8th Gatlinburg

SYMPOSIUM

July 26 - 29, 1998
Knoxville, TN

7th Biennial Conference

MOLECULAR & CELLULAR BIOLOGY OF THE SOYBEAN

ABSTRACT BOOK

The University of Tennessee, Knoxville
Center for Legume Research
Knoxville, Tennessee

Table of Contents

Welcome

July 26, 1998

Welcome to the 7th Meeting on the 'Molecular and Cellular Biology of the Soybean', held in conjunction with the 8th Gatlinburg Symposium and 'The Forum on Transgenic Soybean'.

Faculty members of the Center for Legume Research and UT Conferences staff welcome you to Knoxville and The University of Tennessee. We have planned a highly informative program, amidst a casual atmosphere of discussions, chances for net-working and data evaluation. We have tried to make the social program congenial with the concept of open exchange and cooperation, and hope that the next 3 days will enrich our opportunities to know more about soybean, and plants in general.

It was 10 years ago that I attended the first "soybean" meeting organized in Iowa by Randy Shoemaker. I recall returning to Knoxville and speaking to an editor of a major journal about the great advances in soybean. 1988 seemed to be a water-shed year as the first RFLP mapping was completed, transposable elements were found, symbiotic mutants were being characterized, storage protein genes were cloned, and soybean transformation was reported by both *Agrobacterium* and biolistic transformation. **Soybean had come of age!**

Here we are 10 years on, and the young field of 1988 has grown into a powerful movement. I am nearly speechless at the advance in so many areas. Preparing the abstract book, I am astonished at the insights of the genetic and molecular make-up of this important crop. I am impressed by the extensive collaborations between laboratories and states. State and national producer organizations like the United Soybean Board and the North Central Soybean Research Consortium have fostered research. Federal agencies recognize the scientific value as well as the agronomic worth of this important plant.

I am grateful as well as impressed by the level of support for this meeting from a wide range of sponsors. These are listed separately, and they are thanked deeply for making it possible to help some of the younger scientists to attend this meeting.

The following pages of the abstract book provide you with a record of the scientific content of this meeting. Some abstracts may be misplaced in your opinion; this was entirely my responsibility as I wanted to stimulate cross-fertilization, and integration. For example, all 'nematode' papers are not kept in the same section. Additionally, some of you came late and we really did not want to re-index the book. Apologies to those who feel in the wrong spot.

We hope that the meeting will be of benefit to y'all, and that you take good memories with you from Knoxville.

Again, welcome to Knoxville and The University of Tennessee. We will do our best to ensure that you have a wonderful stay.

Peter M. Gresshoff
Gary Stacey
Fred Allen
Center for Legume Research

Sponsors

The Organizing Committee wishes to thank the following sponsors for their contributions to this successful meeting.

AsGrow Seed Company

DuPont

Fisher Scientific

Monsanto

Novartis/Ciba-Geigy Corporation

Owensboro Grain

PE AgGen

Pioneer

Tennessee Soybean

United Soybean Board

US Army Research

US Tobacco

UT Agriculture Experiment Station

UT Center for Legume Research

UT Office of Research

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Local Organizing Committee

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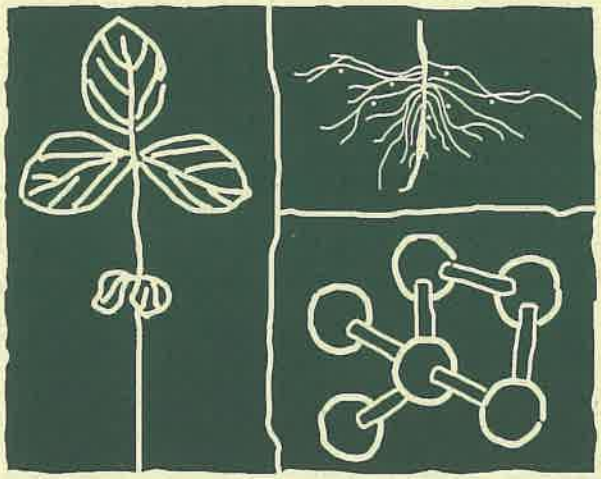
Program



Molecular and Cellular Biology of the Soybean
8th Gatlinburg Symposium

All events other than the dinner on Monday are at the UT Conference Center on Henley Street, Downtown Knoxville, between the Holiday Inn and the Hilton Hotels. The Center is on the 4th Floor.

