboratory, Queensland, Australia บรรยายเรื่อง Breeding for bruchid and mungbean yellow mosaic virus resistance in mungbean using molecular markers

- 17 ตุลาคม 2540 ; Dr. John Kuspira, Professor in Plant Cytogenetics and Breeding, University of Alberta, Canada บรรยายเรื่อง Phylogeny of polyploid wheat

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- 21 พฤษภาคม 2541 ; น.ส.เนตรชนก นุ้ยสีรุ่ง นักวิจัยของสถาบันวิจัยและพัฒนาฯ ม.เกษตรศาสตร์ บรรยายเรื่อง Inheritance of certain characters of mungbean and breeding for resistance to Cercospora leaf spot

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- 30 ธันวาคม 2541 ; Dr. Toshifumi Murakami, Researcher, Nagano Chushin Agricultural Experiment Station, Japan บรรยายเรื่อง Development of low input technology for crop production in Nagano, Japan

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- 23 กรกฎาคม 2542 ; ดร.ธีรยุทธ ตู้จินดา นักวิจัย ศูนย์พันธุวิศวกรรมและเทคโนโลยีชีวภาพแห่งชาติ บรรยายเรื่อง Quantitative traits : From genome to breeding

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7.3 การผลิตบัณฑิต

โครงการฯ ได้รับความนิยมจากนักศึกษาขั้นปริญญาโทและเอกเป็นอย่างมาก จนต้องจำกัดจำนวนนักศึกษาที่ประสงค์จะมาทำวิทยานิพนธ์ในโครงการฯ ขณะนี้มีนักศึกษาจบการศึกษาไปแล้ว 5 คน เป็นระดับปริญญาเอก 1 คน ปริญญาโท 4 คน ผู้ที่จบการศึกษา 3 คน (เอก 1 คน โท 2 คน) กลับไปทำงานในหน่วยงานราชการในสังกัดเดิมและได้รับตำแหน่งสูงขึ้น อีก 2 คนที่ยังไม่มีงานทำก่อนการศึกษา คนหนึ่งได้งานเป็นนักปรับปรุงพันธุ์พืชในบริษัทเจียไต๋เมล็ดพันธุ์ จำกัด ส่วนอีกคนหนึ่งได้งานในตำแหน่งผู้ช่วยนักวิจัย ที่สำนักวิจัยและพัฒนาเทคโนโลยีชีวภาพ กรมวิชาการเกษตร

ขณะนี้มีผู้ที่กำลังศึกษาอยู่ในโครงการฯ เป็นระดับปริญญาเอก 3 คน ปริญญาโท 9 คน ซึ่งคาดว่าเมื่อจบการศึกษาแล้ว จะเป็นกำลังสำคัญในการวิจัยและพัฒนาด้านพันธุศาสตร์และการปรับปรุงพันธุ์พืชของไทยในอนาคตเช่นกัน

7.4 การเผยแพร่ผลงานแบบอื่น ๆ

เสนอผลงานของโครงการฯ ผ่านรายการคลื่นอนาคต ทางวิทยุ จ.ส. 100 จำนวน 2 ครั้ง คือ เรื่องโครงการพัฒนาพันธุ์พืชตระกูลถั่วในประเทศไทย และงานวิจัยจีโนมในพืชตระกูลถั่ว วิทยุประเทศไทยกรมประชาสัมพันธ์ 1 ครั้ง เรื่อง GMOs วิทยุประเทศไทยกาญจนบุรี 1 ครั้ง เรื่องการปลูกถั่วเขียว และวิทยุ มก. 2 ครั้ง คือ เรื่อง บทบาทของโครงการฯ ในการพัฒนาพันธุ์ตระกูลถั่วให้แก่เกษตรกรไทย และบทบาทของโครงการฯ ในการสร้างนักวิจัยด้านการปรับปรุงพันธุ์พืชให้แก่ประเทศไทย

ตีพิมพ์บทความเรื่อง การลงทุนด้านการวิจัย ผลตอบแทนคุ้มเกินคุ้ม ว.ประชาคมวิจัย 22 : 17-21. (เอกสารแนบ 31)

รายชื่อผู้ทำงานในโครงการ

ชื่อ - นามสกุล	ตำแหน่งวิชาการ		ต้นสังกัด			ตำแหน่งในโครงการ	สถานภาพปัจจุบัน
	เมื่อเข้าร่วมโครงการ	ปัจจุบัน	ภาควิชา	คณะ	มหาวิทยาลัย/สถาบัน		
1. ดร.พีระศักดิ์ ศรีนิเวศน์	ศาสตราจารย์	ศาสตราจารย์	พืชไร่นา	เกษตร	เกษตรศาสตร์	หัวหน้าโครงการ	หัวหน้าโครงการ
2. ดร.อภิชาติ วรรณวิจิตร[1/]	ผู้ช่วยศาสตราจารย์	ผู้ช่วยศาสตราจารย์	พืชไร่นา	เกษตร	เกษตรศาสตร์	ผู้ร่วมวิจัย/ที่ปรึกษา	ยังร่วมโครงการ
3. ดร.จุลภาค คุ้นวงศ์[1/]	อาจารย์	ผู้ช่วยศาสตราจารย์	พืชสวน	เกษตร	เกษตรศาสตร์	ผู้ร่วมวิจัย/ที่ปรึกษา	ยังร่วมโครงการ
4. ดร.สมวงษ์ ตระกูลรุ่ง[1/]	นักวิจัย	นักวิจัย	หน่วย DNA fingerprinting	ศูนย์พันธุฯ	สวทช.	ผู้ร่วมวิจัย/ที่ปรึกษา	ยังร่วมโครงการ
5. ดร.ถวัลย์ศักดิ์ เผ่าสังข์	อาจารย์	อาจารย์	พืชไร่นา	เกษตร	เกษตรศาสตร์	นักวิจัย	ขอลาออกไปเป็น หัวหน้าโครงการมะม่วง
6. ดร.ทศพล พรพรหม	อาจารย์	ผู้ช่วยศาสตราจารย์	พืชไร่นา	เกษตร	เกษตรศาสตร์	นักวิจัย	ยังร่วมโครงการ
7. ดร.สนธิชัย จันทร์เปรม	อาจารย์	ผู้ช่วยศาสตราจารย์	พืชไร่นา	เกษตร	เกษตรศาสตร์	นักวิจัย	ยังร่วมโครงการ
8. ดร.วิทิตร ใจอารีย์	อาจารย์	อาจารย์	พืชไร่นา	เกษตร	เกษตรศาสตร์	นักวิจัย	ยังร่วมโครงการ
9. น.ส.อรอุมา รุ่งน้อย	—	อาจารย์	เทคโนโลยีการผลิตพืช	เทคโนโลยีการเกษตร	พระจอมเกล้าลาดกระบัง	ผู้ช่วยวิจัย	ไม่ได้ร่วมโครงการแล้ว
10. น.ส.ลัดดาวัลย์ แจ่มจำรัส	—	นักวิชาการ	—	—	บริษัทลัดดา จำกัด	ผู้ร่วมวิจัย	ไม่ได้ร่วมโครงการแล้ว
11. นางสุมนา งามผ่องใส	นักวิชาการเกษตร 5	นักวิชาการเกษตร 7	ศูนย์วิจัยพืชไร่ชัยนาท	สถาบันวิจัยพืชไร่	กรมวิชาการเกษตร	นักศึกษาปริญญาโท	จบการศึกษาและกลับต้นสังกัดแล้ว
12. นางสมใจ โควสุรัตน์	นักวิชาการเกษตร 5	นักวิชาการเกษตร 6	ศูนย์วิจัยพืชไร่อุบลราชธานี	สถาบันวิจัยพืชไร่	กรมวิชาการเกษตร	นักศึกษาปริญญาโท	จบการศึกษาและกลับต้นสังกัดแล้ว
13. น.ส.นงนุช สาริการินทร์	—	นักปรับปรุงพันธุ์พืช	—	สถานีทดลองกาญจนบุรี	บริษัทเจียไต๋ จำกัด	นักศึกษาปริญญาโท	จบการศึกษาและได้งานภาคเอกชน
14. นายสุรพงษ์ ประสิทธิ์วัฒนเสรี	—	นักวิชาการเกษตร 3	ศูนย์วิจัยพืชไร่นครสวรรค์	สถาบันวิจัยพืชไร่	กรมวิชาการเกษตร	นักศึกษาปริญญาโท	นักศึกษาปริญญาโท
15. น.ส.วารุณี โสมนัส	—	นักศึกษาปริญญาโท	พืชไร่นา	เกษตร	เกษตรศาสตร์	นักศึกษาปริญญาโท	นักศึกษาปริญญาโท
16. น.ส.เสาวณีย์ เหลืองทองอร่าม	—	—	พืชไร่นา	เกษตร	เกษตรศาสตร์	นักศึกษาปริญญาโท	จบการศึกษาและได้งานที่กรมวิชาการเกษตร

(ต่อ)

ชื่อ - นามสกุล	ตำแหน่งวิชาการ		ต้นสังกัด			ตำแหน่งในโครงการ	สถานภาพปัจจุบัน
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17. น.ส.นภาพร หวลอาลัย	—	นักศึกษาปริญญาโท	พืชไร่นา	เกษตร	เกษตรศาสตร์	นักศึกษาปริญญาโท	นักศึกษาปริญญาโท
18. น.ส.ฉัตรนภา ข่มอาวุธ	—	นักศึกษาปริญญาโท	พืชไร่นา	เกษตร	เกษตรศาสตร์	นักศึกษาปริญญาโท	นักศึกษาปริญญาโท
19. น.ส.สุขุมาภรณ์ ศรีเผด็จ	—	นักศึกษาปริญญาโท	พืชไร่นา	เกษตร	เกษตรศาสตร์	นักศึกษาปริญญาโท	นักศึกษาปริญญาโท
20. น.ส.สุภาวินี แสงโชติ	—	นักวิชาการเกษตร 3	ศูนย์วิจัยข้าวปทุมธานี	สถาบันวิจัยข้าว	กรมวิชาการเกษตร	นักศึกษาปริญญาโท	นักศึกษาปริญญาโท
21. น.ส.สมจิตร์ ใจวังเย็น	—	นักศึกษาปริญญาโท	พืชไร่นา	เกษตร	เกษตรศาสตร์	นักศึกษาปริญญาโท	นักศึกษาปริญญาโท
22. นายสุริยน สุภาพ	—	นักศึกษาปริญญาโท	พืชไร่นา	เกษตร	เกษตรศาสตร์	นักศึกษาปริญญาโท	นักศึกษาปริญญาโท
23. นายธีระ สมหวัง	นักวิชาการเกษตร 4	นักวิชาการเกษตร 4	สถานีวิจัยเขาหินซ้อน	สถาบันอินทรีจันทรสถิตย์ฯ	เกษตรศาสตร์	นักศึกษาปริญญาโท	นักศึกษาปริญญาโท
24. น.ส.บัวทิพย์ อุบลประเสริฐ	ผู้ช่วยศาสตราจารย์	ผู้ช่วยศาสตราจารย์	พืชศาสตร์	เกษตรศาสตร์บางพระ	สถาบันเทคโนโลยีราชมงคล	นักศึกษาปริญญาเอก	จบการศึกษา กลับต้นสังกัด
25. นายจิรวัฒน์ สนิทชน[2/]	อาจารย์	อาจารย์	พืชไร่	เกษตรศาสตร์	ขอนแก่น	นักศึกษาปริญญาเอก	นักศึกษาปริญญาเอก
26. น.ส.พัชรินทร์ ตัญญะ[2/]	—	นักศึกษาปริญญาเอก	พืชไร่นา	เกษตร	เกษตรศาสตร์	นักศึกษาปริญญาเอก	นักศึกษาปริญญาเอก
27. Mr. Mohammad Abul Kashem Chowdhury[3/]	Assist. Prof.	Assist. Prof.	Genetics and Plant Breeding	Patuakhali Agric. College	Bangladesh	นักศึกษาปริญญาเอก	นักศึกษาปริญญาเอก
28. นายธีระพล ศิลกุล[4/]	นักวิชาการเกษตร 6	นักวิชาการเกษตร 6	ศูนย์วิจัยพืชไร่ชัยนาท	สถาบันวิจัยพืชไร่	กรมวิชาการเกษตร	นักศึกษาปริญญาเอก AIT	นักศึกษาปริญญาเอก AIT
29. นายศรีปาน เชยกลิ่นเทศ[4/]	อาจารย์	อาจารย์	พืชศาสตร์	วิทยาเขตพระนครศรีอยุธยา หันตรา	สถาบันเทคโนโลยีราชมงคล	นักศึกษาปริญญาเอก AIT	นักศึกษาปริญญาเอก AIT

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ภาคผนวก

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EFFECTS OF GAMMA IRRADIATION OF SOYBEAN COTYLEDON EXPLANTS ON EMBRYO INDUCTION AND REGENERATION IN A SOMATIC EMBRYO CYCLING SYSTEM

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SUMMARY

Soybean cultivars BSR 101, Gasoy 17, and Chiang Mai 60 were used to study the effect of gamma-rays on the induction and growth of soybean embryos *via* somatic embryo cycling. Young soybean pods were irradiated with gamma-rays at 0, 1, 3, 5, 7, 9 and 11 Gy, and immature cotyledons were subsequently cultured on somatic embryo induction medium. Somatic embryogenesis was markedly enhanced by irradiation at 1 to 5 Gy in BSR 101 and Chiang Mai 60, and at 1 to 3 Gy in Gasoy 17. The embryos were multiplied by culturing in liquid medium (pH 5.8) for 5 weeks. To ensure a continuous supply of embryos for *in vitro* selection, somatic embryo cycling was carried out in the MSO medium. The process was performed for four cycles in BSR 101 and one cycle in Gasoy 17 and Chiang Mai 60. The embryos of BSR 101 were able to regenerate into plantlets after each cycle, but at a decreasing rate.

Key words: Gamma radiation, soybean, embryo induction, plant regeneration, somatic embryo cycling.

The earliest attempt to regenerate soybean *via* somatic embryogenesis was reported by Beversdorf and Bingham (1977), but embryos were obtained only sporadically. Several groups of scientists tried to regenerate soybean plants using different explants in combination with modification of the components of the media, primarily the auxin source and concentration. However, little progress was made until 1985 when the first fertile regenerated plants were obtained by Lazzeri et al. (1985) and Ranch et al. (1985). Subsequently, numerous critical factors for somatic embryogenesis have been investigated to optimize the system. Although regenerated plants can be routinely obtained *via* auxin-stimulated somatic embryogenesis, only 2 to 76% of the explants responded, producing 1.00 to 3.14 embryos per responding explant. The efficiency of response depends on genotype (Parrott et al., 1989; Komatsuda and Ko, 1990; Shoemaker et al., 1991). In addition, all successful plant regeneration *via* somatic embryogenesis has been obtained exclusively from immature embryo cotyledons.

Finer and Nagasawa (1988) reported the establishment and maintenance of an embryogenic soybean suspension culture. For some genotypes, this system can provide a large number of somatic embryos each of which originates from 2-4 cells in the surface of embryogenic tissue (Finer and McMullén, 1991). Observation of the secondary somatic embryos or embryogenic tissue arising from primary somatic embryos in soybean (Ranch et al., 1985; Finer and Nagasawa, 1988) indicated that some cells in the embryos are competent for somatic embryogenesis. Maheswaran and Williams (1985) observed that

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both single-cell and multicellular initiation of embryos occurred, depending on the age or degree of maturity of the parental tissues. Older tissues with some immature epidermal cells would give rise to embryos with a single-cell origin. It is, therefore, possible to use properly staged embryos as explants to induce the next generation of embryos arising from a single cell. This so-called embryo cycling system can increase both the frequency of explant response and the number of embryos per responding explant in comparison to those obtained from zygotic explants. Gamma radiation at low doses, 20-30 grays (Gy), was reported to stimulate growth and regeneration in *Atropa belladonna* and *Lavandula angustifolia* organogenic callus, while inhibiting growth and organogenesis in *Datura innoxia* stem-derived callus (Onisei et al., 1992). So far, there has been no report of irradiation effects on somatic embryo induction and regeneration in soybean. Therefore, in this study, immature seeds from gamma-irradiated pods were cultured in a somatic embryo cycling system to observe the frequencies of embryo formation and regeneration. The continuity of embryo production from cycle to cycle also was investigated, because this capability is needed to impose selection stresses on the somatic embryos *in vitro*.

MATERIALS AND METHODS

Young pods (12 to 16 days old) of the three soybean cultivars, BSR 101 and Gasoy 17 from the US and Chiang Mai 60 from Thailand, were irradiated with gamma (γ) rays at 0, 1, 3, 5, 7, 9 and 11 Gy. The exposure was performed in a Gammator (J.L. Sherpard & Associates, Model Mark I-30) having a caesium 137 source with an irradiation activity of 4,500 curies. Pods were placed on a turntable so as to receive a dose rate of 5.58 Gy/min. Immature seeds were then dissected from the pods. Each seed was split into two cotyledons and the embryonic axis removed. Each cotyledon was placed with its abaxial (curved) side on a solid medium for somatic embryo induction (Finer and Nagasawa, 1988) and incubated for four weeks. The somatic embryos were transferred into a liquid medium at pH 5.8 (Finer and Nagasawa, 1988) and incubated for five weeks to increase the number of embryos before their subculture in a somatic embryo cycling medium (MSO) (Liu et al., 1992). Each cycle was initiated by transferring 0.1-0.5 g of globular somatic embryos into a 50 mL flask containing 20 mL of fresh MSO basal liquid media. Cultures were incubated for five weeks. The experiment included four cycles for BSR 101 and one cycle each for Gasoy 17 and Chiang Mai 60. The average data from 10 flasks per treatment/cultivar combination were periodically recorded on the number of explants that produced embryos, the number of embryos formed on each explant, and the number of regenerated plantlets. A two-way analysis of variance was performed to detect significant differences among the levels of each factor in terms of embryo proliferation and plant regeneration, using the 2-factor interaction mean squares as the error terms.

RESULTS

Three weeks after the embryos were transferred from liquid medium to the somatic embryo cycling medium (MSO), new embryos were induced from localized regions of the epidermis of old embryos in the same way as they were formed from immature cotyledons. Most of the young embryos were light green and globular-shaped, while some were heart-shaped (Figure 1a). Some epidermal cells responded earlier than the others and thus embryos at different stages were observed at three weeks after culturing.

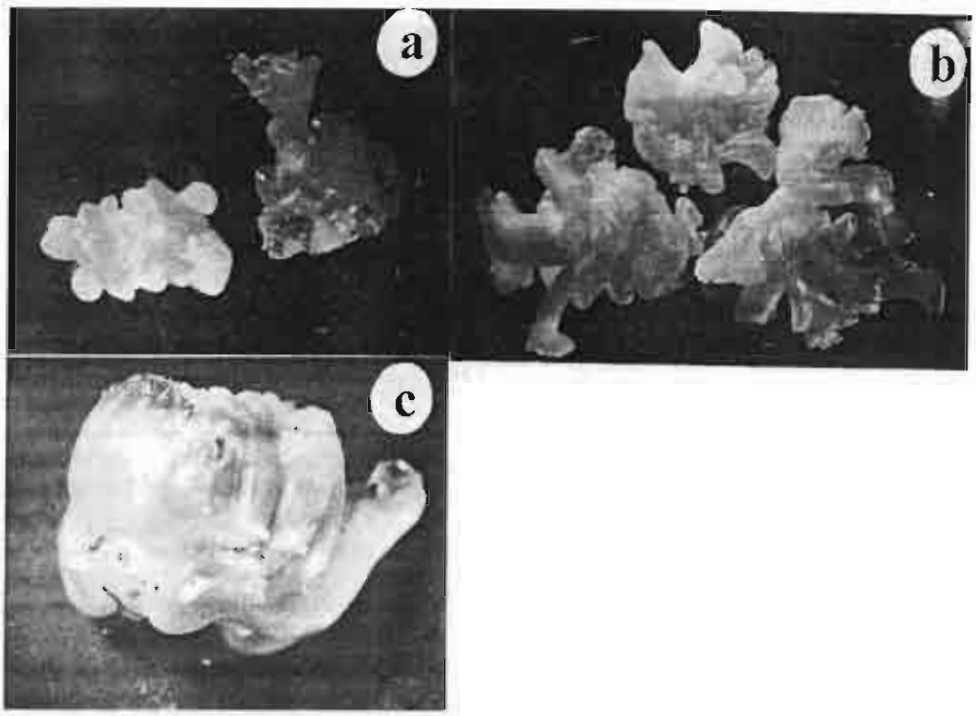

Figure 1. Young somatic embryos proliferated from old embryos cultured in MSO medium; (a) 3 weeks after culturing; (b) 5 weeks after culturing; (c) trumpet-shaped embryos.