Sunday, July 26th

1:00 p.m.	Poster assembly
2:00 p.m. - 6:00 p.m.	Registration
5:00 p.m. - 6:30 p.m.	Conference Mixer and informal get-together

Program: Monday, July 27th

7:30 a.m. Registration

8:30a.m. Introductory remarks: Peter M. Gresshoff

Session 1: Advances in soybean genomics: *Peter Gresshoff, Chair*

8:45 a.m. The development of the soybean physical map: *Ted Hymowitz*

9:30 a.m. Soybean genomics, ESTs and map: *Randy Shoemaker*

10:00 a.m. Poster viewing and refreshment break

10:30 a.m. From genes to chromosomes. *Halina Knap*

10:55 a.m. QTL mapping using recombinant inbred lines. *Levi Mansur*

11:25 a.m. Genomic analysis in the region of *rhg1*, a major soybean cyst nematode resistance gene. *Nevin Young*

11:55 a.m. Lunch, UT Conference Center room 404. (provided by conference)
Poster viewing

Session 2: Marker-technology and gene discovery: *Randy Shoemaker, Chair*

1:35 p.m. Application of DNA markers to soybean breeding. *Roger Boerma*

2:05 p.m. Assessment of single nucleotide polymorphisms in soybean. *Perry Cregan*

2:35 p.m. Soybean-*Phytophthora sojae*, a model plant-fungal interaction. *Madan Bhattacharyya*

3:00 p.m. Poster viewing and refreshment break

3:30 p.m. Molecular analysis of the *nts-1* (*rj7*) supernodulation gene in soybean. *Artem Men*

3:55 p.m. Isolation of ethylene-insensitive mutant of soybean that display altered responses to soybean pathogens. *Andrew Bent*

4:20 p.m. Genetic analysis of SCN resistance in soybean introductions: an overview. *Prakash Arelli*

4:40 p.m. Molecular characterization of root-knot nematode resistance in soybean. *Jiansheng Qiu*

5:00 p.m. Marker-assisted selection of Southern Root Knot nematode resistance. *Lakshmi Jakkula*

5:20 p.m. End of formal program

6:30 p.m. Cash bar and dining area open at Hyatt. Remember to bring your two complimentary drink tickets, which you received at registration.

7:00 p.m. Dinner is ready!

Conference Dinner: Hyatt Regency Hotel. Casual dress. Poolside dinner, weather permitting. The dinner will be moved into the Hyatt Regency ballroom if inclement weather occurs. Vans will leave from the east side of the UT Conference Center.

Program: Tuesday, July 28th

Session 3: Soybean biochemistry and molecular physiology:
Perry Cregan, Chair

8:30 a.m. Respiratory gene expression in soybean. *David Day*

9:05 a.m. Soybean-*Bradyrhizobium* nodulation and nitrogen fixation partnership. *Hauke Henneke*

9:40 a.m. The glutamine synthetase gene family in soybean. *Champa Sengupta-Gopalan*

10:05 a.m. Poster viewing and refreshment break

10:35 a.m. Down-regulation of soybean vegetative storage proteins via RNA antisense technology in transgenic soybeans. *Zhanyuan Zhang*

10:55 a.m. Strategies for modifying soybean phytic acid content in soybean seeds. *Elizabeth Grabau*

11:15 a.m. Analysis of the symbiosome membrane of soybeans. *Dietrich Werner*

11:45 a.m. Nodulin 26 confers aquaporin and uncharged solute transport. *Dan Roberts*

12:10 p.m. SAGE analysis of immature cotyledon gene expression. *Paul Keim*

12:30 p.m. Lunch, UT Conference Center room 404. (provided by conference) Poster viewing

Session 4: Transgenic technology: *Madan Bhattacharyya, Chair*

1:50 p.m. Advances in soybean transformation. *Jack Widholm*

2:20 p.m. Isolation and transgenic analysis of a soybean pod-specific promoter. *Martina Stromvik*

2:40 p.m. Identification of critical components for soybean transformation. *Glenn Collins*

3:10 p.m. Poster viewing and refreshment break

4:00 p.m. Current progress towards an efficient regeneration and transformation protocol for soybean. *Wayne Parrott*

4:30 p.m. **Forum** on the uses and export of transgenic soybean. Sponsored by the US Office of Army Research.

5:30 p.m. End of formal program

Dinner on your own. See restaurant suggestions in the Abstract Book.