Five weeks after culturing, the embryos could be classified into normal light yellow and white embryos with a torpedo or leafy shape (Figure 1b), and abnormal embryos having a trumpet shape (Figure 1c). The new embryos from BSR 101 cultured in MSO proliferated up to 189% of the original number, while those of Gasoy 17 and Chiang Mai 60 proliferated to 148 and 80%, respectively (Table 1). Embryo proliferation in BSR 101 was the highest among the three soybean genotypes at all gamma-rays doses, i.e. 203.0%, in contrast to 135.3% in Gasoy 17 and 118.6% in Chiang Mai 60. Immature cotyledons treated with mild radiation doses produced more embryos in every cultivar. The number of embryos proliferating from cotyledons of all cultivars irradiated with 1-7 Gy was higher than that from the non-irradiated ones ($P<0.05$, Table 1).

After passing through the first cycling, the plant regeneration rate of BSR 101 receiving 0 to 5 Gy irradiation was higher than that of Gasoy 17 and Chiang Mai 60. Regeneration rate was not affected by irradiation at the lower doses but it was inhibited at the higher levels. BSR 101 was more sensitive to the higher γ-ray doses than the other cultivars; its regeneration was completely inhibited at 7 Gy and above. Plant regeneration from Gasoy 17 embryos was completely inhibited at 9 and 11 Gy, whereas Chiang Mai 60 embryos produced plantlets at even at the 11 Gy dose (Table 1).

Table 1. Percentages of embryo proliferation and plant regeneration of soybean cultivars BSR 101, Gasoy 17, and Chiang Mai 60, after passing through the first somatic embryo multiplication cycle.

Radiation	Embryo proliferation (%)			Plant regeneration (%)[1]		
dose (Gy)	BSR 101	Gasoy 17	Chiang Mai 60	BSR 101	Gasoy 17	Chiang Mai 60
0	189	148	80	11.8	5.3	5.5
1	213	152	188	9.7	3.3	3.5
3	200	63	142	17.4	3.3	3.3
5	223	148	106	11.8	1.5	0
7	343	286	102	0	3.3	1.5
9	125	85	131	0	0	1.5
11	128	65	81	0	0	1.0
Mean	203.0a[2]	135.3b	118.6b	12.7a	3.3b	3.1b

[1] The cultivar regeneration percentages were averaged over the 0, 1, 3 and 5 Gy treatments only.
[2] Within each of the proliferation and regeneration data sets, cultivar means followed by the same letter are not significantly different. LSDs are 59.7% among cultivar proliferation means (P=0.05) and 5.9% among regeneration means (P=0.01).

The cultivar BSR 101 was chosen for the embryo cycling study because of its high embryo proliferating ability, and its high plant regeneration rate. After the second cycle, the non-irradiated treatment tended to produce more embryos (Table 2), and increasing doses gave progressively fewer embryos. The embryo proliferation rate was significantly and negatively correlated with dose after both the second ($P<0.05$) and third ($P<0.01$) cycles. On average over these cycles, the regression equation was: Embryo proliferation percentage = 408.7 - 23.4×Dose, with a correlation coefficient of $r = -0.94$, $P<0.01$. There was no marked drop in the embryo proliferation percentage from the second to the third cycle, whereas plant regeneration rate decreased significantly during the same period.

Table 2. Percentages of somatic embryo proliferation and plant regeneration for soybean cultivar BSR 101 after passing through the second and third somatic embryo culture cycles.

Radiation	Embryo proliferation (%)			Plant regeneration (%)	
dose (Gy)	2nd cycle	3rd cycle	Mean	2nd cycle	3rd cycle
0	372	418	395.0a	5.5	2.7
1	358	371	364.5a	4.5	2.5
3	360	338	349.0a	8.1	2.0
5	313	270	291.5ab	5.0	1.0
7	391	244	317.5ab	0	0
9	100	287	193.5bc	0	1.0
11	71	147	109.0c	0	2.5
Mean	280.7	296.4	288.6	5.8a[1]	2.1b

[1] Regeneration percentages were averaged over the 0, 1, 3 and 5 Gy doses only. The difference between cycle means was significant at the P=0.05 level.

Plant regeneration frequencies were not significantly affected by radiation treatments of 0-5 Gy (Table 2). However, callus formation increased from the second to

the third cycle; this increased callusing is believed to be responsible for the reduction in the plant regeneration rate. After the fourth cycle, embryo production declined markedly to 2-3 embryos per flask and over 75% of those were trumpet-shaped and callus-forming (data not shown).

DISCUSSION

This study has shown that low doses of γ-irradiation stimulated proliferation of embryos from immature soybean cotyledons cultured firstly on the Finer and Nagasawa (1988) solid induction medium and later in the liquid somatic embryo cycling medium of Liu et al. (1992). The results were similar to those reported earlier in other species by Onisei et al. (1992), who found that gamma-rays could stimulate callus growth and regeneration in *Atropa belladonna* and *Lavandula angustifolia*. In this study, there was genetic variation in the frequencies of embryo proliferation and plant regeneration from the embryos. The cultivar BSR 101 formed more embryos than Gasoy 17 and Chiang Mai 60. The non-irradiated control cotyledons gave similar responses to that reported by Liu et al. (1992). They also reported genetic variation in embryo proliferation capacity in the somatic embryo cycling system. Low doses of γ-rays applied to young pods before cotyledon excision promoted embryo proliferation, but not plant regeneration. The somatic embryos developed from immature seeds treated with higher doses of γ-rays could not germinate into plantlets and those of BSR 101 were the most sensitive. Gamma-rays can affect biochemical processes relating to initiation and development of somatic embryos (Onisei et al., 1992). In this study, γ-irradiated explants combined with the embryo cycling technique could extend embryo formation and plant regeneration of BSR 101 up to the fourth cycle.

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INTERSPECIFIC HYBRIDIZATION OF MUNGBEAN [*VIGNA RADIATA* (L.) WILCZEK] AND RELATED *VIGNA* SPECIES USING EMBRYO RESCUE TECHNIQUES

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ABSTRACT

Interspecific hybridization was performed between *Vigna radiata* and *Vigna* spp. Pod setting percentage was high when using *V. radiata* as female parent. The cross *V. radiata* x *V. radiata* (wild) gave the highest pod setting of 26.1%. Interspecific crosses were successful in *V. radiata* x *V. mungo*, *V. radiata* x *V. mungo* (wild), *V. radiata* x *V. umbellata*, *V. radiata* x *V. umbellata* (wild), having F_1 seed germination rate of 94.1, 40.9, 6.4, 17.5, and 2% with the number of F_1 seedlings produced were 46, 10, 7, 7 and 1, respectively. The F_1 seeds were mostly small, shrunken, wrinkled, and broken. Embryo rescues of the seeds having *V. radiata* as female parent were all successful, except only in the cross with *V. aconitifolia*. The embryo rescued percentages from the crosses having male parent as *V. radiata* (wild), *V. mungo*, *V. mungo* (wild), *V. umbellata*, *V. umbellata* (wild), *V.angularis*, and *V. glabrescens* were 79.0, 72.5, 79.8, 51.3, 43.1, 40.0 and 4.0, while the percentage of seedlings produced were 65.7, 36.7, 49.5, 43.6, 39.2, 0, and 2.0, respectively. Percentages of F_1 plants that grew until flowering were 22.8, 1.8, 2.1, 17.9, 9.8, 0 and 1.0, producing 24, 2, 4, 7, 5, 0 and 1 plants, respectively. Most of the F_1 plants were sterile with low pollen viability. They set flowers profusely without pod setting. Pollen staining percentage was the highest (20.6%) in the interspecific cross of *V. radiata* x *V. mungo*.

Key Words : Hybridization, Mungbean, *Vigna* spp., Embryo Rescue.

INTRODUCTION

The subgenus *Ceratotropis* of the genus *Vigna* includes five important Asian pulses i.e. mungbean, black gram, rice bean, adzuki bean, and moth bean. Mungbean *(V. radiata)* and black gram (*V. mungo*) have been consumed in South and Southeast Asian countries since the ancient times. Rice bean (*V. umbellata*) is widely cultivated in Asia and the Pacific islands in a small scale. Moth bean (*V. aconitifolia*), which shows a high level of drought tolerance, is cultivated in the semi-arid areas of India and Pakistan (Jain and Mehra, 1980). *V. glabrescens*, a wild species possessing resistance to major mungbean pests and diseases, has been used as sources of the resistance in AVRDC mungbean inprovement program (AVRDC, 1986).

To promote an effective mungbean breeding program, a broader genetic base is required to increase the potential for crop improvement. Interspecific hybridization can be used to increase genetic variation. Although the exact causes of the failure of interspecific hybridization in *Vigna* species are not fully understood, many researchers indicated that the immature embryo rescue technique could be applied to obtain hybrid plants (Ahn & Hartmann 1978; Egawa *et al.*, 1988; Rashid *et al.*, 1988; Chen *et al.*, 1989). Interspecific hybridization between mungbean and blackgram was first reported by Sen and Ghosh (1960). They attempted to incorporate the following characteristics exhibited by *V. mungo* to *V. radiata*; large amount of sulfur-containing amino acids, plant vigor, resistance to diseases and insects, and tolerance to adverse environmental conditions. The cross between *V. radiata* and *V. mungo* was successful when *V. radiata* was used as the female parent while the reciprocal cross was not successful. Similarly, the cross between *V. radiata* and *V. umbellata* was successful only when *V. radiata* was the female parent. Interspecific hybrids between *V. radiata* and *V. angularis* were successfully produced by Ahn and Hartmann (1978) and Chen *et al.*, (1989) using embryo culture techniques. The hybrids were highly sterile and no F_2 offspring seeds were obtained. Interspecific hybridization from *V. angularis* x *V. umbellata* was reported to be successful by embryo culture. In the case of reciprocal cross, however, no pod setting was observed (Siriwardhane *et al.*, 1991). When a tetraploid species, *V. glabrescens* was crossed with diploid Asian *Vigna* species - *V. radiata, V. mungo,* and *V. umbellata* - hybrid plants were obtained by culturing embryos on White's medium supplemented with 200mg/1 of yeast extract, although the hybrid seeds died two or three weeks after pollination (Egawa *et al.*, 1988).

The objective of the present study was to explore the success of interspecific hybridization among major *Vigna* spp. with and without embryo rescue techniques.

MATERIALS AND METHODS

The *Ceratotropis* species are conventionally divided into mungbean group and adzuki bean group based on their germination habit. These two groups are phylogeneti-cally differentiated (Egawa 1988). Mungbean (*Vigna radiata*) and blackgram (*V. mungo*) belong to the former group, while rice bean (*V. umbellata*), adzuki bean (*V. angularis*), mothbean (*V. aconitifolia*), and *V. glabrescens* belong to the latter group (Table 1).

Vigna strains were grown for crossing in pots in a glasshouse at Chai Nat Field Crops Research Center, Chai Nat, Thailand. Flowers were emasculated just before the buds opened. They were immediately pollinated and covered with paraffin-paper bags to prevent contamination. Hundreds of flowers were crossed in each combination and divided into 2 groups. One group was allowed for natural pod development while the other was undergone embryo rescuing. The rescue techniques began from cleaning immature pods (about 2 weeks after pollination) with detergent and tap water, then rinsed in 70% ethyl alcohol for 5 minutes. The pods were sterilized with 15'% Clorox ® solution added with 2 to 3 drops of Tween 20 for 15 minutes in a laminar flow. The pods were washed with sterilized distilled water for 3 times every 5 minutes and dried in Petri dishes. The embryos were removed and kept on White's agar medium (White, 1963) supplemented with 200 mg/1 of yeast extract for shoot induction. The shoots were then transferred to B5 medium (Gamborg *et al.*, 1968) with 2 mg/1 of IBA to promote rooting. Plantlets were transferred into vermiculite bed in a growth chamber for acclimatiza-

Table 1 Strains of *Vigna* species used in interspecific hybridization

No.	Species name	Common name	2n chromosome	Variety/Accession
1.	Mungbean group species			
	V. radiata var. *radiata*	mungbean	22(2x)	Chai Nat 36
	V. radiata var. *sublobata*	Wild mungbean	22(2x)	TC 1966
	V. mungo var. *mungo*	black gram	22(2x)	Phitsanulok 2
	V. mungo var. *silvestris*	wild black gram	22(2x)	TC 2211
2.	Adzuki bean group species			
	V. umbellata	rice bean	22(2x)	80015
	V. umbellata	wild rice bean	22(2x)	80021
	V. angularis var. *angularis*	adzuki bean	22(2x)	Fukuoka
	V. aconitifolia	moth bean	22(2x)	80053
	V glabrescens	(wild species)	44(4x)	V1160

tion. Later, they were transplanted into pots filled with soil and kept in a glasshouse.

RESULTS AND DISCUSSION

Results of the interspecific crossing are presented in Table 2. Percentage of pod setting was high when *V. radiata* was used as female parent. The crossed between *V. radiata* and *V. mungo*, and between *V. radiata* and *V. umbellata* were successful when *V. radiata* was used as female parent. The cross *V. radiata* x *V. radiata* (wild) gave the highest pod setting of 26.1%. Interspecific crosses were successful in *V. radiata* x *V. mungo*, *V. radiata* x *V. mungo* (wild), *V. radiata* x *V. umbellata*, *V. radiata* x *V. umbellata* (wild), having F_1 seed germination rate of 94.1, 40.9, 6.4, 17.5, and 2% while the number of F_1 seedlings produced were 46, 10, 7, 7 and 1, respectively. The F_1 seeds were mostly small, shrunken, wrinkled, and broken. The crosses between *V. radiata*, *V. mungo* and *V. aconitifolia* were not successful and pod setting did not take place.

The results of embryo culture and hybrid plants obtained are presented in Table 3. Rescues of the F_1 seeds having *V. radiata* as female parent were all successful, except only in the cross with *V. aconitifolia*. The embryo rescued percentages from crosses having male parent as *V. radiata* (wild), *V. mungo*, *V. mungo* (wild), *V. umbellata*, *V. umbellata* (wild), *V. angularis*, and *V. glabrescens* were 79.0, 72.5, 79.8, 51.3, 43.1, 40.0 and 4.0, while the percentage of seedlings produced were 65.7, 36.7, 49.5, 43.6, 39.2, 0, and 2.0, respectively. Percentages of F_1 plants that grew until flowering were

Table 2 Results of interspecific crosses and germination test of the resulting F_1 seeds

Cross combination	No. of flowers pollinated	No. of pods set (%)	No. of seeds sown	No. of seeds germinated (%)	No. of plants obtained
V. radiata x *V. radiata* (w)	180	47 (26.1)	51	48 (94.1)	46
V. radiata x *V. mungo*	207	50 (24.1)	44	18 (40.9)	10
V. radiata x *V. mungo* (w)	191	25 (13.1)	124	8 (6.4)	7
V. radiata x *V. umbellata*	46	8 (17.4)	40	7 (17.5)	7
V. radiata x *V. umbellata* (w)	100	13 (13.0)	51	1 (2.0)	1
V. radiata x *V. aconitifolia*	74	0 (0.0)	0	0 (0.0)	0
V. radiata x *V. angularis*	234	5 (2.1)	4	0 (0.0)	0
V. radiata x *V. glabrescens*	87	14 (16.1)	59	0 (0.0)	0

(w) = wild species

Table 3 Results of embryo culture of interspecific hybrids among *Vigna* species

Cross combination	No. of embryos cultured	No. of embryos germinated (%)	No. of seedlings obtained (%)	No. of plants obtained (%)
V. radiata x *V. radiata* (w)	105	83 (79.0)	69 (65.7)	24 (22.8)
V. radiata x *V. mungo*	109	79 (72.5)	40 (36.7)	2 (1.8)
V. radiata x *V. mungo* (w)	188	150 (79.8)	93 (49.5)	4 (2.1)
V. radiata x *V. umbellata*	39	20 (51.3)	17 (43.6)	7 (17.9)
V. radiata x *V. umbellata* (w)	51	22 (43.1)	20 (39.2)	5 (9.8)
V. radiata x *V. aconitifolia*	-	-	-	-
V. radiata x *V. angularis*	10	4 (40.0)	0 (0.0)	0 (0.0)
V. radiata x *V. glabrescens*	101	4 (4.0)	2 (2.0)	1 (1.0)

22.8, 1.8, 2.1, 17.9, 9.8, 0 and 1.0, producing 24, 2, 4, 7, 5, 0 and 1 plants, respectively. Hybrids between tetraploid and diploid species such as between *V. radiata* and *V. glabrescens* could not be obtained by conventional crosses, due to wilting of the hybrid pods at 2 weeks after pollination. The present experiment showed that young triploid hybrid seedlings could be obtained through embryo culture.

Morphological and agricultural characteristics are studied on F_1 of the cross *V. radiata* x *V. mungo* and certain variation is presented in Table 4. The hybrid plants were similar to female parent or intermediate between the two parental species. The size of F_1 plants were small having hypocotyl length of about 4.0 cm which was longer than both parents. Primary leaf, terminal leaflet, and lateral leaflet were intermediate between parents while growth habit was semi-erect. F_1 plants produced many flowers but most of them were abortive. Even if the F_1 plants could bear some pods, they contained only one or two seeds. Pod length was intermediate between the parents, while seeds per pod, pods per plant, seed coat color, seed size, seed weight, and plant height at maturity were lower than both parental species. The normal fertile pollen were large and stained red by acetocarmine while the abnormal sterile ones were small and not stained. Most pollen of the F_1 plants were sterile having stainability of 20.6%. Under field condition F_1 plants were found resistant to Cercospora leaf spot and powdery mildew diseases. This suggested potential uses of interspecific cross between both species to exchange desirable characters among them in a mungbean breeding program.

Table 4 Variation in morphological characteristics of *V. radiata* (ChaiNat 36), *V. mungo* (Phitsanulok 2) and their interspecific F_1 hybrid

Characters	*V. radiata* (female)	Hybrid	*V. mungo* (male)
Color of upper epicotyl	green	purple	purple
Hypocotyl length (cm)	3.0	4.0	3.0
Primary leaf L/W (cm)1/	8.7/11.7	5.9/2.5	7.3/3.6
Terminal leaflet shape	deltoid	deltoid	cuneate
Terminal leaflet L/W (cm)	8.2/5.9	5.7/4.3	7.3/6.4
Lateral leaflet L/W (cm)	8.2/5.8	4.3/3.3	8.1/5.2
Days to first flowering (dap)2/	29±0.5(28-30)3/	30±0.3(29-31)	29±0(29-29)
Pollen stainability (%)	94.1	20.6	83.9
Pod pubescence	intermediate	intermediate	heavily pubescent
Days to pod maturity (dap)	60	65	60
Pod length (cm)	7.2±0.6(4.2-9.0)	5.3±0.6(4.3-6.2)	3.1±0.4(1.9-3.9)
Seeds/pod	7.4±0.8(4-10)	2.5±0.4(2-4)	4.4±0.8(1-7)
Pods/plant	43±0.5(42-44)	13±0.9(10-15)	106±0.5(105-107)
Seed coat color	green	brown	black
Seed size L/W (mm)	6.0/4.5	3.5/2.2	4.8/3.5
Seed weigth (gm/plant)	25.5±2.0(20-32)	1.8±0.1(1.4-2.0)	21.1±0.5(20.0-22.2)
100 seeds weight (gm)	6.9±0.01(6.8-7.1)	5.5±0.4(4.8-6.8)	5.6±0.05(5.4-5.7)
Heigth at maturity (cm)	58.5±2.35(52-65)	37.5±0.9(35-40)	53.1±2.3(48.5-57.7)
Cercospora leaf spot 4/	moderately resistance	resistance	resistance
Powdery mildew 4/	moderately resistance	resistance	resistance

1/ L/W = length/width
2/ dap = days after planting
3/ Average ± standard error (range)
4/ Field rating without inoculation

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Identification of RAPD Marker Associated with Downy Mildew Resistance in Soybean Using Bulked Segregant Analysis of Near-isogenic Lines

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Abstract

Downy mildew caused by the fungus *Peronospora manshurica* is a worldwide distributed foliar disease of soybean. In Thailand, the disease infests one of the popular soybean cultivars, 'Nakhon Sawan 1' ('NS1'), and causes yield loss of up to 25%. A study was conducted to identify RAPD markers associated with the gene conferring resistance to this disease, especially to the fungus strains prevailing in Thailand. Leaf DNA from recurrent parent NS1, donor parent AGS129, near isogenic line (NIL) BC5-76-3 F5, was extracted and amplified by 80 Operon primers. Totally 375 bands, 67 of which revealed polymorphism, were amplified. Two polymorphic bands, designated as $OPH02_{710}$ and $OPH02_{800}$, were considered as potential RAPD markers linked to the resistant gene.

Key words : bulked segregant analysis, downy mildew resistances, NIL, RAPD markers, soybean

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Introduction

Soybean downy mildew is a worldwide foliar disease caused by the fungus *Peronospora manshurica*, which is detrimental to soybean production (Yang, 1980). The symptoms of downy mildew appear firstly on the upper surface of young leaves as pale green to light yellow spots, which enlarge into pale to bright yellow lesions of indefinite size and shape, depending on leaf age. The disease causes mild defoliation, lowers seed quality, and reduces seed size. It may cause yield losses of up to 8% (Sinclair, 1989). The climatic condition of high humidity and temperature in Thailand is favorable for spreading of the disease, thus it becomes a major constraint to soybean production. Nuntapant *et al.* (1986) reported yield loss of up to 25% in 'Nakhon Sawan 1' ('NS1'), one of Thailand's popular cultivars.

Use of resistant soybean cultivars has been the most effective means to combat against the disease. However, pathogenic variability of the fungus which always creates new races represents a permanent challenge to plant breeders. Geeseman (1950a, 1950b) designated three races on three differential cultivars and reported the mode of inheritance reaction to them as due to three gene pairs. Bernard and Cremeens (1971) identified a gene for general resistance to the disease. However, new races gradually overcame these resistant genes. By 1977, the number of races of *Peronospora manshurica* increased to 32, and the gene *Rpm* was shown to impart resistance to all known races of the fungus. Lim *et al.* (1984) reported that the resistance was overcome by the new race 33 in 1981. Later, they showed that the gene *Rpm2* conditioning resistance to race 33 segregated independently of *Rpm* (Lim 1989). When a resistant variety with new resistant gene is developed and released to the farmers, the pathogen always creates a new race or changes the prevalent race against the resistance. Race surveys in the US indicated changes in the prevalence between 1971 to 1976. To circumvent this problem, the idea of pyramiding different genes into the same cultivar has been suggested and used in the improvement of resistant plants (Lim *et al.*, 1984). The possibility now exists for combining two or more genes to provide broader and longer lasting resistance.

In recent years, the development of molecular marker technology has considerably expanded the potential for pyramiding disease resistant genes. Of these molecular markers, RFLP, RAPD, and AFLP are the most used ones in plant breeding. Compared with the other molecular markers, Williams *et al.* (1990) concluded that RAPDs have several advantages, viz. (1) a universal set of primers can be used and screened in a short period of time, (2) no isolation of cloned DNA probes or preparation of

hybridization filters is required, (3) only small quantities of DNA are needed, allowing the use of simple and rapid methods for DNA isolation, and (4) it is relatively less expensive than others. For the reasons above, RAPD markers have high potential for use in marker-assisted selection.

Inheritance studies indicated that resistance to the disease in Thailand in the line AGS129 was controlled by a single dominant gene (Juwattanasamran *et al.*, 1988). So, it is relatively easy to develop downy mildew resistant isogenic lines in soybean by backcross method. Using the susceptible variety NS1 from Thailand as recurrent parent, and the resistant line AGS129 from the Asian Vegetable Research and Development Center (AVRDC) as donor parent, Jene-kritiya (1991) developed 15 downy mildew resistant near-isogenic lines (NILs) through 5 cycles of back-crossing. Although race of the fungus and resistant gene in the host plant have not been identified, the NILs were identical in agronomic characters and all immune to the disease. Thus, in the current experiment, the donor parent, the recurrent parent, and one near-isogenic line were picked up as plant materials to identify RAPD markers associated with soybean downy mildew resistance. The suitable marker, if available, can assist selection for resistant progenies without the need of the time-consuming artificial inoculation in a disease nursery.

Materials and Methods

Plant materials

Plant materials used in this study were developed by Jene-kritiya (1991) which included resistant donor parent AGS129, susceptible recurrent parent NS1, a near isogenic line (NIL) BC5-76-3 F5 obtained from five cycles of back-crossing. The seeds were sown in the rainy season of 1997 in the nursery of Asian Regional Center - Asian Vegetable Research and Development Center (ARC - AVRDC), Kasetsart University, Kamphaengsaen Campus. One week after emergence, the first leaflets of seedlings from each of the three genotypes were bulk collected and extracted for DNA.

DNA extraction

The total genomic DNA extraction procedure was modified from Doyle and Doyle (1987). The sampled leaves were frozen in liquid nitrogen in a mortar, ground with a pestle, then added with 1 ml CTAB buffer. The paste was poured to a 1.5 ml micro-centrifuge tube, incubated in water bath at 65°C for 30 min, then added with chloroform-isoamyl alcohol (24 : 1 v/v) to fill up the tube. The content was mixed by gentle inversion before being centrifuged at 1.5 G (15,000 rpm) for 5 min. The upper aqueous phase was transferred to a new tube with 600 µl cold isopropanol. The precipitated DNA was washed in 800 µl of 75% ethanol with 10 mM ammonium acetate, and

again in 800 µl of 75% ethanol. The DNA was then air-dried and resuspended in TE buffer (10 mM Tris and 1 mM EDTA). The final concentration of sample DNA was adjusted to 5 ng/µl.

PCR amplification

Amplification reaction was done using 10 µl of the final volume added with 10x buffer (100 mM Tris buffer pH 8.3, 500 mM KCl, 20 mM MgCl2), 0.1 mM dNTPs, 0.6 (M primer, 0.02 unit/(µl Taq DNA polymerase (Promega), and 1 ng/µl genomic DNA. Each reaction mixture was overlaid with a drop of mineral oil. DNA amplification was performed in a DNA Thermal Cycler (BIO Oven III). The thermal cycles used were 1 cycle for 1 min at 90-95°C, then 44 cycles for 1 min at 95°C, 1 min at 36°C, 2 min at 70°C, followed by 1 cycle for 7 min at 72°C as the final extension. Following amplification, the RAPD products were size-separated using electrophoresis in 1.6% agarose gel with 1x TAE buffer. The gels were stained with ethidium bromide, then viewed and photographed under ultraviolet light. In this study, a total of 80 primers of Operon products Kit E, F, G, H, and N were used to identify the RAPD markers associated with downy mildew resistance in soybean.

Results and Discussion

The number of amplification products produced by each primer varied from as few as zero (OPF17, OPG01, OPG20 and OPH10) to as many as 9 (OPE16). The size of amplified DNA fragments ranged from less than 100 bp to more than 2 kb. Out of the 80 primers used, 40 primers revealed polymorphism ranging from 12.5% to 100%. A total of 375 amplification products were obtained, out of which 67 showed polymorphism. Two of the 67 polymorphic bands were shared by AGS129 and NIL (97.02% polymorphism), suggesting that the NIL carried only a small segment of the donor parent genome. Meanwhile, only five polymorphic bands (or 7.45%) appeared between NS1 and NIL, which revealed that the NIL shared much genetic background with the recurrent parent. Among these five polymorphic bands, only one band that produced by primer OPH02 was present in the donor parent and NIL, but not in the recurrent parent. This band appeared to be approximetely 710 bp in size and was designated $OPH02_{710}$. Another band generated from the primer OPH02 was also observed and nominated $OPH02_{800}$ from its size of around 800 bp. This band was found in the recurrent parent but not in the donor parent and NIL. Considering that the NIL BC5-76-3 F5 was obtained through five cycles of backcrossing and selfing, the undesired donor DNA was removed by backcrossing while the resistance to downy mildew was maintained by selfing and selec-

tion. Theoretically, the NIL had the most background of the recurrent parent. They are just different by the presence and absence of the resistant gene and its flanking sequences. Thus, the polymorphism between NIL and recurrent parent should be represented in the region surrounding the resistant gene, i.e. the RAPD markers are linked to resistance to the disease.

This finding is in general agreement with that theoretically proposed by Muehlbauer *et al.* (1988). They calculated that if a BC_5S_1 NIL was screened with 100 randomly chosen loci, assuming polymorphisms existed between the recurrent parent and the donor parent at all loci, four loci should detect donor DNA and two or three of them could be expected to be genetically linked to the introgressed gene.

Although a set of RAPD marker was identified to be linked with the resistant gene(s) found in AGS129, more sources of downy mildew resistance should be investigated in order to recombine more resistant genes into a cultivar. More genotypes can also be screened to verify the reliability of the RAPD markers through retrospective analysis of F2 population, by bulk segregant analysis using the progenies from cross between recurrent parent and NIL.

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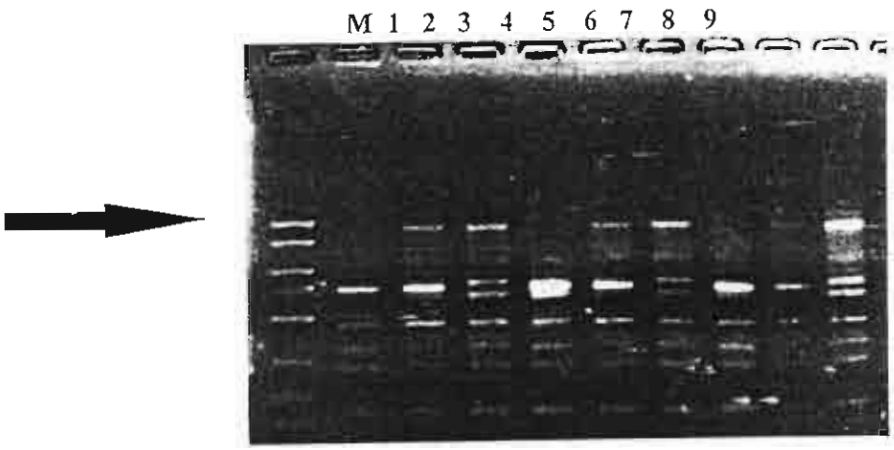

Figure 1 A 1.6 % agarose gel showing the results of PCR amplification with the primer OPH02 of the donor parent AGS129, NIL BC5-76-3 F5, the recurrent parent NS1. The markers fall between 50 to 2000 bp ladder. The amplified products were determined in triplicate where lanes 1, 4, 7 were from AGS 129, lanes 2, 5, 8 were from NIL, and lanes 3, 6, 9 were from NS1, respectively. Lane M indicates the standard markers with the arrow pointing at the marker $OPH02_{710}$.

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Effects of the multiple leaflet gene on agronomical and physiological characters of mungbean (*Vigna radiata*)

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SUMMARY

Near-isogenic lines of mungbean (*Vigna radiata*) 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 the two recipients at Kasetsart University, Thailand, in the rainy season of 1996 and the dry season of 1997. The effect of the gene controlling the multiple leaflet on physiological and agronomical characters was investigated. Seed yield, plant height, and number of pods per plant were greater in the trifoliate leaflet cultivars than in the multiple leaflet near-isogenic lines, whereas the numbers of seeds per pod and 1000-seed weights were not significantly different. Light saturation and photosynthetic rate did not differ, while light interception and dry matter accumulation were greater in the trifoliate leaflet cultivars than in the multifoliate lines. The leaf area index (LAI) of the trifoliate parents was greater at the vegetative stage but lower at the flowering and young pod stages, due to the greater number of leaflets per plant of the multifoliate lines. After the beginning of the podding stage, the LAI of the multifoliate lines was less than that of the parents, as the number of leaflets per plant of these lines hardly changed while that of the trifoliate parents increased.

INTRODUCTION

Mungbean (*Vigna radiata* (L.) Wilczek) is an important pulse crop and is widely cultivated in Thailand. In 1997, it occupied a planted area of 350000 ha which produced 202500 t of grain (Anon, 1998). Mungbean can be sown for up to three crops per year and it fits well into cropping systems. Mungbean seed has a high nutritive value. Domestic and international demand is high. However, the average yield is still low due to indeterminate growth habit, photoperiod sensitivity, late and non-synchronous maturity, susceptibility to lodging, pod shattering, and losses due to pests and diseases (Fernandez & Shanmugasundaram 1988). In order to raise the yield per unit area, new cultivars must be developed and cultural practices improved. Physiological studies have revealed that mungbean yield bears a close relationship to the duration and rate of photosynthesis (Kuo *et al.* 1978). Mungbean lines with more leaflets per leaf produce greater leaf area which can intercept more sunlight and thus give greater yield. Sripisut & Srinives (1986) reported that the multiple leaflet character is controlled by a single recessive gene. This character can be transferred to other mungbean lines by backcrossing. Isogenic lines carrying the gene can bear up to nine leaflets. AVRDC (1988) observed that among F_2-segregants, the multiple leaflet plants tended to yield less than the normal trifoliate ones. However, AVRDC did not form isogenic lines to assess the effects of the multiple leaflet gene. Thus the present work was designed to investigate possible benefits of the multiple leaflet *v.* normal trifoliate genes.

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MATERIALS AND METHODS

Plant material

Isogenic mungbean lines were produced from nine consecutive backcrosses having two recommended cultivars, Chai Nat 36 (CN36) and Kamphaeng Saen 1 (KPS 1), as recurrent parents. Both cultivars are popular in Thailand due to their desirable determinate growth habit and high yield. Four multifoliate BC9 lines, two from each parent, were sown along with the trifoliate cultivars in an experiment of randomized complete block design with three replicates. Each plot consisted of eight rows, each 5 m long. The spacing between rows and hills was 50 × 12·5 cm, with two plants per hill. Ten days after emergence, a compound

fertilizer was applied providing, in kg/ha, 18·75 N, 37·50 P_2O_5, 18·75 K_2O. Weeds were controlled by alachlor, a pre-emergence herbicide, and by hand hoeing. Insect pests were controlled by spraying with monocrotophos and triazophos.

Agronomic data

When 80% of the total pods had turned brown, tan, or black, ten plants were sampled to measure plant height, number of pods per plant, number of seeds per pod and 1000-seed weight. Pods from four centre rows of each plot were harvested, dried, and threshed to determine seed yield.

Physiological data

Light interception

Light interception was measured at 7 day intervals beginning 14 days after emergence (DAE) up to harvesting using a Sunlink Linear PAR probe (Decagon, Washington, US) which has 80 independent sensors for photosynthetically active radiation (PAR). Sunlink was fabricated for use with a user-provided 21X Micrologger. PAR Interception was estimated by comparing paired measurements made above and below the mungbean canopy. Three paired measurements were taken weekly from each plot on each date, whenever the incident PAR was equal to or greater than 1400 μmol/m²/s.

Leaf area index (LAI)

The LAI was measured on the same dates as light interception, using a LAI-2000 Plant Canopy Analyzer connected with a LAI-2050 Optical Sensor (LI-COR, Lincoln, Nebraska, US). The measurements were made in the early morning or in the evening, using techniques recommended for row crops. In each plot, the sensor was pointed once above and four times below the canopy across a pair of crop rows. Two measurements were made in each 8-row plot, between rows 3 and 4, and 5 and 6. The average LAI was recorded.

Leaf photosynthetic rate (P_{max})

Leaf photosynthetic rate at the light saturation (P_{max}) was measured with a LI-6200 Portable Photosynthesis System (LI-COR, Lincoln, Nebraska, US) at 7 day intervals from 14 days after emergence (DAE) until the onset of maturity. Four plants were chosen per line for the measurements. Measurements were taken on all fully expanded leaves of the sample plants whenever the PAR $\geq$ 1400 μmol/m²/s. During measurement, the central leaflet of each leaf was enclosed in a one-litre gas exchange chamber. A measurement was made when the chamber attained CO_2 concentration of 350 ppm and 40–50% relative humidity.

Dry matter

Dry matter was measured on six occasions. On each occasion, six plants were sampled and separated into root, stem and petiole, leaf, flower, pod and seed. All samples were dried at 70 °C for 72 h in a hot air oven and dry weights were recorded.

Data analysis

From data of a trait or measurement, a RCBD analysis of variance of six treatments in three replicates was used in assessing the significant difference among the mungbean genotypes. Mean comparisons were performed using the Least Significant Difference (LSD) at $P < 0{\cdot}05$. Each standard error of mean (S.E.) was estimated at 10 degrees of freedom (D.F.).

RESULTS AND DISCUSSION

Yield and yield components

Means of yield and yield components are presented in Table 1. Their respective LSD at $P < 0{\cdot}05$ revealed that CN36 and KPS1 gave higher yields than their BC9 isogenic lines. The results agreed with the speculation of AVRDC (1988). Plant height and number of pods per plant in the normal leaflet cultivars were also greater than in the corresponding multiple leaflet lines. However, the lines did not differ in number of seeds per pod or 1000-seed weight.

Light interception

Light interaction percentage differed ($P < 0{\cdot}05$) between normal and multiple leaflet mungbeans at 14, 21 and 28 DAE (Fig. 1). Light interception was similar among the six lines after 35 DAE. The leaflets in the normal plants, although trifoliate, were large and together absorbed more light than the multiple leaflet types. Light interception percentage during the vegetative stage increased rapidly until the beginning of pod and seed filling stages. At 56 DAE, all entries reached their greatest light interception. At harvesting, light interception percentage decreased as leaves at lower canopy senesced. Thus, normal lines which produced greater yield had a greater light interception during the vegetative stage. This finding is in agreement with the association between light interception during the vegetative stage and the beginning of the reproductive stage and seed yield of soyabean (Board *et al.* 1992).

Leaf area index (LAI)

On several sampling dates the LAI of the cultivars was significantly greater ($P < 0{\cdot}05$) than that of their BC9 isogenic lines (Fig. 2). At 21 and 28 DAE, the cultivars had a greater LAI than the multifoliate lines.

Table 1. *Mean yield and yield components of the trifoliate mungbean cultivar Kamphaeng Saen 1 (KPS1) and Chai Nat 36 (CN 36) and the multifoliate BC9 isogenic lines*

Lines	Yield (t/ha)	Plant height (cm)	Pods/plant	Seeds/pod	1000 seed weight (g)
KPS 1	1·42	84·7	13·3	10·9	56·9
CN 36	1·45	91·0	15·3	11·5	57·6
KPS 1 BC9-1	1·12	81·5	13·0	11·6	55·7
KPS 1 BC9-2	1·16	85·7	12·0	11·4	53·7
CN 36 BC9-1	1·26	82·1	13·3	10·6	55·0
CN 36 BC9-2	1·18	81·0	12·7	11·0	56·8
S.E. ($P < 0·05$) D.F. = 10	0·058	1·69	0·38	0·20	0·86

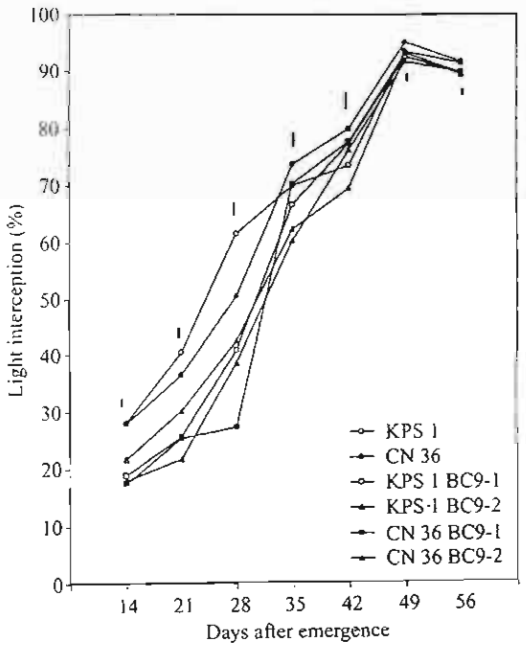

Fig. 1. Light interception percentage of Kamphaeng Saen 1 (KPS1) and Chai Nat 36 (CN36) mungbeans and their BC9 isogenic lines. S.E. ($P < 0·05$, D.F. = 10) at each date is represented by a vertical bar.