Program: Wednesday, July 29th

Session 5: Soybean cultivar development: *Roger Boerma, Chair*

8:30 a.m. Development of White Mould Resistant soybean. *Daina Simmonds*

8:55 a.m. Genetic modification of soybean oil. *Vincent Pantalone*

9:20 a.m. Genetic analysis of *Heterodora glycines* parasitism. *Charlie Opperman*

9:45 a.m. New Sources of resistance for soybean cyst nematode. *Brian Diers*

10:05 a.m. Poster viewing and refreshment break

Session 6: Model legumes and plant-microbe interactions: *Gary Stacey, Chair*

10:35 a.m. Comparative legume biology in *Medicago truncatula* and possible connections to *Arabidopsis thaliana*. *Doug Cook*

11:05 a.m. The model legume *Lotus japonicus* as a way into soybean. *Peter Gresshoff*

11:25 a.m. Lotus japonicus, insertional mutagenesis and mapping. *Jens Stougaard*

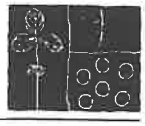
11:55 a.m. Generation and characterization of expressed sequence tags (ESTs) of the model legume *Lotus japonicus*. *Krzysztof Szczyglowski*

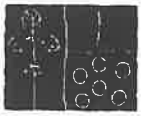
12:20 p.m. A high affinity binding site for N-Acetyl-Chitooligosaccharides. *Brad Day*

12:45 p.m. Soybean nodulation control-genetics and physiology. *Jim Harper*

1:05 p.m. Cultivar-specific nodulation of soybean. *Steven Pueppke*

1:30 p.m. End of conference.





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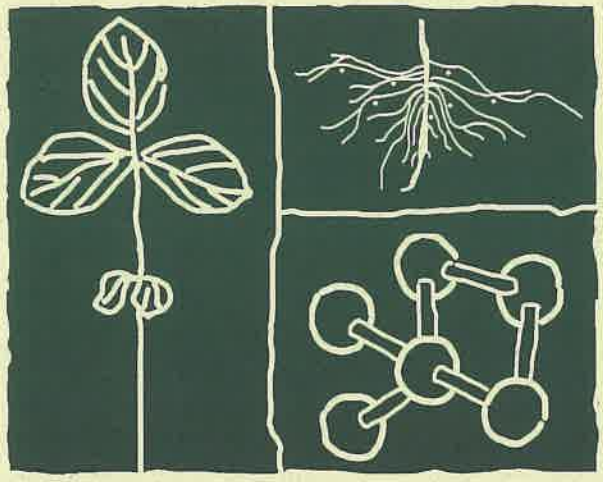


Meeting Information

WELCOME TO KNOXVILLE!

All conference sessions will take place on the 4th floor of the UT Conference Center; all contributed papers will be presented and posters displayed. The barbecue on Monday evening will be located at the Hyatt Regency hotel with transportation provided to and from the Conference Center and the Hyatt.

UT Conference Center
600 Henley Street, 4th floor
Front Desk Phone: 423.974.0250
Office FAX: 423.974.0290

REGISTRATION AND INFORMATION DESK

The registration desk, in the atrium of the Conference Center, will be open during the symposium hours.

MESSAGE BOARD

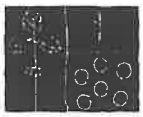
This board will be located near the registration desk. Supplies will be provided for you to leave messages. All incoming messages to our office will be posted on this board. Fax capabilities are available, please check at the registration desk.

PARTICIPANT LIST

The list at the time of printing is included in this book. An addendum will be available on Wednesday morning.

SPEAKERS

Persons making presentations should bring their slides to the projection preview room, room 417, 30 minutes before the start of the session. Speakers are encouraged to introduce themselves to the person presiding over the session in which they will speak. This will allow the presiding person to know who will be presenting the paper and to be certain of correct pronunciation and affiliations. Speakers are requested to pick up their slides within 30 minutes after the session is adjourned.



SPEAKER PREP ROOM

Room 417 is set aside for speakers' preparation. A 35 mm slide projector, overhead projector and screens will be available in the room. Carousel trays and laser pointers will also be available and can be checked out by speakers at the registration desk.

POSTERS

Posters will be displayed in the hallways throughout the Conference Center. Posters may be set up, Sunday 3pm-7pm and Monday – Tuesday 7am – 6pm. The posters should be removed by 1pm on Wednesday. Tacks will be provided to assist in displaying your posters.