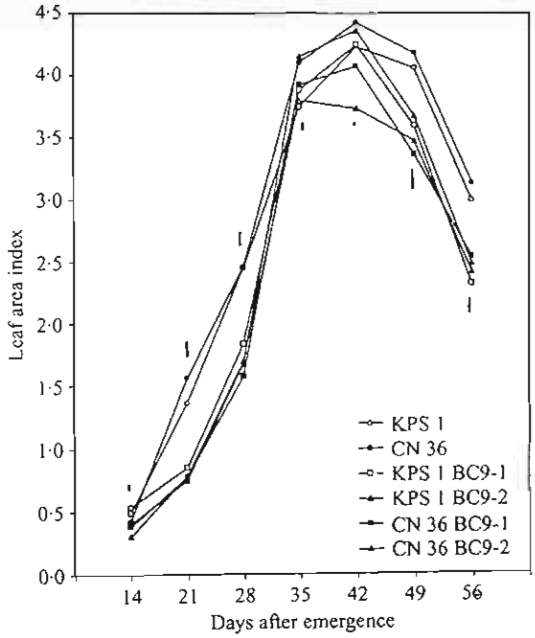

Fig. 2. LAI of Kamphaeng Saen 1 (KPS1) and Chai Nat 36 (CN36) mungbeans and their BC9 isogenic lines. S.E. ($P < 0·05$, D.F. = 10) at each date is represented by a vertical bar.

After flowering, the number of leaflets per plant increased in the normal cultivars but remained constant in the multifoliate lines. This trend continued until maturity, and is thus another possible reason for greater seed yield in the normal cultivars. The LAIs were easier to measure with high precision during flowering and pod setting (35 and 42 DAE). This resulted in smaller standard errors on both occasions.

Leaf photosynthetic rate (P_{max})

Both cultivars tended to retain leaf photosynthetic ability well until 53 DAE, whereas multifoliate lines had significantly reduced ability on the same date. These were the expected temporal changes in P_{max}, but no significant difference ($P < 0·05$) was observed among lines on any measurement occasion (Fig. 3). Failing to detect the difference may be due to too few plants (four in this case) measured per line and thus caused a high S.E. for this trait. The P_{max} values were in general agreement with those reported by Salisbury & Ross (1992) for soyabean (10–20 μmol/m²/s), and by Greer (1995) for dwarf bean (12 μmol/m²/s).

Dry matter

Dry matter per plant was greater for the cultivars than for the isogenic lines especially after later

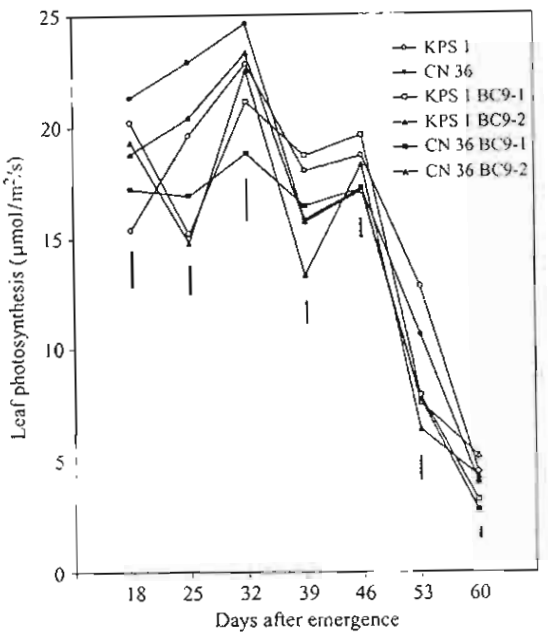

Fig. 3. Leaf photosynthesis (μmol/m²/s) averaged from central leaflets of trifoliate leaves of Kamphaeng Saen 1 (KPS1) and Chai Nat 36 (CN36) mungbeans and the corresponding leaflets in the BC9 isogenic lines. S.E. ($P < 0.05$, D.F. = 10) at each date is represented by a vertical bar.

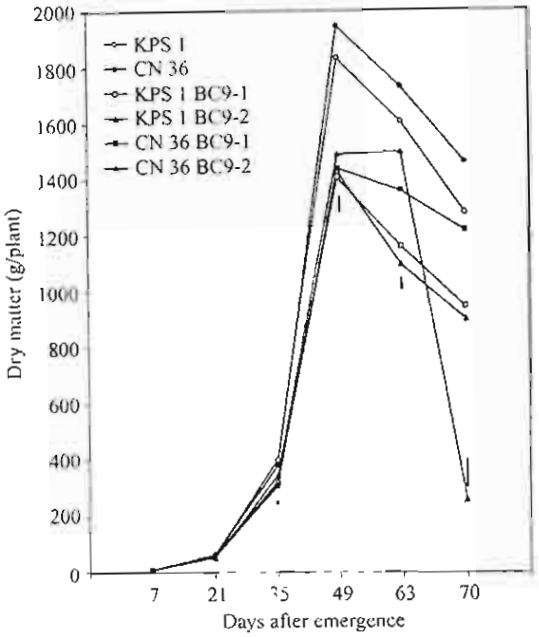

Fig. 4. Dry matter (g/plant) of Kamphaeng Saen 1 (KPS1) and Chai Nat 36 (CN36) mungbeans and their BC9 isogenic lines. S.E. ($P < 0.05$, D.F. = 10) at each date is represented by a vertical bar

sampling occasions (Fig. 4). The greater dry matter of the cultivars was associated with heavier root and stems, especially after flowering. The multifoliate lines had less dry matter at all growth stages until harvesting. All lines had the greatest dry matter at 49 DAE.

The above results reveal inferiority of the multiple leaflet gene. The multiple leaflet isogenic lines yielded less than the trifoliate parents because they had fewer pods per plant. Physiological studies showed that the parents absorbed more light during the vegetative stage, and had greater LAI and dry matter per plant throughout the growth cycle. The cultivars did not differ in leaf photosynthetic rate, however. Thus, it may be concluded that the multiple leaflet trait has no advantage in mungbean yield improvement.

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RESEARCH ARTICLE

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Effect of Seed Texture Layer on Bruchid Infestation in Mungbean *Vigna radiata* (L.) Wilczek

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ABSTRACT An experiment was carried out to evaluate the effect of texture layer of mungbean seed on infestation of bruchids (*Callosobruchus maculatus* and *C. chinensis*) (Coleoptera: Bruchidae). Seventeen BC9 near-isogenic mungbean lines (NILs) which are resistant to the insects, and their parents (KPS 1, CN 60 and the wild mungbean TC 1966), were compared. Each entry was prepared in two sets, one with intact texture layer and the other with texture layer removed by 15% NaOH. Each set was divided into 8 samples of 5 g each to test against the bruchids. The results revealed that both bruchid species laid more eggs on no texture layer seeds than on the intact ones. The difference in egg laying was especially high on seed of the wild mungbean TC 1966. The NILs, KPS 1 and CN 60 harbored several eggs on seed surfaces. However, the resistant lines had almost no damaged seed, regardless the removal of texture layer. The highest damage among the resistant NILs was only 5.5% while the susceptible recurrent parents were completely damaged. The texture layer seemed to affect only insect oviposition but not seed damage. Thus, to evaluate for chemical resistance to bruchids in a mixture of dull- and shiny-seeded mungbean, their texture layer should be removed to normalize the number of eggs laid on seeds.

KEYWORDS: *Vigna radiata, Collasobruchus chinensis, C. maculatus*, mungbean, bruchids, seed texture layer.

INTRODUCTION

Bruchids, *Callosobruchus maculatus* (F.) and *C. chinensis* (L.) (Coleoptera: Bruchidae) are the most devastating and widespread store pests that can infest mungbean in the field as well as during storage. The initial infestation originates in the field, where the adult beetles lay eggs on green pod and the larva bore through the pod and feed in developing seed.[1] When the seeds are harvested and stored, the insects continue to feed, emerge to adults, and cause further infestation which results in total destruction of seed within 3 to 4 months.[2] In storage, the adults lay eggs directly on seed coat. The newly hatched larvae bore through the egg shell and penetrate seed coat to find their food. The female bruchids prefer to lay eggs on smooth surface rather than on rough surface of seeds. The rough (dull) seeds are those covered with inner pod membrane that renders the seed dull. When this membrane is removed, the seed coat underneath is shiny.[3] The pod membrane may contain brown or black pigment through which the seed coat color may not be apparent. Fujii *et al.*[4] observed that seed of the bruchid resistant mungbean TC 1966 is covered with a network of parallel and transverse ridges, compared with smooth surface of commercial mungbean. This characteristic makes the female bean weevil rather hesitant to lay eggs on. Seed size may also play role in oviposition preference. However, the resistant mechanism is reportedly antibiosis type and independent of the physical mechanism.[5] They constituted artificial seeds from varying ratios of flour from resistant and susceptible accessions and found that the number of bruchid adults emerged from the artificial seeds decreased with increasing proportion of flour from the resistant accession. Watt *et al.*[3] reported that texture layer can be removed from mungbean seed surface by washing seeds with 15% NaOH solution. Imrie and Lambrides[6] mass screened mungbean germplasm and found that the presence of texture layer and seed size influenced bruchid damages. Seeds with texture layer intact and smaller seeds harbored less eggs of four major bruchid species, *C. maculatus, C. chinensis, C. phascoli* and *Acanthoscelides obtectus*. They did not assess further for seed damage. They thus recommended to initially screen large number of accessions using seeds with texture layer intact. Only the resistant accessions will be retested using seeds treated with 15% NaOH to remove the layer. Then resistant accessions from the latter step are identified for further tests or uses.

The current experiment was conducted to identify effect of texture layer on egg laying, number of adults emerged and percent seed damaged from two bruchid species, *C. maculatus* and *C. chinensis*.

Materials and Methods

This experiment was carried out in the insect laboratory of the Asian Regional Center of the Asian Vegetable Research and Development Center (ARC-AVRDC), Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand. Mungbean seed of 17 BC_9 near-isogenic lines (NILs) and their parents, 'Kamphaeng Saen 1' (KPS 1), 'Chai Nat 60' (CN 60) and TC 1966 were used to test the effect of texture layer on bruchid infestation. The BC_9 NILs were developed by backcross breeding method using the high yielding bruchid susceptible cultivars KPS 1, and CN 60 as recurrent parents and the wild mungbean TC 1966 as the donor of bruchid resistance gene. Backcrossing was performed for nine generations plus one selfing generation to establish each NIL.

Procedure for removing texture layer

1. Add 125 to 150 ml of 15% NaOH to a clean 250 ml glass beaker.
2. Add 50 g mungbean seed with a magnetic stirring rod, put on a stirrer to allow stirring at 475-500 rpm for 3 minutes.
3. Pour off the NaOH, then wash seed twice with 125-150 ml distilled water, stirring for 30 sec each time.
4. Pour off water and blot dry the seed immediately with tissue paper.

Procedure for assessment of bruchid resistance

Seeds from KPS 1, CN 60 and each NIL were divided into two sets, one with the texture layer, the other with texture layer removed. Each set was subdivided into 8 samples of 5 g each to test against *C. maculatus* and *C. chinensis*. Each sample was put in a 50 cm^3 plastic box with 30 adult insects of each species having roughly equally males and females. One week later, the insects were removed from the box and the number of eggs laid on the seed coat were counted. Seeds were kept in the box for 40 days more to allow emergence of new adults. Data were recorded on number of emerged adults, number of good seeds and damaged seeds from which the % damaged seed was calculated.

Data analysis

The data on number of eggs laid on 5 g seed were analyzed following the factorial arrangement of treatment combinations in a Completely Randomized Design consisted of three factors, viz. 20 mungbean genotypes x 2 bruchid species x 2 texture layers. Since this experiment has no real replication, the 2- and 3-factor interactions were combined and used as a pooled error to test for significance of the main effects. Data on number of adults emerged and % seed damaged were not normally distributed, however clearly different among the entries, thus they did not require statistical analysis and testing.

Results and Discussion

The NILs had slightly different weight of 1000 seeds as shown in Table 1. This variation was too small and can be neglected. The number of eggs counted in each seed box revealed that the bruchids laid significantly more eggs on the no texture layer seed than on the texture layer one. The contrast was especially clear in TC 1966. *C. maculatus* and *C. chinensis* laid only 70 and 127 eggs per 5 g intact seeds, while they laid 507 and 311 eggs per 5 g on no texture layer seeds, respectively. Variation in number of eggs laid by different bruchid species and on different seed texture layers were clearly confirmed in the analysis of variance table (Table 2). With the intact-dull TC 1966 seed, the bruchids laid eggs mainly on the plastic box, which is glossier. Although less pronounced, *C. maculatus* females laid more eggs on NIL seeds in the absence of texture layer. The NILs of KPS 1 and CN 60 harbored on the average 344.5 and 365.1 eggs per 5 g seeds as compared to in the presence of texture layer, which harbored 305.3 and 297.1 eggs per 5 g seeds, respectively. Similarly, *C. chinensis* females laid 260 and 272.6 eggs on the no texture layer seeds of NILs of KPS 1 and CN 60, as compared to 166.5 and 184.8 eggs on the texture layer intact seed. Although the NILs had as large and shiny seed as their recurrent parent, eggs laid on the NaOH washed seeds tended to be slightly greater than on the intact ones (Table 1). Almost all eggs were laid on shiny seeds of these lines, only few eggs were found on the plastic box.

Many adults emerged from seed of the two susceptible cultivars, KPS 1 and CN 60 regardless the presence or absence of texture layer, while only few adults were observed from the NIL and TC 1966 seed. Thus there was no relationship between the number of eggs laid and the number of adults emerged. This fact also holds on percent of seed damaged. The damage varied between 0 to 5.5 % in the NILs, whereas 88.9 to 98.7 % were detected in the susceptible cultivars, regardless number of eggs laid. Seed damage percentage was clearly associated with number of adults emerged. Thus it is conclusive that the presence or absence of texture layer on

Table 1. Effect of mungbean seed texture layer on reaction to bruchids, *C. maculatus* and *C. chinensis*

Entry no.	Cultivars/ lines	1000-seed weight (g)	*C. maculatus*						*C. chinensis*					
			No. eggs laid		No. adults emerged		% damage		No. eggs laid		No. adults emerged		% damage	
			Texture	No texture	Texture	No texture	Texture	No texture	Texture	No texture	Texture	No texture	Texture	No texture
1	KPS1-2-7-29	66.6	299	378	0.0	0.3	0.0	0.3	98	226	0	0	0	0
2	KPS1-3-6-37	64.0	251	283	0.7	0.0	0.9	0.0	135	201	0	0	0	0
3	KPS1-1-4-29	67.3	322	293	2.3	0.7	2.3	0.6	149	287	0	0	0	0
4	KPS1-4-4-41	64.5	311	277	0.5	0.0	0.6	0.0	177	260	0	0	0	0
5	KPS1-3-1-35	65.7	306	436	0.3	0.5	0.3	0.6	251	266	0	0	0	0
6	KPS1-2-9-31	66.8	238	358	0.7	0.5	0.9	0.6	242	284	0	0	0	0
7	KPS1-3-3-37	65.4	348	399	0.7	0.5	0.9	0.6	135	306	0	0	0	0
8	KPS1-4-2-39	66.4	367	332	0.5	0.0	0.6	0.0	145	250	0	0	0	0
Average (KPS 1 NILs)		**65.8**	**305.3**	**344.5**	**0.7**	**0.3**	**0.8**	**0.3**	**166.5**	**260.0**	**0.0**	**0.0**	**0.0**	0.0
9	CN60-3-1-13	69.6	274	350	0.5	2.0	0.6	2.5	161	291	0	0	0	0
10	CN60-5-7-24	62.2	264	357	3.5	2.5	5.5	3.0	203	254	0.3	0.3	0.3	0.3
11	CN60-2-2-11	63.8	299	342	3.5	0.7	3.5	0.8	145	310	0.3	0	0.3	0
12	CN60-6-7-17	74.7	289	357	1.7	0.5	2.0	0.7	118	245	0	0	0	0
13	CN60-5-3-20	59.6	275	409	0.3	2.5	0.3	2.3	205	240	0	0	0	0
14	CN60-6-6-16	75.4	316	289	3.3	0.3	3.7	0.3	214	324	0	0	0	0
15	CN60-6-3-16	79.1	314	487	0.3	0.0	0.3	0.0	256	265	0	0	0	0
16	CN60-2-3-12	62.8	321	373	1.3	3.3	1.4	2.6	225	241	1	1.7	0.3	0.8
17	CN60-2-1-10	63.6	322	322	0.3	4.5	0.3	4.9	136	283	0.3	0.7	0.3	0.9
Average (CN 60 NILs)		**67.9**	**297.1**	**365.1**	**1.6**	**1.8**	**2.0**	**1.9**	**184.8**	**272.6**	**0.2**	**0.3**	**0.1**	**0.2**
18	KPS1	68.4	178	299	130.0	171.3	98.7	97.9	133	260	142.5	174.5	99.7	98.1
19	CN60	69.2	292	306	105.7	153.0	98.1	91.1	185	286	154	157.5	97.7	88.9
20	TC 1966	16.7	70	507	1.3	3.7	0.4	1.1	127	311	1.3	1.3	0.1	0.3
Mean			**282.8**	**357.7**	-	-	-	-	**172.0**	**269.5**	-	-	-	-

Table 2. Analysis of variance in number of eggs laid by *C. maculatus* and *C. chinensis* on 5 g mungbean seeds with and without texture layer.[a]

Sources of variation	Degrees of freedom	Mean squares
Mungbean lines	19	5,304[ns]
Bruchid species	1	181,832**
Seed texture layer	1	163,262**
Pooled error[2][b]	58	21,113

[a] Raw data were obtained from Table 1.
[b] Pooled error is obtained by combining 2- and 3- factor interactions.
ns = non-significant; ** significant at P = 0.01

mungbean seed has nothing to do with seed damaged by *C. maculatus* and *C. chinensis*. The resistance is purely of antibiosis type as reported by AVRDC.[5]

Conclusion

The presence or absence of texture layer affect oviposition of both bruchids, *Callosobruchus maculatus* and *C. chinensis*, especially in dull texture layer seed of the accession TC 1966. Although texture layer has nothing to do with mungbean antibiosis resistance to the bruchids, it is often necessary that the number of eggs laid on seeds be normalized under a more precise selection process. In this case, the texture layer of the dull-seeded accessions should be removed before use in screening for bruchid resistance to increase the number of eggs laid so that the selection pressure is uniformly imposed on all accessions under selection.

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Perspectives and Economic Analysis of Virus-free Garlic in Commercial Production

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Abstract

An improved scheme for propagation of the virus-free garlic was proposed. With this method, it took only 4 years for the bolting type and 5 years for the non-bolting type from meristem culture to the release of elite seed garlic. Based upon an economic analysis, net profits ranging from 129.1% to 244.5% for the bolting type and from 59.3% to 164.5% for the non-bolting type were obtained from selling various seed grades during propagation, beginning from nucleus to elite seeds. By using the virus-free garlic, both yield and quality of the bulbs and pedicels clearly increased. This procedure promotes garlic production and its processing industry. In addition, application of the virus-free garlics can help create jobs, reduce the use of production area and chemical inputs, and thus provide beneficial effects to the environment.

Key words : *Allium sativum,* economic analysis, garlic propagation, virus-free.