SESSIONS

Please see the program/agenda. Monday 8a.m.-5p.m., Tuesday 8a.m.-5p.m. and Wednesday 8a.m.-12noon

PHONES

Pay phones are located on the 4th floor of the Center. Local calls can be made from the house phones.

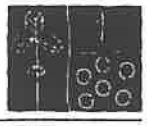
SMOKING is not allowed in the UT Conference Center Building.

RETURN AIRPORT SHUTTLE

Shuttles will be available on Wednesday. You must stop by the registration desk to sign up for the return shuttle Sunday - Tuesday. The shuttle will pick you up 2 hours before your flight departure time. Guests of the Hilton receive complimentary shuttle service, please check with their front desk. Taxi numbers can be found in the HELPFUL PHONE NUMBERS section.

PARKING

All permits are available at the Registration Desk.
Hilton guests: you can park in the Hilton Garage for $3 per day.
Holiday Inn guests: you can park in the Holiday Inn Garage at no charge.
On campus guests: If you are parking on campus, you must have a parking permit in the dashboard of your car. This permit allows you to park in the garage behind Clement Hall.
Commuters: You can park in the UT Conference Center Garage. Stop by the Registration Desk or the Front Desk to receive a complimentary sticker for one day of parking. This does not allow in and out privileges. You must pick up one per day.



RESTAURANTS

A variety of eateries are located within walking distance to the Conference Center. Please see the KNOXVILLE RESTAURANTS section for a listing and description of area restaurants.

REFRESHMENTS and LUNCH

Beverage breaks are provided during the meeting. Noon luncheons will be in the dining room (room 404) on Monday and Tuesday.

BANKING

Campus and downtown banks include: First American, First Tennessee, UT Credit Union, Nation's Bank, Home Federal, SunTrust Bank, Union Planters and BankFirst. ATM machines can be found at most of the above listed banks and at the campus University Center.

SPECIAL EVENTS

Sunday reception at the UT Conference Center.
Monday barbecue at the HYATT hotel.
Wednesday after the conference, opportunities for campus tours and excursions.

EVALUATIONS

Please assist us in future planning by completing the evaluation forms included in the conference materials. Completed evaluations can be dropped into the evaluation box located at the registration desk when the meeting has concluded.

CONFERENCE HOUSING

Knoxville Hilton, 501 W. Church Ave., Knoxville, TN 37902, phone: 523-2300

Holiday Inn World's Fair, 525 Henley St., Knoxville, TN 37902, phone: 522-2800

Campus housing: Clement Hall, 1629 Cumberland Ave., Knoxville, TN 37996, phone: 974-5201

TRANSPORTATION

KAT Bus Service 637-3000, Knoxville Area Transit (KAT) provides service to Kingston Pike and local malls.

Downtown and Campus Trolley Service, 637-3000, offers free service to downtown, the Old City and campus areas.

COMPUTERS

The lobby computer has internet access and is available to you. Please check with the registration desk if you need use of a printer.

Helpful Phone Numbers

Medical Assistance: Call the following number for non-emergency illness.
University of Tennessee physicians referral 544-9640. Hours are 7:00am-7:00pm.

Area Hospitals:

UT Medical Center	544-9000	(4 miles from UT Conference Center)
Ft. Sanders Regional Medical Center	541-1111	(1 mile from UT Conference Center)
Baptist Hospital	632-5011	(1 mile from UT Conference Center)
St. Mary's Medical Center	971-6011	(2 miles from UT Conference Center)
Children's Hospital	541-8000	(1 mile from UT Conference Center)
Park West Hospital	693-5151	(8 miles from UT Conference Center)

Emergency Telephone Numbers:

Fire, ambulance, etc.	911
Knoxville Police Department	521-1200
Knox County Sheriff	521-2432
Rural Metro Ambulance Service	675-0775
Poison Control Center	1-800-288-9999
Knoxville Rescue Squad	546-4821
University of Tennessee Police Dept.	974-3111
Non-Emergency- UT Police	974-3114

Cabs/Taxis/Airport Shuttle:

AAA Airport Taxi	531-1930
Benchmark Taxi	567-0035
Big Orange Express	523-3400
Yellow Cab	523-5151

Area Laundry & Dry Cleaning Service:

Big Orange Cleaners, pick-up and delivery	688-0270
King Cleaners (campus)	522-0211
Buds and Suds (campus)	524-5702
Prestige Cleaners	584-7701

Area Restaurants

Consult the Knoxville Visitors Mini-Guide for more choices.