Introduction

Garlic is an important vegetable worldwide. Annually, it has a total growing area of over 800,000 ha with a total production of almost 8,000,000 tons. The current average yield of garlic in the world is 9.5 t/ha whereas that in Asia is 10.2 t/ha (Anon, 1998). Achievement in rapid multiplication of virus-free garlic were reported by many scientists (Mori, 1971; Ayuso and Pena-Iglesias, 1981; Bertaccini *et al.*, 1986; Xu *et al.*, 1987, 1994; Messiaen *et al.*, 1994). Using

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virus-free garlic is a powerful technique to protect the crop from damage caused by virus diseases in production fields. Several schemes for propagation of the virus-free garlic have been proposed. However, only a few of them are put into large scale production, considering the production cost and benefit. In this study, the efficiency of an improved scheme for propagation of virus-free garlic as advocated by Xu *et al.* (1994) was proposed. Economic benefit of the propagation and possible impacts on society and environment were discussed based on both experimental and practical operations.

Materials and methods

The experiments were carried out partly at the Asian Regional Center of the Asian Vegetable Research and Development Center (ARC-AVRDC), Kasetsart University, Kamphaeng Saen, Thailand, and partly at the Yanggu Experimental Base for Elite Seed Propagation of the Biotech Center of the Yellow River Group Company and the Institute for Vegetable Research of Shandong Academy of Agricultural Sciences (SAAS), both in Shandong, China. Three garlic cultivars used in this experiment were obtained from Shandong Province. They were Jiaxiang Zasuan (Jx Za), Cangshan Gaojiao (Cs Ga) and Jiaxiang Redskin (Jx Re).

The experiments on yield comparison were conducted in an experimental field in SAAS by randomly assigning the virus-free and virus-infected garlics as paired comparison in two replications. Calculations on production cost and income benefits were based primarily upon production status and current market price of garlics in China.

The scheme for obtaining virus-free galic was modified from the one proposed by Xu *et al.* (1994) by separating the explants with light virus infection from those with severe infection. For the lightly infected garlic the same scheme could be applied to both bolting and non-bolting types. For the severely infected one, different schemes were used on different bolting types as shown in Table 1.

For inflorescence meristem culture, pedicels with floral sets were sterilized with 95% ethanol for 1 sec., then with 0.2% (w/v) mercuric chloride solution for 3-6 min., and finally rinsed thrice with sterilized water. The meristems were excised from the floral apex and placed on the inflorescence meristem culture (IMC) liquid medium filled in 50 ml flask. The medium was a modified MS medium in which the N-salts were replaced by 5.7 g NO_3 and 0.14 NH_4, 5 mg/l

kinetin (KT), 0.1 mg/l indole butyric acid (IBA), and 40 mg/l gibberellic acid (GA). The flasks were incubated at 23-25 °C under 5,000 lux light intensity, and 16 h day length.

For multi-bulbil multiplication, the basic salts conld be either from B5 or MS medium with 7 g/l agar content, 1 mg/l naphthalene acetic acid (NAA), 70 g/l sugar content. The medium was put in 100 ml baby food jar, 20-25 ml/jar, and kept under supplemented light of 5,000 lux and 18 h day length. The incubating temperature was 23-25 °C.

Under this scheme the virus-free garlic was available for production one or two years earlier than those produced from the original method proposed by Xu *et al.* (1994). The other culture media as stated in Table 1 followed those advocated by Xu *et al.* (1994). The presence or absence of virus in each seed class was assessed following Xu *et al.* (1987).

Results

Economic analysis on propagation of virus-free garlics

Production cost and income benefit for both bolting and non-bolting types in the course of the propagation process for virus-free garlics are shown in Tables 2a and 2b.The farmers who produced the elite seed garlics (ES) gained the highest profit at 389% for the bolting type and 191% for the non-bolting type. The prestock (PS) and elite seed garlics produced in both the isolated field (IF) and the seed field (SF) gave the second highest benefit, with 141% and 245% for the bolting type and 88% and 165% for the non-bolting type, respectively. Lower profits were obtained from the nucleus seed (NS) and prestock seed (PS) at 127 and 129% for the bolting type and 59 and 62% for the non-bolting type, respectively. The benefits from the *in-vitro* bulbil (IB) for bolting type was 214%, which was greater than that for the non-bolting type (108%).

Comparison between the benefits obtained from production of each seed grade of both garlic types is summarized in Figure 1.

Yield performance of the virus-free garlics and the production benefits

Bulb and pedicel yields of the virus-free crops were 54-183% and 117-200%, respectively higher than those of the virus-infected crops as shown in Figure 2. The input-output for production of virus-free and virus-infected crops of the cultivars Jiaxiang Zasuan (Jx Za), Cangshan Gaojiao (Cs Ga) and Jiaxiang Redskin (Jx Re) revealed difference between the two

types of garlic, with 1-3 fold higher output values from the virus-free crops. Monetary values of both bulbs and pedicels produced by the virus-free garlic were even higher. Proportion of large bulbs produced by the virus-free crops was as high as 95%, while the virus-infected lines did not produce large bulbs at all (data not shown).

The using the virus-free garlic as planting materials, the yield of commercial garlic could be increased up to 14,251 kg/ha for the world's average and up to 15,297 kg/ha for Asia's. Total production of the world could reach up to 11,429,700 tons, and that of Asia up to 9,423,000 tons. In addition, 5,884,200 person-days of jobs could be created in the process of producing the virus-free seed garlic. If the proposed system was used in garlic production throughout the world an equivalent arable land area of 400,600 ha could be reduced or used for producing other crops. This estimate was derived by converting the total world garlic increase of 3,805,700 tons from the virus-free garlic production into the land areas through a conversion factor of 9.501 tons of garlics being equivalent to 1 ha of land. Furthermore, the use of virus-free garlic for production can decrease the worldwide production cost due to less land required to produce the same amount of garlics. This land reduction could help saving up to 300,800 tons of chemical fertilizers, or an equivalent of 36,234,900 US$, not consider the other costs of production.

Discusson and Conclusion

Application of the scheme for propagation of virus-free garlic could produce garlics for commercial production within 4-6 generations (Table 1). If 100,000 *in-vitro* cultured bubils are produced in the first year, then a production acreage of 670 ha can be planted with the elite virus-free seed garlic in the fourth or fifth year. Once the system is established, the garlies can be routinely supplied to the growers each season. Propagation and production of the virus-free garlic using the afore-mentioned scheme and techniques are economically feasible at a ratio of cost increase ranging from 57% to 389% (Tables 2a and 2b). The farmers who use the elite virus-free seed garlic get the highest return, although they invest less. Producers of the *in-vitro* bubils and the nucleus seed garlic invest the most, and get a reasonably high profits. Yield and quality of both bulbs and pedicels of the virus-free garlic were superior to those of the virus-infected one and favored agricultural development and processing of garlic. Furthermore, the system provides beneficial effects to society, ecology and environment by creating jobs, increasing efficiency of

arable lands and decreasing area planted to garlics and thus decreasing the use of chemical inputs.

Several plans have bean proposed to organize the use virus-free garlics (Xu *et al.*, 1994; Messiean *et al.*, 1994). The French experts preferred to extend the use of virus-free garlic through farmer organizations rather than the governmental agencies. We suggest that research institution, governmental extension service, and the farmers' organization play combined roles in promotion of the virus-free garlics. Since the amount of investment at the stage of producing the *in-vitro* cultured (IB) and nucleus seed (NS) is relatively higher than that of any other stages, it may be difficult for general farmers to invest. Research institutions and the governmental extension service may take charge in producing IB and NS as well as setting up a technical training course for garlic producers. Thereafter, the farmers can produce the stock seed (SS) and elite seed.

Finally, a system for certification of the seed garlic should be initiated by the government, which also provides the inspection service for testing viruses in each seed grade in order to secure the quality of the commercial garlics.

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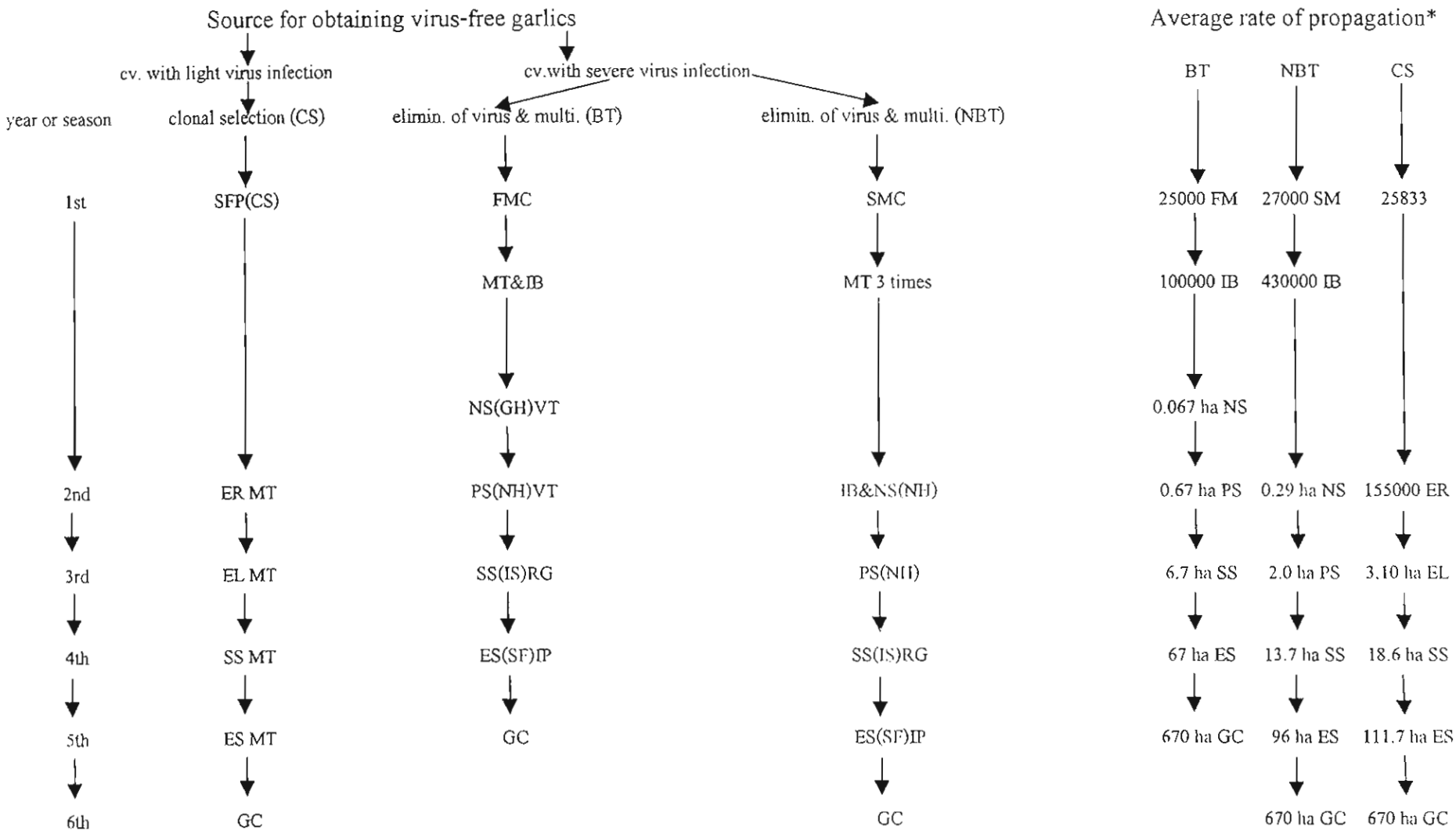
Table 1 The proposed scheme for elite virus-free seed garlic production.



BT	bolting type
CS	clonal selection
cv.	cultivar
EL	elite line
ER	elite row
ES	elite seed
FMC	inflorescence meristem culture
GC	garlic for commercial production
GH	greenhouse
IB	*in-vitro* cultured bulb
IP	inspection
IS	*in-vitro* cultured seedling
MT	multiplication
NBT	non boting type
NH	nethouse
NS	nucleus seed
PS	prestock seed
RG	rogue
SF	symptom free plant
SMC	stem-tip meristem culture
SS	stock seed
VT	virus test

* Average rate of propagation
in the vegetative generation

bolting type:	10 times
non-bolting type:	7 times
clonal selection:	6 times

Table 2a Economic analysis on multiplication and utilization of virus-free bolting garlic in Shandong, China.

Year	Planting	Input (US$)[4]			Output (US$)			Profit (US$)		Seed
	date	S&M[1]	Processing[2]	Total input	Product	Price/unit[3]	Total output	(output-input)	% profit on input	grade
1st	Jul	1108.4	482	1590	100000 bulbil	0.05	5000	3410	214	Lab (IS)
2nd	Jan	5000.0	325	5325	45 (kg) aerial clove	120.00	12200	6875	129	NH (NS)
					400 (kg) bulb	17.00				
2nd	Jun	1700.0	256	1956	70 (kg) aerial clove	30.12	4442	2486	127	IF (PS)
					600 (kg) bulb	3.89				
3rd	Jun	486.3	72	559	100 (kg) aerial clove	3.89	1349	790	141	SF (ES)
					1200 (kg) bulb	0.80				
4th	Jun	100.0	60	160	800 (kg) pedicel	0.24	552	392	245	SF (ES)
					1500 (kg) bulb	0.24				
5th	Jun	36.0	40	76	800 (kg) pedicel	0.24	372	296	389	PF (GC)
					1500 (kg) bulb	0.12				

Lab (IS) *in-vitro* seedlings produced in lab

Lab (IB) *in-viro* bulbils produced in lab

NH (NS) nucleus seed garlics produced in nethouse

NH (PS) prestock seed garlics produced in nethouse

IF (SS) stock seed garlics produced in isolated field

SF (ES) elite seed garlics produced in seed field

PF (GC) garlic for commercial production in planting field

Table 2b Economic analysis on multiplication and utilization of virus-free non-bolting garlic in Shandong, China.

Year	Planting	Input[4] (US$)			Output (US$)			Profit (US$)		Seed
	date	S&M[1]	Processing[2]	Total input	Product	Price/unit ($)[3]	Total output ($)	(output-input)	% profit on input	grade
1st	Oct	37	240	277	25000 seedling	0.02	575	298	108	Lab (IS)
	Dec	575	482	1057	100000 seedling	0.03	2700	1643	155	Lab (IS)
1st-2nd	Feb	2700	482	3182	100000 bulbil	0.05	5000	1818	57	Lab (IB)
	Jul	5000	253	5253	500 (kg) bulb	17.00	8500	3247	62	NH (NS)
	Jun	1700	253	1953	800 (kg) bulb	3.89	3112	1159	59	NH (PS)
3rd-4th	Jun	389	36	425	1000 (kg) bulb	0.80	800	375	88	IF (SS)
4th-5th	Jun	100	36	136	1500 (kg) bulb	0.24	360	224	165	SF (ES)
5th-6th	Jun	36	36	72	1500 (kg) bulb	0.14	210	138	191	PF (GC)

[1] Seeds and mevistem, Including the costs of garlic cloves as source for obtaining meristems, *in-vitro* seedlings, and seed garlics

[2] Including the costs of culture medium, glassware, electricity, water, labors, use of equipment, rent of rooms and land, fertilizer and chemicals

[3] Based on the 1998 Chinese market price

[4] Planting acreage for each of the NS, PS, SS, ES, and CG was 0.067 ha

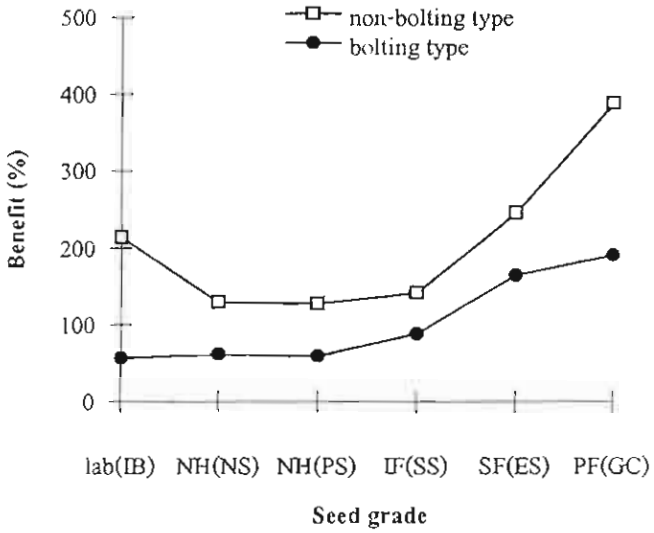


Figure. 1 Benefits obtained from producting different grades of virus-free garlics in bolting and non-bolting types in Shandong, China.

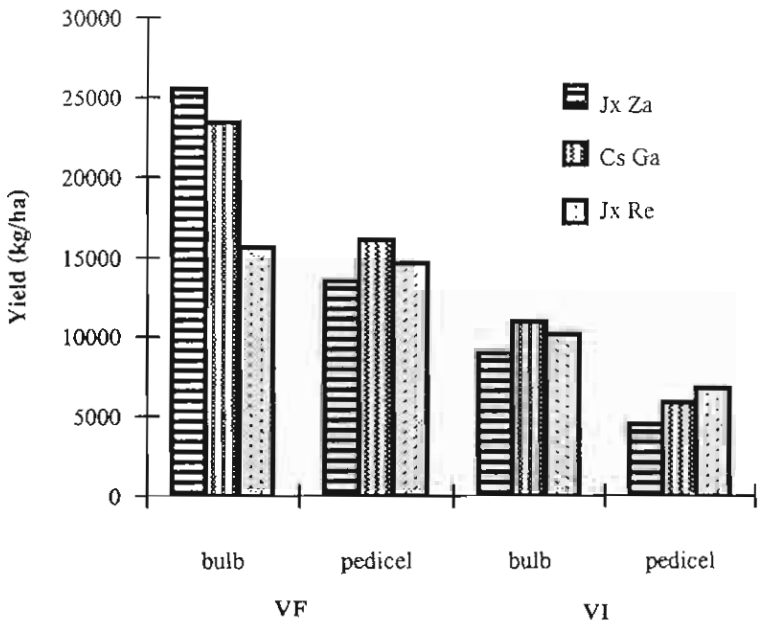


Figure. 2 Comparison on bulb and pedicel yield between the virus-free (VF) and the virus infected (VI) garlics in 3 cultivars tested in paired plots in Shandong, China.

Rapid Multiplicaton of Virus-free Garlic by Inflorescence Meristem Culture and Induction of Multi -bulbils

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Abstracts

A total of 5,000 multi-shoots or multi-bulbils were obtained from one garlic pedicel in one year through four cycles of inflorescence meristem culture. Garlic genotype and culture medium influenced the number of multi-shoots significantly, while the daylength influrenced multi-bulbil induction. An optimum medium for multi-shoot production was the MS having the N-salts replaced with 5.7 g NO_3 and 0.14g NH_4. The growth regulators, kinetin, indole butyric acid, and gibberellic acid were essential in maintaining high rate of multiplication. Multi-bulbils were obtained from the high sugar content (70 g/l) medium at long artificial daylength of up to 18 hr. Using the suggested method, major garlic viruses were eliminated by 60-100%. Garlic crops grown from bubils yielded as large bulbs as those grown from seed bulbs whereas those grown from seedlings yielded significantly smaller bulbs.

Key words : *Allium sativum*, garlic, inflorescence, multi-bulbils, multi- shoots.

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Introduction

Virus diseases are major constraints of garlic production throughtout the world. They cause severe reduction in yield and quality of the garlic products (Messiaen *et al.*, 1994, Xu, *et al.*, 1991, 1994). Using virus-free garlics as planting materials increased the yield of both bulbs and pedicels by 30-180%, and the ratio of big bulbs (>5 cm in diameter) by 20-90% (Xu, *et al.*, 1991; 1994). *In-vitro* culture of stem-tip meristems and lateral buds in garlics were reported by several authors (Mori, 1971; Harvranek, 1972; Ayuso and Pena-Iglesias, 1981; Bhojwani *et al.*, 1982; Bertaccini *et al.*, 1986; Xu *et al.*, 1987; Verbeek *et al.*, 1995). These methods can be applied to produce virus-free garlics, either through *in-vitro* seedlings or *in-vitro* multi-bulbils. However, to produce the virus-free planting materials for a large scale production, the multiplication rate needs to be accelerated and the period during which the *in-vitro* cultured materials growing into the conventional cultivated bulbs for field planting needs to be shortened.