☑ Campus and Downtown locations

American

☑ *BW-3*: 109 South Central Avenue; 522-4293
M-Su 11am-3am; Eight styles of wings and fifteen beers on tap; billiards

☑ *The Tomato Head*: 12 Market Square; 637-4067
M-F 11am-2pm; F-Sa 5:30-11pm; Gourmet pizzas, homemade bread, sandwiches, salads.

☑ *Hawkeye's*: 1717 White Avenue; 524-5326
Salads, steaks, alfredos, burgers, pitas, and sandwiches.

☑ *O'Charley's Sports Grille*: 1915 West Cumberland Avenue; 525-7665
M-Sa 11am-3am; Su 11am-10pm; Entertainment; steaks, chicken, sandwiches, and salads.

☑ *Spicy's*: 1931 West Cumberland Avenue; 523-4723
M-Su; Best wings in town; largest beer selection

☑ *Copper Cellar*: 1807 Cumberland Avenue; 673-3411
M-Th 11am-10:30pm; F-Sa 11am-11:30pm; Prime Rib, lobster, seafood

☑ *Charlie Pepper's*: 716 Cumberland Avenue @ 20th Street; 524-8669
M-Su 11am-12am; TexMex

☑ *Calhoun's on the River*: 400 Neyland Drive; 673-3355
M-Th 11am-10:30pm; F-Sa 11am-11pm; Su 11am-10pm; Best ribs in America!

☑ *Lucille's*: 106 South Central Avenue; 546-3742
T-Su 5pm-late; Live outside jazz and blues

☑ *Windows Over The Park*: 525 Henley Street; 522-2800
Holiday Inn Select-World's Fair Park

Darryl's Restaurant: 6604 Kingston Pike; 584-1879
M-Su 11am-11pm; Sandwiches, ribs, salads

☑ *Old College Inn (OCI)*: 2204 Cumberland Avenue; 523-4597
M-Su 11am-3am; American food and drinks.

Mexican

Garcia's Mexican Restaurant: 1516 Downtown West Blvd.; 690-9910
A fresh tradition in Mexican food.

El Charro Mexican Restaurant, Inc.: 10400 Kingston Pike; 693-9660

Cozymel's Mexican Grill: 7727 Kingston Pike; 694-9811
Coastal specialties with all your traditional favorites.

Italian

Italian Market and Grill: 9648 Kingston Pike, Franklin Square; 690-2600
Casual, contemporary Italian restaurant.

Naples Italian Restaurant: 5500 Kingston Pike; 584-5033
Homemade pasta, award winning wine list and service, nightly seafood and pasta specials.

Romano's Macaroni Grill: 7723 Kingston Pike; 691-0809

Chinese/Japanese

Mandarin House: 314 Merchants Drive; 689-4800
Voted best buffet in Knoxville.

Szechwan Garden: 4211 Chapman Hwy; 579-0889

Kyoto: 8207 Kingston Pike; 691-3121
Steak, seafood, and sushi bar

Stir-Fry Café: 7420 Kingston Pike; 588-2064
Thai dishes and oriental food

Indian

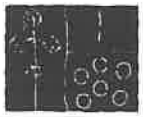
☑ *Kashmir Indian Restaurant*: 711 S. 17th Street; 524-1982
Sunday buffet noon-3p.m.

Steaks

☑ *Butcher Shop Steakhouse*: 806 World's Fair Park Drive; 637-0204
Dinner only, 5 p.m. Grill the perfect steak, or let the chef do it for you! Home of the largest steaks in Knoxville.

The Chop House: 9700 Kingston Pike, Franklin Square Center; 531-2467
Specializing is serving chops, steaks, and other quality meats.

Ye Olde Steakhouse: 6838 Chapman Hwy; 577-9328
Specializing in filets and charcoal broiled shrimp.



Seafood

☑ *Chesapeake's*: 500 Henley Street; 673-3433
M-F 11am-2:30pm; F-Sa 5-9:30pm; Featuring fresh seafood and Eastern shore specialties.

Tjaarda's: 118 South Central Avenue; 637-8702
M-Sa 5-11pm; seafood and fine vegetarian fare

Specialty

☑ *Great Southern Brewing Company*: 424 S. Gay Street; 523-0750
M-Su 11am-2:30am; Seafood, steaks with authentic Micro Brewery

Blackhorse Pub and Brewery: 4429 Kingston Pike, Western Plaza; 450-9312
Gormet pizza, burgers, steaks, and 6 freshly brewed beers. Billiards and games also.