This study was conducted to identify various factors influencing the efficiency of rapid multiplication of virus-free garlics by inflorescence meristem culture and multi-bulbil induction. The major factors studied included garlic cultivar, stage of pedicels used as explants, composition of the culture media, light intensity and duration. Finally, the efficiency of virus elimination was also determined.

Materials and Methods

The experiments were carried out partly in Thailand at the Asian Regional Center of Asian Vegetable Research and Development (ARC-AVRDC) located in Kasetsart University, Kamphaeng Saen, and partly in China at the Institute for Vegetable Research of Shandong Academy of Agricultural Sciences (SAAS), Yanggu Experimental Base for Elite Seed Propagation of the Biotech Center of the Yellow River Group, and the Institute for Plant Genetics and Breeding of the Agricultural University of Shandong.

Materials

Nine garlic lines and cultivars were used in the experiments. Six of them were from Shandong Province, one from Jiangsu Province of China, while the rest two were from Thailand as listed in Table 1.

Table 1 Garlic cultivars and lines used in the experiments.

No.	Name	Origin	Abbreviation
1	Cangshan Puke	Cangshan County of Shandong Province, China	Cs Pu
2	Cangshan Gaojiao	Cangshan County of Shandong Province, China	Cs Ga
3	Cangshan Caosuan	Cangshan County of Shandong Province, China	Cs Ca
4	Caohe Zasuan	Yanzhou County of Shandong Province, China	Ch Za
5	Jiaxiang Redskin	Jiaxiang County of Shandong Province, China	Jx Re
6	Jiaxiang Zasuan	Jiaxiang County of Shandong Province, China	Jx Za
7	Taicang Whiteskin	Taicang County of Jiangsu Province, China	Tc Wh
8	Thai Cm	Chiang Mai Province, Thailand	Th Cm
9	Thai Ks	Nakhon Pathom Province, Thailand	Th Ks

Methods

1. Media used

2.1 *In-vitro* culture media

Media for 3 types of culture were formulated, viz. inflorescence meristem (IM), stem tip meristem (SM), and *in-vitro* cultured bulbil (IB). Each type comprised different basic medium with varying concentration of plant growth regulators, adenine, activated charcoal, and sucrose. Altogether 19 types of media were formulated from different kinds and amount of ingredients as shown in Table 2.

1.2 Transplanting medium

Soil medium used for growing *in-vitro* cultured seedlings was a mixture of garden soil, sand, and compost at the volumetric ratio of 2:1:2, and the complete fertilizer, $N:P_2O_5:K_2O$=12:24:12, was added to the mixture at the rate of 1:1000 w/w.

Table 2 Media used in *in-vitro* culture of inflorescence meristem (IM), stem tip meristem (SM), and in-vitro cultured bulbil (IB).

Formula no.	Type of culture	Basic medium	Plant growth regulator (mg/l)[2/]					Adenine (mg/l)	Activated charcoal (g/l)	Sucrose (g/l)
			BA	KT	NAA	IBA	GA			
1	IM	B5	1.0		0.1			20		30
2	SM	B5		2.0		0.02	40			30
3	SM	B5		3.0		0.05	0.0			30
4	SM	B5		5.0		0.10	20			30
5	SM	MS		2.0		0.05	20			30
6	SM	MS		3.0		0.10	40			30
7	SM	MS		5.0		0.02	0.0			30
8	SM	MSs[1/]		2.0		0.10	0.0			30
9	SM	MSs		3.0		0.02	20			30
10	SM	MSs		5.0		0.05	40			30
11	IB	MS	0.01		0.05				0.0	30
12	IB	B5	0.01		0.05				0.1	50
13	IB	MSs	0.01		0.05				0.2	70
14	IB	B5	0.01		0.5				0.2	30
15	IB	MSs	0.01		0.5				0.0	50
16	IB	MS	0.01		0.5				0.1	70
17	IB	MSs	0.01		1.0				0.1	30
18	IB	B5	0.01		1.0				0.2	50
19	IB	MS	0.01		1.0				0.0	70

[1/] MSs: The N-salts in MS were replaced by 5.7 g of NO_3 and 0.14 g of NH_4

[2/] BA: butyric acid; KT : kinetin; NAA : naphthaleneacetic acid; IBA : indole butyric acid ; GA : gibberellic acid

2. *In-vitro* culture

2.1 Effect of garlic cultivar and pedicel stage on inflorescence meristem culture

The pedicels with floral sets were sampled from 4 garlic cultivars (Cs Pu, Cs Ga, Cs Ca, and Jx Re) at 3 stages, viz. at "bolt tail" emerging, and at 3 and 10 days after emerging. The sampled floral sets were initially sterilized with 95% ethanol for 1 sec., then with 0.2% w/v mercuric chloride solution for 3-6 min. After being rinsed thrice in sterilized water, the meristems were excised from the floral apex. Every six apex were inoculated on the inflorescence meristem medium (IM) as indicated in Table 2, filled in a 50 ml flask, 20 flasks per treatment. Number of inflorescence meristems obtained and rate of multi-shoot initiation observed were examined one month later. Thus each observation was a record from 120 pieces of the meristem. The meristems in the flasks were kept in a culture room with supplemented light at 5000 lux intensity, and 16 h daylength at 23-25°C.

2.2 Factors affecting induction of multi-shoots and multi-bulbils

Shoots derived from the above meristem culture were cut at 1 cm above their basal parts for virus testing. The lower parts with the growing points were transferred on to different types of medium (SM or IB), depending upon the culture purposes. The inoculated flasks were then put into a culture room supplemented with light at an intensity of 1000, 3000 and 10000 lux, and a photoperiod of 13 h for multiple-shoot initiation and 8, 13, 18 h for multi-bulbil induction and development.

The same 4 cultivars as in 2.1 were observed for multi-shoots initiated. For bulbil induction, the cultivars studied were Cs Pu, Th Cm, Tc Wh, Jx Za, and Th Ks.

3. Transplanting of *in-vitro* cultured seedlings/bulbils

3.1 Planting of *in-vitro* cultured seedlings

The seedlings from the latter 5 cultivars were taken from the flasks and washed with tap water to remove the culture medium. They were transplanted into a nursery bed filled with the soil medium in a plastic covered greenhouse under a natural light condition. The greenhouse had 60-80% relative humidity (RH) and 30-35°C temperature. The transplanted seedlings were watered and then covered with plastic film for 5-7 days

to prevent evaporation. The seedlings were sprayed with the diluted solution of macro-elements of the B5 medium at a concentration of 1/5 for the first week from the third day after transplanting. When the temperature was high the plastic film covering the greenhouse was replaced with plastic saran for cooling down the temperature. Four months after culturing, the bulbs were harvested and weighted.

3.2 Planting of *in-vitro* cultured bulbils

The bulbils obtained from the cultivars Cs Pu, Tc Wh, Jx Za, Th Cm and Th Ks from experiment 2.2 were sown in nursery beds in a nethouse having 50% light intensity 60-80 % RH and 30-35°C temperature. Using the Chinese government's recommendation for the cultural practices, the same experiment was conducted in 3 sets. The bulbs were harvested within 3 months and weight of 100 bulbils of each cultivar was recorded in grams.

4. Virus testing

Intensive virus testing was performed at the stage of *in-vitro* seedlings by observation on symptoms as well as using the sandwich ELISA test. Antisera against the onion yellow dwarf virus (OYDV), leek yellow stripe virus (LYSV), and shallot latent virus (SLV) were used in the test. All plants from both nucleus seeds in the greenhouse and prestock seeds in the nethouse were tested for viruses by observation of the symptoms. However, 10% of these plants were retested using the ELISA technique.

5. Experimental design and data analysis

The culture treatments were arranged in a factorial manner and randomly assigned in complete blocks. Data were analyzed on a PC computer using the Statistical Analysing System (SAS) program. After an F-test revealed treatment difference, individual mean comparison was performed using least significant difference (LSD) at 0.05 level of probability. Due to complicated treatment combinations, interaction between factors were not considered in the analysis and only the main effect of each treatment would be emphasized.

Results and Discussion

Inflorescence meristem culture and multi-shoot initiation

1. Factors affecting meristem culture and multi-shoot initiation

1.1 Effect of garlic cultivar and pedicel stage

The number of obtainable inflorescence meristems varied, depending on garlic cultivar and the stage at which the floral set was sampled, as shown in Table 3. The highest number of up to 51.3 inflorescence meristems was obtained from the cultivar Jiaxiang Redskin (Jx Re) at 3 days after the pedicel tail emerged. This developmental stage of the pedicel (stage II) also yielded highest meristem number across the four cultivars. In addition, a significant difference in the rate of multi-shoot induction between the cultivars was also observed . The cultivar Cangshan Puke (Cs Pu) gave the highest rate of multi-shoot induction at 4.9 per meristem.

Three kinds of meristems were found on inflorescence of a floral apex of garlics. The first kind differentiated into floral primodia while the other two meristems surrounding it remained undifferentiated. They may later develop into lateral buds and the aerial cloves. Only the undifferentiated meristems were excised for *in-vitro* culture to obtain the virus-free plants. No callus formation were found in the course of culture but organogenesis. The vegetative offsprings multiplied from this study were also uniform in the field with no mutant observed, even among several thousand plants studied.

1.2 Factors affecting induction of multi-shoots

The media expressed the most significant influence on the rate of multi-shoots developed from the inflorescence meristem (Table 4). The highest rate of shoot multiplication (RM) was obtained from the basic medium no. 3 (MSs which is MS medium with the N-salts replaced with 5.7 g NO_3 and 0.14 g NH_4) in both experiments. The cultivar Cangshan Puke (Cs Pu) gave the best RM (4.34) among all three cultivars. Light intensity between 1,000 to 10,000 lux did not affect the RM whereas the growth regulators (KT, IBA, and GA) significantly increased the RM throughout the increasing concentrations under study. No interaction between the factors was detected.

1.3 factor affecting multi-bulbils

Formation and development of the multi-bubils were found to be conditioned by several factors as summarized in Table 5. Only results from the cultivars Cs Pu and Th Cm were presented.

1.3.1 Effect of photoperiod

Survival rates (SR) of the bulbils were not different throughout all the factors under study, except at different photoperiod. The rate of bulbing reached as high as 79% at 18 h artificial photoperiod across all other factors, while no bulbil was observed at 8 h photoperiod. A rather low rate of bulbing was found at the photoperiod of 13 h.

1.3.2 Effect of composition of the culture medium

Sugar content in the medium was more important in enhancement of bulbing rate and bulb weight than the plant growth regulators and the basic medium under study (Table 5). A high rate of bulbing (BR), up to 94%, and the largest size of 27.8 g/100 bulbils were obtained on the medium with sugar content of 70 g/l. Significant effects of NAA and basic medium were also observed on bulbing rate. The basic media expressed no effect on weight of 100 bulbils.

1.3.3Effect of garlic genotype

Weight of *in-vitro* cultured bulbils differed significantly depending on garlic cultivars. The cultivar Jiaxiang Zasuan (Jx Za) gained the highest weight of bulbil, averaging 34g/100 bulbils, while the Kamphaeng Saen line from Thailand (Th Ks) gave the lowest weight of 16g/100 bulbils (Table 6).

Rate of multiplication from inflorescence meristem differed with garlic genotypes and the culture cycle. Averaging from 6 cultivars, each meristem explant initiated *in vitro* 4.26, 4.93 and 2.21 from the lst, 2nd, and 3rd culture cycle, respectively (Table 7). From the average of 35 inflorescence meristems excised from each pedicel, they would produce 149.1 (35 x 4.26) shoots at the end of the first culture cycle. Then, they would be

multiplied to 735.1 (149.1 x 4.93) shoots after the second cycle, and again to 1624.5 (735.1 x 2.21) multi-shoots by the third cycle, and finally 4,841 (1624.5 x 2.98) bulbils would be obtained. Since one culture requires 3 months, a total number of 4841 multi-bulbils could produced from one garlic pedicel in one year. This rate of multiplication was over 20 fold of that using the stem tip meristem culture reported by Xu *et al.* (1994). They were able to produce 232 seedlings from one garlic plant in one year.

Survival rate of the bulbils was better than the seedlings. Yet the bulbs that resulted from bulbil crop were always bigger than from the seedling crop. The average bulb weight of 5.37 g obtained from the bubil crops was about the regular size of seed bulbs while that of the seedlings was only 3.20 g (Table 8). Plant height of the crop grown from both bulbils and seedlings differed depending on garlic genotypes. However, averaging across the cultivars, both planting materials resulted in the same plant height in spite of the shorter growth period of the bulbils. The bubil crops grew significantly faster than the seedling crops. In addition, the bulbils had better survival rate and were more hardy and thus became a productive crop with lower inputs than the seedling crop.

2. Efficiency in elimination of viruses by means of inflorescence meristem culture

At least five viruses infected the garlic crops in the area of Shandong province, China (Xu *et al.*, 1991, 1994). They were onion yellow dwarf virus (OYDV), leek yellow stripe virus (LYSV), shallot latent virus (SLV), potato virus Y (PVY), and garlic mosaic virus (GMV). From several lots of seedlings obtained from this experiment, those free from the OYDV, LYSV and SLV were found between 50-90%, 70-100% and 60-80%, respectively (data not shown). Thus, on the average, the efficiency of virus elimination by inflorescence meristem culture was around 78.6%. This was close to the results reported by Verbeek *et al.* (1995).

Conclusions

Inflorescence meristems of garlic could be cultured to partilly eliminate major garlic viruses at the rate ranging from 60 to 100%. On the average, 35 meristems were obtained from each pedicel for culturing, depending upon the garlic genotypes and the stage at which the flora set was sampled. Rate of multi-shoot initiation varied according to the ingredients in the culture medium, particularly the basic medium and plant growth regulators.

Multi-bulbils were obtained by culturing the multiplied garlic shoots derived from the inflorescence meristems. It is essential that sucrose content in the medium be around 70 g/l and photoperiod in the culture room be around 18 h/day.

In this study, a high rate of multiplication at almost 5000 bubils per year was obtained from one garlic plant. The *in-vitro* cultured bulbil crop produced the bulbs as nearly the same size as the crop from the regular seed bulbs at low cost of production. This study showed clearly that it is possible to produce the virus-free garlic in a large scale production.

The technique on inflorescence meristem culture and induction of multi-bulbils helped accelerating multiplication of virus-free garlics, shortening the period of turning the in-vitro cultured materials to marketable bulbs, and decreasing the production cost. However, the inflorescence meristem culture technique was limited to the bolting type garlics, while the multi-shoot and multi-bulbil culture can be applied to the non-bolting type as well.

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Table 3 Number of inflorescence meristems and rate of multi-shoot initiation observed from culturing of four garlic cultivars, using pedicels at different develpmental stages as explants.

Cultivars	No. of inflorescence meristems obtained			RM
	I	II	III	
Cs Pu	2.0	22.0	9.0	4.9 a[1/]
Cs Ga	3.0	23.7	13.0	3.8 b
Cs Ca	5.0	46.0	26.0	4.0 b
Jx Re	5.0	51.3	19.0	3.1 b
Total	15.0	143.0	67.0	15.7
Average	3.8 c[1/]	35.8 a	16.8 b	3.9

[1/] Means followed by the same letter in the same row (no. of inflorescence meristems obtained) or column (rate of multi-shoot initiation) are not significantly different as tested by $LSD_{.05}$.

Stage I: at the emerging of pedicel tail

Stage II: three days after emerging of pedicel tail

Stage III: ten days after emerging of pedicel tail

Table 4 Orthogonal presentation of the rate of multiplication (RM) from inflorescence meristem of three garlic cultivars cultured in combinations of media and light intensities, conducted in two separated experiments.

Treatment	Experiment I			Experiment II			
	Basic medium	Cultivar	Light Intensity (lux)	Basic mediu m	KT (mg/l)	IBA (mg/l)	GA (mg/l)
1	B5	Tc Wh	1000	B5	2.00	0.02	40.0
2	B5	Cs Pu	3000	B5	3.00	0.05	20.0
3	B5	Jx Za	10000	B5	5.00	0.10	0.0
4	MS	Tc Wh	3000	MS	2.00	0.05	0.0
5	MS	Cs Pu	10000	MS	3.00	0.10	40.0
6	MS	Jx Za	1000	MS	5.00	0.02	0.0
7	MSs	Tc Wh	10000	MSs	2.00	0.10	20.0
8	MSs	Cs Pu	1000	MSs	3.00	0.02	0.0
9	MSs	Jx Za	3000	MSs	5.00	0.05	40.0
t1[1/]	1.79 c[2/]	3.10 b	3.06	1.53 c	1.60 b	2.13 b	3.37 a
t2	2.73 b	4.34 a	2.96	2.50 b	1.63 b	3.97 a	2.40 b
t3	4.28 a	3.05 b	3.97	3.50 a	5.10 a	4.30 a	1.87 c

[1/] In experiment I; t1, t2 and t3 represent the single factor average of RM of the three treatments in that column (i.e. B5, MS and MSs for basic medium, etc.). In experiment II; t1, t2 and t3 follow the order of the first three treatments (i.e. 2.00, 3.00, 5.00 mg/l KT, etc.).

[2/] Means followed by the same letter in the same column are not significantly different as tested by $LSD_{.05}$.

Table 5 Orthogonal presentation of the rate of bulbing from multi-shoots of two garlic cultivars cultured in combinations of media, daylengths, and light intensities, conducted in two experiments.

Treatment	Experiment I[1/]		Agar	Activated		Experiment II		
	Day length (hr)	Light intensity (lux)	(g/l)	charcoal (g/l)		NAA (mg/l)	Sugar (g/l)	Basic Medium
1	8	1000	7.0	0.00		0.05	30	MS
2	8	3000	7.50	0.10		0.05	50	B5
3	8	5000	8.0	0.20		0.05	70	MSs
4	13	1000	7.5	0.20		0.50	30	B5
5	13	3000	8.0	0.00		0.50	50	MSs
6	13	5000	7.0	0.10		0.50	70	MS
7	18	1000	8.0	0.10		1.00	30	MSs
8	18	3000	7.0	0.20		1.00	50	B5
9	18	5000	7.5	0.00		1.00	70	MS
t1[3/]	0.00 c[4/]	0.35	0.51	0.46	SR[2/]	0.90	0.85	0.91
t2	0.53 b	0.43	0.38	0.50		0.90	0.93	0.90
t3	0.79 a	0.53	0.43	0.36		0.86	0.85	0.86
t1					BR	0.55 b	0.53 c	0.60 b
t2						0.81 a	0.62 b	0.59 b
t3						0.77 a	0.94 a	0.77 a
t1					BW	12.08 b	9.57 b	18.93
t2						26.18 a	23.78 a	19.23
t3						24.58a	27.77a	22.95

[1/] The cultivars used in experiment I and II were Cs Pu and Th Cm, respectively.

[2/] SR, BR and BW are survival rate, bulbing rate, and weight of 100 bulbils, respectively.

[3/] t1, t2 and t3 represent the single factor average of SR, BR or BW of the three treatments in that column.

[4/] Means followed by the same letter in the same column are not significantly different as tested by $LSD_{.05}$.

Table 6 Weight of in-vitro cultured bulbils (g/100 bulbils) from 5 garlic cultivars cultured in 3 sets.

Cultivar	Set no.			
	I	II	III	Mean
Cs Pu	27	23	34	28.0 ab[1/]
Tc Wh	21	23	19	21.0 cd
Jx Za	33	31	38	34.0 a
Th Cm	25	29	27	27.0 bc
Th Ks	15	16	15	15.3d

[1/] Cultivar means followed by the same letter are not significantly different as tested by LSD_{05}.

Table 7 Number of muti-shoots obtained through rapid multiplication of virus-free garlics from six cultivars in three vegetative generations.

Cultivar	Generation I		Generation II		Generation III		Total	Mean
	No. of shoots	RM[1/]	No. of shoots	RM	no. of shoots	RM	no. of shoots	RM
Cs Ga	54.0	5.40	248.7	4.61	669.3	2.69	972.0	4.23 ab[2/]
Jx Za	37.0	3.70	169.0	4.57	214.0	1.27	420.0	3.18 c
Ch Za	28.0	2.80	78.7	2.81	154.7	1.97	261.4	2.53 c
Cs Pu	47.3	4.73	383.0	8.10	967.3	2.53	1397.6	5.12 a
Cs Ca	58.7	5.87	278.0	4.74	631.3	2.27	968.0	4.29 a
Jx Re	30.3	3.03	145.0	4.79	370.3	2.55	545.6	3.46 bc
Mean	42.6	4.26	217.1	4.93	501.2	2.21	760.8	3.80

[1/] RM : Rate of multiplication = no. of shoots mutiplied from one explant

[2/] Mean RM followed by the same letters are not significantly different as tested by LSD_{05}

Table 8 Comparison of efficiency between the crops produced from in-vitro cultured seedlings and bulbils of five garlic lines.

Treatment	Rate of survival	Bulb weight (g)	Plant height (cm)
Planting material			
Bulbil	0.99 a[1/]	5.37 a	58.46
Seedling	0.91 b	3.20 b	55.59
Cultivar			
Cs Pu	0.96	5.49 a	60.12 a
Jx Re	0.94	2.40 c	43.76 c
Cs Ga	0.95	5.12 a	64.93 a
Cs Ca	0.94	4.04 b	57.56 b
Ch Za	0.96	5.26 a	60.98 a

[1/] Means followed by the same letter in the same column are not significantly different as tested by LSD_{05}.

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Ammonia Accumulation as an Index of Glufosinate-Tolerant Soybean Cell Lines

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Selection of soybean (*Glycine max* L. cv. SJ 4) cell lines tolerant to glufosinate was attempted using cell suspension cultures induced from hypocotyls of young seedlings. The cell suspension was cultured 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 tolerant to 10^{-7} M glufosinate was obtained through direct selection of diploid cells in the suspension culture. It is referred to as 10^{-7} M glufosinate-tolerant soybean cell line. Determination of ammonia concentration in the herbicide-treated cells indicated that the normal (nontolerant) cells accumulated up to 15-fold more ammonia than the tolerant cells. This information suggested that lower ammonia accumulation in the tolerant cells can be used as an indicator to select for glufosinate-tolerant soybean cell lines. © 2000 Academic Press

Key Words: ammonia accumulation; cell suspension culture; glufosinate; herbicide-tolerant soybean.

INTRODUCTION

Glufosinate is a foliar-applied contact herbicide that acts faster than glyphosate but slower than paraquat. It is a toxicologically and environmentally benign herbicide that does not persist in the environment. Although some plant species are more tolerant to glufosinate than others, it is generally used as a nonselective herbicide in much the same way as glyphosate. Thus, resistant crops would greatly increase the utility of this herbicide.

Glufosinate is a potent inhibitor of the biosynthetic enzyme glutamine synthetase (GS), which is involved in general nitrogen metabolism in plants, including the assimilation of ammonia accumulated as a result of photorespiration and nitrate reduction (1, 2). The accumulation of ammonia caused by GS inhibitors is thought to be the principal cause of phytotoxicity in plants (3).

Selection for herbicide-tolerant crops can be accomplished by both traditional plant breeding (4–7) and biotechnolgical techniques. Some of the recent examples include maize and barley plants with improved glyphosate tolerance (8, 9) and herbicide-tolerant transgenic sugarcane plants (10). Currently, research in this area emphasizes physiological and biochemical mechanisms of natural and evolved tolerance to herbicides. There are several reports on the mechanism of herbicide tolerance, both in whole plants (7, 11) and in cultured cells (8, 12, 13).

In the current study, glufosinate-tolerant soybean cell lines were obtained from suspension cultures. Whereas the properties of ammonia accumulation in plant cells are important factors affecting differential response to the herbicide in normal (nontolerant) and tolerant cells, no information is available on the derivation of glufosinate tolerance from selected cell lines. The availability of glufosinate-tolerant soybean cell lines should be of great agronomic interest and should expand the use of the herbicide, which is now limited due to its nonselective mode of action. The present study reports the results from selection of soybean cell lines tolerant to glufosinate and their ammonia accumulation as an indicator of glufosinate-tolerant soybean cell lines.

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MATERIALS AND METHODS

Herbicide Treatment

Technical-grade glufosinate (2-amino-4-(hydroxymethylphosphinyl) butanoic acid; purity 94.4%), the ammonium salt, was dissolved in ethanol and sterilized with a membrane filter. The herbicide as selective agent was added to the sterilized culture medium immediately before subculturing of the cells, with a final ethanol concentration in the medium at the maximum of 0.1% (v/v).

Cell Suspension Culture

Soybean cultivar 'SJ 4' was used in the experiment. SJ 4 was selected from the progenies of a cross between 'Acadian' and 'Tainung 4.' It was released to Thai farmers in 1976 and is still grown in many soybean-growing areas throughout the Kingdom. Callus was induced from hypocotyls of young seedlings. A friable callus was selected from compact organogenetic calli and subcultured on the fresh medium every 2 weeks. After four subcultures, about 10 g of fast-growing callus was transferred into the MS (14) liquid medium supplemented with B5 vitamins, 0.3% sucrose, and 10 mg/l NAA (pH 5.8) on a gyrating shaker (120 rpm) at 28 °C. Subculturing was done at 10-day intervals by transferring 1 ml of packed cell volume (PCV) cells to 50 ml fresh medium. Cell growth reached the maximum after 10 days. A 10-fold increase in PCV during each 10-day period was repeatedly obtained.

Selection of Herbicide-Tolerant Cells

Initially, the lowest concentration of the herbicide was used to treat the cells. Surviving cells were subcultured at 10-day intervals by transferring them to fresh medium with the same concentration of the herbicide. When the cells had reached the same growth rates as that of non-treated cells, they were transferred into a medium containing a progressively higher concentration of the herbicide. This process was repeated for several passages until highly tolerant cells were obtained.

Ammonia Accumulation Test

Ammonia accumulation and its inhibition by glufosinate were compared in normal and tolerant cells according to the modified procedures of Weatherburn (15). Both types of cells were treated with glufosinate at the concentration of 10^{-10} to 10^{-7} M. After subculturing, the cells obtained from day 0, 3, 5, 7, and 10 were used for the assay. All extraction procedures were carried out according to Desmaison *et al.* (16). Briefly, 2 g of fresh soybean cell material was extracted in 10 mM HCl, 0.18 g of sulfosalicylic acid and then centrifuged at 3000 rpm for 15 min. The upper 200 μl of supernatant was diluted with 800 μl distilled water. Reagent A (1 g phenol, 5 mg sodium nitroprusside, 100 ml distilled water) at 2.5 ml was added to 20 μl of the diluted cell extract, followed by adding 2.5 ml reagent B (0.5 g NaOH, 0.84 ml NaOCl, 100 ml distilled water). The reaction mixture was incubated for 20 min at 37°C, and the absorbance was measured at 625 nm. The ammoniacal nitrogen was determined on a standard curve (micrograms of ammoniacal nitrogen per gram of fresh weight = micrograms of determined ammoniacal nitrogen × 450). The standard curve was made using NH_4Cl at concentrations ranging from 0.5 to 4 μl ammoniacal nitrogen. Ammonia accumulation was expressed as micrograms of ammoniacal nitrogen per gram of fresh weight and converted to percentages of the control. Data were analyzed using analysis of variance, and means were separated by least significant difference at $P = 0.05$.

RESULTS AND DISCUSSION

Selection of Herbicide-Tolerant Cells

Initial attempts to select for glufosinate-tolerant cell lines were performed on soybean cell suspension cultures. The relative growth rate of the cell lines at various herbicide concentrations was estimated by measuring the increase in PCV over a culture period of 10 days. Growth of cells was inhibited in accordance with herbicide concentration. Growth was completely stopped by 10^{-5} M glufosinate. When the cells were

treated with 10^{-9} M glufosinate, their growth rates were slightly reduced. This concentration was used as the initial selection pressure. The tolerant cell lines were isolated by sequencial transfers into a medium with the same or higher concentration of the herbicide. Cells that could grow in 10^{-7} M glufosinate were successfully isolated. The period required for selection was 8 months (data not shown). The resulting cell line is referred to as the 10^{-7} M glufosinate-tolerant soybean cell line. It was developed by simple selection for tolerance in the cell cultures.

The growth response of glufosinate-tolerant cells was determined at different concentrations ranging from 10^{-9} to 10^{-5} M (Fig. 1). In normal cells, 50% growth inhibition occurred at 5×10^{-8} M, whereas tolerant cells grew successfully in the presence of 10^{-6} M glufosinate, a concentration at which the normal cells did not survive. This suggested that the tolerant cells were 20-fold more tolerant to the herbicide than the non-selected normal cells (as measured by the ratio of tolerant I_{50} to normal I_{50}, Table 1). These results were consistent with those of previous reports in which tolerant soybean cells were selected from cell cultures subjected to clomazone (17), atrazine (18), fluazifop-*P*-butyl, imazaquin, and oxyfluorfen (19).

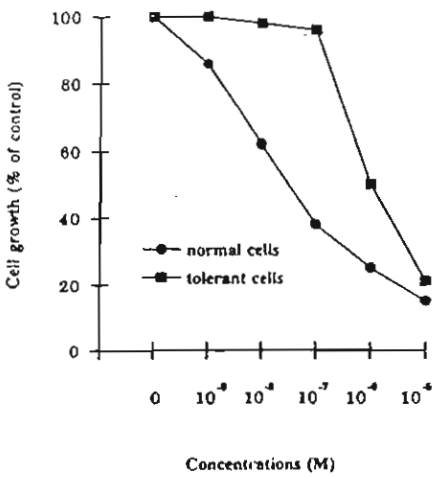

FIG. 1. Growth response of normal and tolerant soybean cells to glufosinate was determined 10 days after herbicide treatment. Each data point represents the average of three replicates.

TABLE I

I_{50} for Glufosinate Determined from the Growth Response and Ammonia Accumulation of Normal and Tolerant Soybean Cells

	I_{50} (M)[b]	
Cells	Growth response	Ammonia accumulation
Normal	$5 \times 10^{-8} \pm 0.75$	$5 \times 10^{-9} \pm 0.55$
Tolerant	$10^{-6} \pm 1.05$	$7.5 \times 10^{-8} \pm 0.90$
T/S[a]	20	15

[a] T/S = I_{50} tolerant/I_{50} normal was calculated to indicate the degree of tolerance.

[b] Values represent the I_{50} mean ± SE of three independent experiments.

Ammonia Accumulation Test

Glufosinate is an inhibitor of glutamine synthetase (GS), preventing incorporation of ammonia into amino acids. Inhibition of GS by glufosinate results in toxic accumulation of ammonia in plant cells (1–3). A complete understanding of the mechanism of the glufosinate-tolerant soybean cell lines begins at the level of ammonia accumulation. Due to numerous concerns about herbicide-tolerant crops (12, 17–19), much current research is focusing on strategies for developing and identifying the mechanisms of herbicide tolerance. In this study, the glufosinate-tolerant soybean cell lines were obtained through direct selection of diploid cells in the suspension cultures. As more glufosinate-tolerant lines become available, the mechanism of herbicide tolerance in relation to ammonia accumulation can be compared in the cell lines.

The effect of glufosinate on ammonia accumulation in the normal and tolerant cell lines was determined (Fig. 2). Accumulation of ammonia in both types of cell lines increased with increasing concentration of glufosinate herbicide throughout the time course of the experiment. However, the tolerant cells were less sensitive than the normal cells. The I_{50} concentrations of glufosinate in the normal and tolerant cells were 5×10^{-9} and 7.5×10^{-8} M glufosinate, respectively (Table 1). This indicated that the accumulation of ammonia differed by a factor of 15-fold between the normal and the tolerant cells. It is reasonable to suggest that less

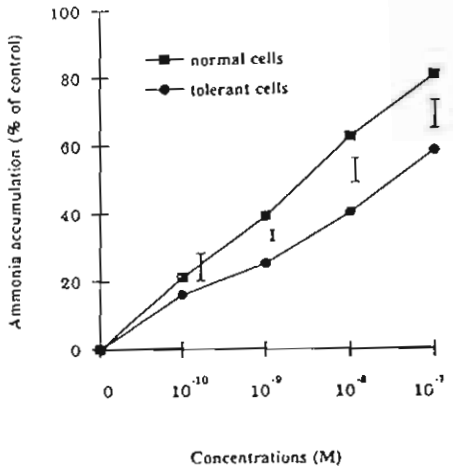

FIG. 2. Effect of glufosinate on ammonia accumulation in normal and tolerant soybean cells. Each data point represents the average of three replicates. The vertical bars represent LSD.05 at each concentration.

sensitivity to glufosinate confers the tolerance to these tolerant cells.

Variation in ammonia accumulation in the cells contributed to alteration in the target site in the more tolerant cell lines. These effects may be directly related to accumulation of ammonia by glufosinate-tolerant lines. These results were consistent with the reports on several inhibitor-tolerant crops, including soybean cells tolerant to clomazone (17) and oxyfluorfen (12). In addition, there were several reports on the mechanisms of herbicide tolerance in both whole plants (7, 11) and cultured cells (8, 13). In these studies, the tolerance apparently was due to an alteration in the target site, which correlated with a physiological and biochemical basis for the tolerance. This finding supports the view that accumulation of ammonia caused by GS inhibitors may be the principal cause of cytotoxicity in the plant cells. This suggests that one of the mechanisms for glufosinate-tolerant soybean cell lines is reduced ammonia accumulation in the tolerant cells. However, there may also be other mechanisms.

Biotechnology is now providing a method to discover tolerant genotypes against postemergence, nonselective herbicides, such as glyphosate and glufosinate (20). The current study indicated that soybean cultivar SJ 4 could give rise to cell lines tolerant to glufosinate. The cell lines were determined by their high tolerance ratios for plant cell response to the herbicide and reduced ammonia accumulation in the cells. With regard to tolerance ratios, I_{50} tolerant/ I_{50} normal was calculated to indicate the degree of tolerance (Table 1). Regeneration of the toterant cell line is being undertaken. Use of glufosinate herbicide will greatly improve the flexibility of current soybean weed management programs. Furthermore, it can provide growers with a safe and effective weed management option for late-emerging weeds and provides a different mode of action in a crop and/or weed resistance management plan.

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Asian Vegetable Research and Development Center

Collaborative Mungbean Breeding Research between AVRDC and its Southeast Asian Partners with Emphasis on Thailand

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Importance of Mungbean in Southeast Asia

Mungbean is an old crop of the Southeast Asian region. Besides growing mungbean for domestic consumption, Myanmar, Thailand, and Vietnam export their surplus to other countries in Asia and Europe. Thailand, in particular, had been the world's largest mungbean exporter for over 30 consecutive years (Srinives and Yang 1988). In recent years, the use of high-yielding cultivars in China, Myanmar, and Vietnam, coupled with expansion of planted areas, allowed them to be more competitive. High production cost was also blamed as a reason for Thailand losing its position as an exporter. The mungbean statistics in Table 1 show that Southeast Asian countries grew around 1.2 million t of mungbean on some 1.4 million ha yearly, with an average yield ranging from 0.5 to 1.3 t/ha. In addition, Laos PDR and Cambodia have a potential to become mungbean exporters by adopting cultivars and production technology from Thailand.

Table 1. Status of mungbean production in Southeast Asian countries[a]

Country	Area planted ('000 ha)	Average yield (t/ha)	Production ('000 t)	Crop year
Cambodia	20	0.5	10	1994/95
Indonesia	390	1.3	500	1993/94
Lao PDR	3.3	0.7	2.3	1994/95
Malaysia	NA	NA	NA	
Myanmar	447	0.6	268	1994/95
Philippines	44	0.7	32	1993/94
Thailand[b]	335	0.8	256	1994/95
Vietnam	172	0.7	119	1993/94
Total Asean	1,411.3	0.5-1.3	1,187.3	

[a] Statistics obtained from FAO Year Books and country reports of the participants of AVRDC-ARC Regional Training Course in Vegetable Production and Research
NA = Data not available
[b] Including blackgram (~ 20% of total planted area and production)

Utilization of AVRDC Breeding Lines in the Region

Since 1971, AVRDC researchers have been developing improved mungbean lines with high yield, short stature, early and uniform maturity, bold seeds, and resistance to diseases and insect pests. Yang (1996) updated a long list of AVRDC mungbean lines released in major producing countries, beginning from the first line, VC 1089A (released as "ASVEG 78" in 1978 in Costa Rica) until VC 2778 B (released as VN 93-1 in 1994 in Vietnam). A total of 46 cultivars developed through AVRDC's mungbean improvement program have been released officially in 20 countries. VC 1973A, probably the most widely grown mungbean line in the world, was released in China, Korea, Thailand, and the US. VC 1960 D became a recommended cultivar in Australia, Vietnam, and Laos. VC 1628A, VC 2768A, and VC 2768D, were each recommended in at least two countries. The remaining lines found their niche in specific countries. Tay (1993) reported that the top 11 parents of improved high-yielding varieties accounted for 76.6% of the elite gene pool. He concluded that, in general, the Indian materials were donors of disease resistance while the Philippine materials were sources of high yield, early and uniform maturity, and photoperiod insensitivity. Of the materials used in crosses by AVRDC mungbean breeders, up to 57.4% were from the Philippines, and 38.2% from India. The remainder of the germplasm came from USA, Korea, Taiwan, and Sri Lanka. Tay (1993) facilitated the use of AVRDC's mungbean collection by tabulating accessions carrying specific gene/traits to suit various breeding objectives.

AVRDC-improved lines serve as excellent parents in breeding programs, especially when the lines' merits are all combined with local breeding objectives. For example, the Nuclear Institute for Agriculture and Biology (NIAB) at Faisalabad, Pakistan has incorporated mungbean yellow mosaic virus resistance into large-seeded high-yielding AVRDC lines carrying resistance to Cercospora leaf spot. As a result, "NIAB Mung 51" and "NIAB Mung 54" (NM 51 and 54), both derived from V 6601 x VC 1973A, were recommended for release in 1990. Later, Pakistan's most popular "NM 92" was selected from progenies of the cross NM 36 x VC 2768B and released in 1993. In 1996, the cultivar "NM 52" accounted for over 60% of the mungbean-growing area in Pakistan (Malik 1996, pers. comm.). Another example of exploiting AVRDC lines in a country program was advocated by the Rural Development Administration (RDA) of Korea. Chonnam Provincial RDA recommended the cultivar "Nampyeong nogdu" from a selection of the cross VC 1089 B x Kyeongijaerae # 5 in 1992 (AVRDC 1992).

Following the establishment of the Asian Regional Center of AVRDC (AVRDC-ARC) in Thailand in 1992, mungbean research, particularly improvement research, 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.H. Kim from Korea, the first mungbean breeder assigned to Thailand, led the ARC mungbean staff in developing more crosses from superior genotypes. Dr. I.A. Malik from Pakistan, who succeeded him, continued to work along the same lines. By mid-1997 ARC had produced about 300 more VCs.

Superior lines derived from the recent crosses were included in the International Mungbean Nursery (IMN) and dispatched to collaborators in various countries. The 20th IMN was distributed in 1994 and 1995 during which 18 sets were sent to six countries. Ten partial sets were also sent to eight countries. In 1996, the 21st IMN was composed and by mid- 1997, 46 trials altogether had been dispatched to 21 countries (Table 2). All South and Southeast Asian countries with the exception of Cambodia have received at least one complete set. During the same period, particular breeding lines were also requested by collaborating scientists in 13 countries, including Cambodia. Over the past 15 years, several additional breeding lines were also hand-carried by participants of the 5-month Regional Training Course in Vegetable Production and Research conducted at ARC. Thus, AVRDC mungbean lines are essentially utilized in all major mungbean breeding programs in the world.

Table 2. Number of mungbean germplasm sets dispatched from ARC/AVRDC to country programs during 1994 - June 1997

Country	1994 - 1995		1996		Jan. - June 1997		Total
	20th IMN	Others	21st IMN	Others	21st IMN	Others	
Australia	-	1	-	-	-	-	1
Bangladesh	-	-	1	-	-	-	1
Bhutan	-	-	1	-	-	-	1
Cambodia	-	-	-	1	-	1	2
China	10	-	-	-	6	1	17
Egypt	-	-	1	-	-	-	1
El Salvador	-	-	-	-	-	1	1
Ghana	-	-	-	-	1	-	1
Guyana	-	-	-	1	-	-	1
India	-	1	7	2	-	-	10
Indonesia	-	-	2	-	-	-	2
Iran	-	-	1	-	-	-	1
Italy (FAO)	1	-	-	-	-	-	1
Korea	-	1	-	-	-	-	1
Laos	-	-	1	-	1	1	3
Malaysia	-	1	-	-	1	-	2
Myanmar	3	1	1	-	-	-	5
Nepal	-	-	1	-	-	-	1
Nigeria	-	-	-	-	-	1	1
Pakistan	-	1	3	3	-	-	7
Philippines	-	-	2	1	-	1	4
Syria	-	-	-	1	-	-	1
South Africa	-	-	1	-	-	-	1
Sri Lanka	-	-	1	-	-	-	1
Taiwan	-	1	1	2	-	3	7
Thailand	1	3	1	5	2	3	15
Uganda	-	-	-	-	1	-	1
USA	1	-	1	-	1	1	4
Vietnam	2	-	6	-	1	-	9
Total	18	10	32	16	14	13	103

Collaborative Mungbean Breeding Research

Shuttle breeding between AVRDC and NIAB

Although mungbean lines developed from AVRDC did well in Southeast and East Asian countries, they did not find big niches in South Asia where over half of the world mungbean is produced. All AVRDC lines developed so far are susceptible to MYMV, the most devastating disease in the region. MYMV is widespread within South Asia, thus AVRDC breeders need to identify resistant mungbean parents in the endemic area. The parents are crossed with AVRDC advanced lines to combine the resistance genes and superior agronomic characters. The segregated population is then included in the shuttle breeding program, wherein resistance screening and selection for certain agronomic traits are normally performed at NIAB in the summer. Selected materials are sent back to AVRDC-ARC for generation advancement and further evaluation through the winter, and then sent back again to NIAB for another screening generation. A number of elite lines are included in the IMN for international evaluation, while others are made available by AVRDC-ARC upon request.

Collaborative program between the Philippines and AVRDC

In recent years, fewer mungbean lines from the Philippines were developed and production also decreased. However, the germplasm developed by Philippine mungbean breeders continue to be used by AVRDC. New AVRDC lines derived mainly from the Philippines and Indian germplasm have been released in many Asian countries, including the source countries, the Philippines and India (Yang 1996). For example, the latest AVRDC mungbean line released in the Philippines (in 1988) was "BPI Mg 9". The cultivar was the elite line VC 2768D, known as "Taiwan Green" in Taiwan, which yielded up to 2.6 t/ha in farmers' fields in northern Philippines. Yet it matured within 55 days (10-20 days earlier than most conventional cultivars) and set most pods above the canopy so that only one picking was needed to harvest them (AVRDC 1993). Without superior germplasm stored at AVRDC it might not have been possible to develop such a good mungbean cultivar for the Philippines.

Line VC 2768 D was developed from the cross VC 1482A x VC 1628A and tested throughout Asia, including the Philippine Bureau of Plant Industry (BPI) and the Institute of Plant Breeding (IPB), University of the Philippines Los Baños. Results from the tests encouraged several Philippine agencies, both government and private, to launch the Mungbean Development Action Project (MDAP) which pilot-tested VC 2768 D in farmers' fields. The success of the test resulted in the establishment of a Mungbean Technology Commercialization Program (MTCP). MTCP linked with AVRDC in a project which consequently published the Philippines Recommends for Mungbean and disseminated various promotional materials.

A field day was also organized in Urdaneta, Pangasinan in northern Philippines by the Philippine Council for Agriculture, Forestry and Natural Resources Research and Development (PCARRD) and AVRDC, the Philippine Department of Agriculture - Region I, and office of the Mayor, Urdaneta. It was funded by the Japan Shipbuilding Industry Foundation (JSIF) through a project implemented by AVRDC (AVRDC 1993).

Collaborative program between Thailand and AVRDC-ARC

AVRDC-ARC has three major collaborators in Thailand. In addition to Kasetsart University (KU) where AVRDC-ARC is located, the Department of Agriculture (DOA) and the Department of Agricultural Extension (DOAE)— both attached to the Ministry of Agriculture and Cooperatives—benefit from the following collaborative activities:

Utilization of mungbean germplasm. Thailand has so far released six mungbean and two blackgram cultivars. Prior to the establishment of AVRDC's outreach program in Thailand in 1981 DOA had released "U-Thong 1" from a collection of mungbean germplasm introduced by the University of Missouri in the US. The cultivar is most likely one of the CES series developed from the University of the Philippines Los Baños. U-Thong 1 helped to stabilize the Kingdom's position as the biggest mungbean exporter in the world for several consecutive years. Five cultivars were introduced AVRDC elite lines. "Kamphaeng Saen 1" (VC 1973A) and "Kamphaeng Saen 2" (VC 2778A) were released in 1986 whereas "Chai Nat 60" (VC 1178), "Prince of Songkla University 1" (VC 2768A), and "Chai Nat 36" (VC 1628A) were officially released in 1987, 1988, and 1991, respectively. The two blackgram cultivars "U-Thong 2" and "Phitsanulok 2" were both of Indian origin. They were officially recommended in Thailand in 1978 and 1990, respectively. After a cultivar was released, the DOAE took the responsibility of increasing the seed and making them available to farmers. This was done by establishing seed centers throughout the kingdom's mungbean growing areas.

Research collaboration with Thai programs. Thailand's mungbean programs capitalized on AVRDC germplasm to suit its own breeding objectives. Key mungbean research staff at Chai Nat Field Crops Research Center (CNFCRC) exchanged information with AVRDC breeders, sought collaboration, and signed a memorandum of understanding. For example, the most recent memorandum with CNFCRC summarizes the planned collaborative activities (Malik 1996, pers. comm.):

- Constitution of a regional yield trial comprising 10-12 elite lines plus check cultivars. The entries would be nominated by AVRDC-ARC and the country's program—mainly from CNFCRC and KU. Eight experiment stations were listed as test sites in each season. CNFCRC agreed to increase the seed and prepare the trials. Methods of data collection were standardized among researchers at the sites. Superior lines were chosen for further tests in the experiment station as well as in farmers' fields.

- Evaluation of materials for insect pest resistance, emphasizing resistance to bruchids and pod borers. Testing for resistant lines against bruchids will be done in the laboratory as well as in the field in both CNFCRC and AVRDC-ARC at Kamphaeng Saen. Ten resistant lines from KU and AVRDC-ARC plus two lines from CNFCRC were included in the trial. The pod borer resistance screening, however, required more information from mass screenings at AVRDC headquarters.

Agreements on the collaboration between AVRDC-ARC and KU were also spelled out by mungbean breeders in both institutes. They agreed to jointly explore the following areas:

- Resistance to major diseases such as CLS, PM, MYMV.
- Resistance to major insect pests such as bruchids and pod borers.
- Genotypes with high yield potential, improved plant type, uniform maturity, and high nutritional seed content
- Tolerance to alkaline soils that are deficient in micronutrients

A collaborative research on "Breeding for Bruchid and Mungbean Yellow Mosaic Virus Resistance in Mungbean Using Molecular Markers" has been jointly conducted by CSIRO Division of Tropical Crops and Pastures and AVRDC-ARC during the past 2 years. ARC is responsible for providing bruchid-resistant materials, specific mapping populations, and Asian bruchids (*Callosobruchus maculatus* and *C. chinensis*). ARC also coordinates sending of segregating populations for MYMV evaluation to NIAB, Pakistan. Parts of ARC's contribution came from the work of two graduate students at KU. One of them monitored resistance of several mungbean lines across bruchid species and growing seasons. The other tried to establish an RFLP mapping technique for the resistance gene, following the protocols advocated by Young et al. (1992).

Manpower development. National programs in Thailand have sent several mungbean scientists for training at AVRDC headquarters. Upon their return to Thailand, most scientists continued to contribute to the kingdom's mungbean research. AVRDC-ARC has grown to become a regional training site but it still lacks manpower to accept medium-term research fellows. The KU mungbean project foresees this activity and is sending a staff member for training on mungbean genetic engineering with support from AVRDC.

AVRDC-ARC has accepted four 1-year research fellows so far. One came from Vietnam to work on soybean while the others were from China to work on garlic, mungbean, and soybean. The fellows worked in the ARC field and KU laboratory, which allowed them to interact with university staff and graduate students in related fields. Some students from the Asian Institute of Technology (AIT) also conducted parts of their theses with KU and ARC to take advantage of the combined facilities.

Extension of AVRDC mungbeans. Researchers at AVRDC-ARC and KU jointly prepared two brochures introducing AVRDC mungbean varieties released in Thailand and their cultural practices. These are extensively used by extension staff and farmers.

Breeder seed and pure cultures of improved cultivars have been maintained by AVRDC-ARC. CNFCRC makes them available upon request from DOAE's seed centers. Seed centers contract certain farmers to produce foundation and certified seeds for extension and sell them to mungbean growers. Seeds of some cultivars are also available across the borders of Cambodia, Laos, and Myanmar. The Thai government has no special restriction on selling mungbean seed overseas.

Conclusion

Collaboration between AVRDC and programs of Southeast Asian countries is strong and successful. Tickoo (1992) stated that mungbean yield levels in these countries are double that of India, partly due to strong AVRDC support and the absence of MYMV. Since South Asia, especially India, is a major producer of mungbean, strengthening cooperation between AVRDC and the national programs in the South Asian region would produce a strong impact on the production of this crop. Tickoo proposed major areas of cooperation to include germplasm exchange, IMN evaluation, and MYMV research. Minor areas include low input production systems, seed quality, rice-based cropping systems, biotechnological research, generalizing research findings across species, overcoming socioeconomic constraints, diversification of mungbean products, training of technical and research personnel, and exchange of information.

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INHERITANCE OF MUNGBEAN TOLERANCE TO MICROESSENTIAL ELEMENT DEFICIENCY IN TAKHLI SOIL SERIES

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Calcareous soils in Thailand belong to Takhli soil series which cover some 300,000 ha in the arable upland central plain. Most part of the area is considered the country's mungbean belt. However, the high yielding cultivars "Kamphaeng Saen 1" (KPS1) and "Kamphaeng Saen 2" (KPS2) yield poorly when grown in this soil due to their inability to utilize low cation trace elements, especially Fe. An immediate recommendation against the problem is to treat the mungbean with Fe-chelate as foliar or soil application. The more desirable and long term measure is to develop tolerant mungbean cultivars that can be grown in such area. Lawn *et al.* (1988), and Oonkasem and Thavarasook (1990) reported genetic variation of mungb tolerance to calcareous soil. Ohwaki *et al.* (1996) capitalized information from the latter report and picked up 6 mungbean genotypes to test for detail reaction of the tolerance in solution as well as in the field. Based on degrees of chlorosis they classified the genotypes into 4 groups. KPS2 is considered highly susceptible, KPS1 as susceptible, Pag-asa1 and VC1163B as moderately tolerant, and "U-Thong1" (UT1) and "Chai Nat 36" as highly tolerant to iron-deficient Takhli soil. They reported that UT1 had an ability to lower the pH of the nutrient solution in response to cation deficiency stress which may contribute to solubilization of iron from calcareous soils. To transfer the tolerant character to susceptible lines, mode of inheritance of the trait should be investigated so that an effective breeding plan can be set up accordingly.

Materials and Melhods

Fifty-one mungbean accessions from major growing areas in Asian countries were screened for tolerance to Takhli soil as compared to growth in normal (Kamphaeng Saen) soil. Five seeds from each accession were sown in each of 2 pots filled with Takhli soil and 1 pot filled with normal soil. Chlorosis was periodically observed until flowering. To determine the responsible microessential element(s) in Takhli soil, 0.5% solution of Fe SO_4, Zn SO_4, Cu SO_4 or Mn SO_4 was brushed on to one half of a yellowish leaflet to observe its greenish recovery. Only the leaflet half brushed with Fe SO_4 became green 3-5 days later. Soil analysis also confirmed that Fe SO_4 in Takhli soil was 2.40 mg Fe/kg soil, which is lower than the critical value of 2.5-4.5 mg Fe/kg soil. By the end of the screening 2 most tolerant lines were identified as NM 6-97 and NM 10-12 from Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan, while the 2 most susceptible lines were KPS1 and KPS2. Four generations, viz. F1, F2, BC1, and BC2 were then developed from individual cross of KPS1 x NM 6-97, KPS1 x NM 10-12, KPS2 x NM 6-97, and KPS2 x NM 10-12. Whereas BC1 and BC2 were backcross progenies of female and male parents, respectively. The four generations plus 2 parents from each cross were sown in a Takhli soil field of Nakhon Sawan Field Crops Research Center, Department of Agriculture. Number of plants showing chlorosis were counted at 10, 20, 30, and 40 days after planting. A number of agronomic traits were also recorded. Observed number of chlorotic plants in each generation was tested against expected number, using X^2 goodness-of-fit test as advocated by Mather (1951).

Results and Discussion

Number of chlorotic plants observed were almost constant during 10 to 40 days after planting (DAP). Thus only data from 10 DAP are employed in the goodness-of-fit test. Considering

all 6 generations, number of chlorotic vs normal plants fitted well with the theoretical inhibition action of 2 loci as exemplified by data obtained from the cross KPS2 x NM 10-12 shown in Table 1. In this type of action gene in one locus inhibits expression of gene in the other. Given that a dominant allele "A" expresses no chlorotic symptom when "I" allele exists, then the parental genotypes for tolerant and chlorotic response are aa II and AA ii, respectively. The F1's (genotype Aa Ii) are all normal. BC1 (Aa Ii x AA ii) gives a 1:1 ratio of A- I- (normal) and A- ii (chlorotic), while BC2 (Aa Ii x aa II) gives all normal plants A- I-. The F2 segregated at the ratio of 13 normal ($^{9}/_{16}$ A- I-, $^{3}/_{16}$ aa I-, $^{1}/_{16}$ aa ii) vs 3 chlorotic ($^{3}/_{16}$ A- ii). Reaction to Fe-deficiency soil clearly reflected agronomic traits of these mungbeans. Data from the same cross revealed that KPS2 grew slowly, displayed poor yield components, and finally accumulated least dry matter (Table 2). The other generations expressed the traits following what observed in NM 10-12, confirming that there were more normal plants produced in each cross than the chlorolic ones as indicated in Table 1. Segregation ratios and reaction to Takhli soil in the other 3 crosses were similar to those of KPS2 x NM 10-12 (data not shown here). Thus it can be concluded that chlorosis developed from iron deficiency in mungbean is governed by 2 loci of genes with inhibition action. Chlorotic plants are very poor yielding and a new cultivar should be incorporated with the tolerant genes for normal growth and yield.

Table 1 Segregation of 10 days old chlorotic and normal plants in 6 mungbean generations observed from the cross KPS2 x NM 10-12 grown in Takhli soil.

Generation	Observed plants		Expected plants		Expected	X^2	Probability
	chlorotic	normal	chlorotic	normal	ratio		
P_1	60	0	60	0	1:0	0.00	≥ 0.99
P_2	0	62	0	62	0:1	0.00	≥ 0.99
F_1	0	41	0	41	0:1	0.00	≥ 0:99
F_2	24	143	31.3	135.7	3:13	2.09	0.10-0.25
BC_1	77	61	69	69	1:1	1.86	0.10-0.25
BC_2	0	143	0	143	0:1	0.00	≥ 0.99

Table 2 Generation means of yield components and some other agronomic characters observed from the cross KPS_2 x NM 10-12 grown in Takhli soil.

Generation	Plant height (cm)		No. pods	No. seeds	100 seed	Dry weight
	30 DAP	60 DAP	per plant	per pod	weight (gm)	per plant (gm)
P_1	6.1	11.7	2.4	3.9	4.9	2.5
P_2	8.3	19.0	8.5	7.3	5.6	7.2
F_1	8.7	23.7	8.9	8.2	6.7	8.0
F_2	8.2	21.4	8.9	8.1	6.0	8.6
BC_1	7.1	19.6	6.9	6.9	5.9	6.7
BC_2	6.5	19.2	7.1	6.8	5.7	6.5

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In Vitro Selection for Tolerance to Iron Deficiency in Soybean

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In Thailand, it is estimated that about 336, 500 ha. of land is covered with calcareous soils. In calcareous soils, the availability of Fe^{3+} is controlled largely by the soil pH, therefore, it is markedly decreased through precipitation of Fe-compounds when the pH value of soil is above 7. Iron deficiency is thus a major factor limiting the growth and production of crop plants on calcareous soils. The purpose of this study is to observe the effect of low dose of gamma irradiation on somatic embryogenesis and to develop an *in vitro* selection technique for soybean tolerance to iron deficiency by subjecting somatic embryos to high pH medium.

Materials and Methods

Soybean cultivar BSR 101 was used in the following experiments:

1. Gamma irradiation : Young pods of BSR 101 were treated with gamma rays at 1-11 Gy. Immature cotyledons were cultured in the induction medium for 4 weeks (1).
2. *In Vitro* selection : Somatic embryos were cultured in the liquid medium at pH 5.8 (2) for 5 weeks before subjecting to high pH media for iron deficiency tolerance screening. A gradual selection was performed by transferring somatic embryos from low pH (6.0) to higher pH (6.5, 7.0, 7.5 and 8.0). Duration of selection in each step was one week interval. Somatic embryos in each selection were partly taken to test their regeneration ability in the regeneration medium.
3. Embryo cycling : Somatic embryos cultured in the liquid medium (pH 5.8) were transferred to three different cycling media (MSO, MSM and IB) (3). The experiment ran for three cyclings and each cycling took five weeks interval.

Results and Discussion

1. Gamma irradiation : After 4 weeks in the induction medium, somatic embryos developed on cotyledons, were observed and percentage of somatic embryogenesis at each radiation dose was calculated (Fig. 1). Somatic embryogenesis was markedly enhanced by radiation doses at 1-5 Gy (Table 1). The result obtained was similar to previous report (4)
2. *In Vitro* selection : After passing soybean somatic embryos to each selection medium at different pH, somatic embryos had changed in color from light green (Fig 2) to yellow and brown (Fig 3). However, somatic embryos at each selection step were able to regenerate but at a very low percentage (Table 2). Regenerated plants were transferred to soil and grown to maturity for further investigation for iron deficiency in their progenies (Fig. 4)
3. Embryo cycling : Among three cycling media, MSO was found to be the best in promoting the growth of somatic embryos by increasing somatic embryo fresh weight at each cycling. Plant regeneration was observed in all three cyclings. However, percentage of plant regeneration was decreased while the number of cyclings was increased. (Table 3)

Table 1. Percentage of somatic embryogenesis in BSR 101 irradiated with gamma-rays.

Radiation dose (Gy)	% Somatic embryogenesis
0	20.74
1	27.31
3	40.34
5	44.47
7	19.26
9	5.97
11	8.35

$$\% \text{ Somatic embryogenesis} = \frac{\text{no. of cotyledons with somatic embryos}}{\text{Total cotyledons cultured}} \times 100$$

Table 2. Percentage of plant regeneration from somatic embryos of BSR 101 selected at different pH.

Radiation Dose (Gy)	pH	% plant regeneration
0	6.0	6.0
5	6.0	2.0
3	7.0	1.0
7	7.5	0.5
5	8.0	1.0

Table 3. Percentage of plant regeneration in MSO after passing through three different cyclings.

Radiation	% Plant regeneration		
	1 st Cycling	2 nd Cycling	3 rd Cycling
0	11.7	5.5	2.6
1	9.6	4.5	2.5
3	17.3	8.1	2.0
5	1.7	5.5	1.0

Fig 1. Somatic embryos from immature cotyledons

Fig. 3. Somatic embryos in selective culture (pH 8)

Fig 2. Somatic embryos in suspension culture (pH 5.8)

Fig 4. Regenerated plant after passing through selective culture

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