☑ *Regas-Restaurant and Gathering Place*: 318 N. Gay Street; 637-9805
Dinner M-Sa 5-10pm; Their Prime Rib is considered to be "as good as it gets."

☑ *Melting Pot*: 111 North Central Avenue; 525-5858
Dinner only; a fondue restaurant

Baker Peters Jazz Club: 9000 Kingston Pike; 690-8110
Great steaks, cigars, and martinis. "All that jazz!" Reservations recommended.

Copelands of New Orleans: 6400 Kingston Pike, on Bearden Hill; 584-5255
Cajun Creole restaurant

Area Attractions

Historic Homes

Blount Mansion 423/525-2375
200 West Hills Avenue, Knoxville, TN 37901
The circa 1792 home of Territorial Governor William Blount, the first and only governor of the Territory Southwest of the Ohio River, is a National Historic Landmark.

James White Fort 423/525-6514
205 E. Hill Avenue, Knoxville, TN 37915
Located on a bluff above the Tennessee River near downtown Knoxville, the fort was built in 1786 by General James White, Knoxville's founder.

Ramsey House 423/546-0745
2614 Thorngrove Pike, Knoxville, TN 37914
Built in 1797, Ramsey House is the first stone house in Knox County and is listed on the National Historic Register.

Governor John Sevier Home (Marble Springs) 423/573-5508
1220 West Governor John Sevier Highway, Knoxville, TN 37920
John Sevier, Tennessee's first governor, built his house when he came to the state capital in 1796.

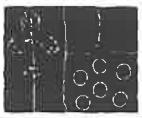
Mabry-Hazen Home 423/522-8661
1711 Dandridge Avenue, Knoxville, TN 37915
This antebellum home served as headquarters for both Union & Confederate Forces during the Civil War.

Shops/Galleries/Museums

Beck Cultural Exchange Center 423/524-8461
1927 Dandridge Avenue, Knoxville, TN 37915
Museum for the research, preservation, and display of the achievements of African Americans in Knoxville.

Candy Factory 423/522-2049
1060 World's Fair Park Drive, Knoxville, TN 37916
The building was built circa 1917. See the chocolatiers at work and stroll through the many unusual shops and galleries.

The Frank H. McClung Museum 423/974-2144
1327 Circle Park, Knoxville, TN 37996-3200 (campus)
McClung is a general museum with collections in anthropology, ancient Egypt, archeology, decorative arts, medicine, local and natural history.



Victorian Houses 423/525-7619
11th Street & Laurel Avenue, Knoxville, TN 37916
These quaint, brightly hued houses, listed on the National Historic Register, were built in the 1920's and are now home to antique and curiosity shops as well as studios and galleries.

Knoxville Museum of Art 423/525-6101
1050 World's Fair Park Drive, Knoxville, TN 37916-1653
Located downtown in the World's Fair Park, the Knoxville Museum of Art features permanent collections, traveling exhibitions, tours, and concert performances.

Volunteer Landing, Downtown Waterfront
500 Neyland Drive, Knoxville, TN 37916
One mile of paved riverwalk, fishing piers, boat docks, picnic facilities, roofed tower with observation deck, and interpretive historical markers. The complete history of Tennessee waterways.

MISCELLANEOUS

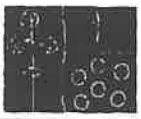
Old City District
Located at the intersection of Jackson Avenue and Central Street in downtown Knoxville, this historic downtown warehouse district welcomes you to dining, shopping and entertainment.

Tennessee RiverBoat Company 423/525-7827
300 Neyland Drive, Knoxville, TN 37902
This genuine 325 passenger sternwheel riverboat offers sightseeing, lunch, dinner, entertainment, and moonlight cruises.

Ijams Nature Center 423/577-4717
2915 Island Home Avenue, Knoxville, TN 37920
Ijams Nature Center is an eight acre city park and community nature center. Walk along foot trails that wind across streams, meadows, fern banks and bluffs overlooking the Tennessee River.

East Tennessee Discovery Center 423/594-1480
516 N. Beaman Street, Knoxville, TN 37914
Exciting science center for children of all ages.

East Tennessee Historical Society 423/544-5732
600 Market St, Knoxville, TN 37914
ETHS fulfills its mission of preserving and promoting East Tennessee history through a variety of programs including exhibits, tours, genealogy conferences, community history projects, publications and lectures.

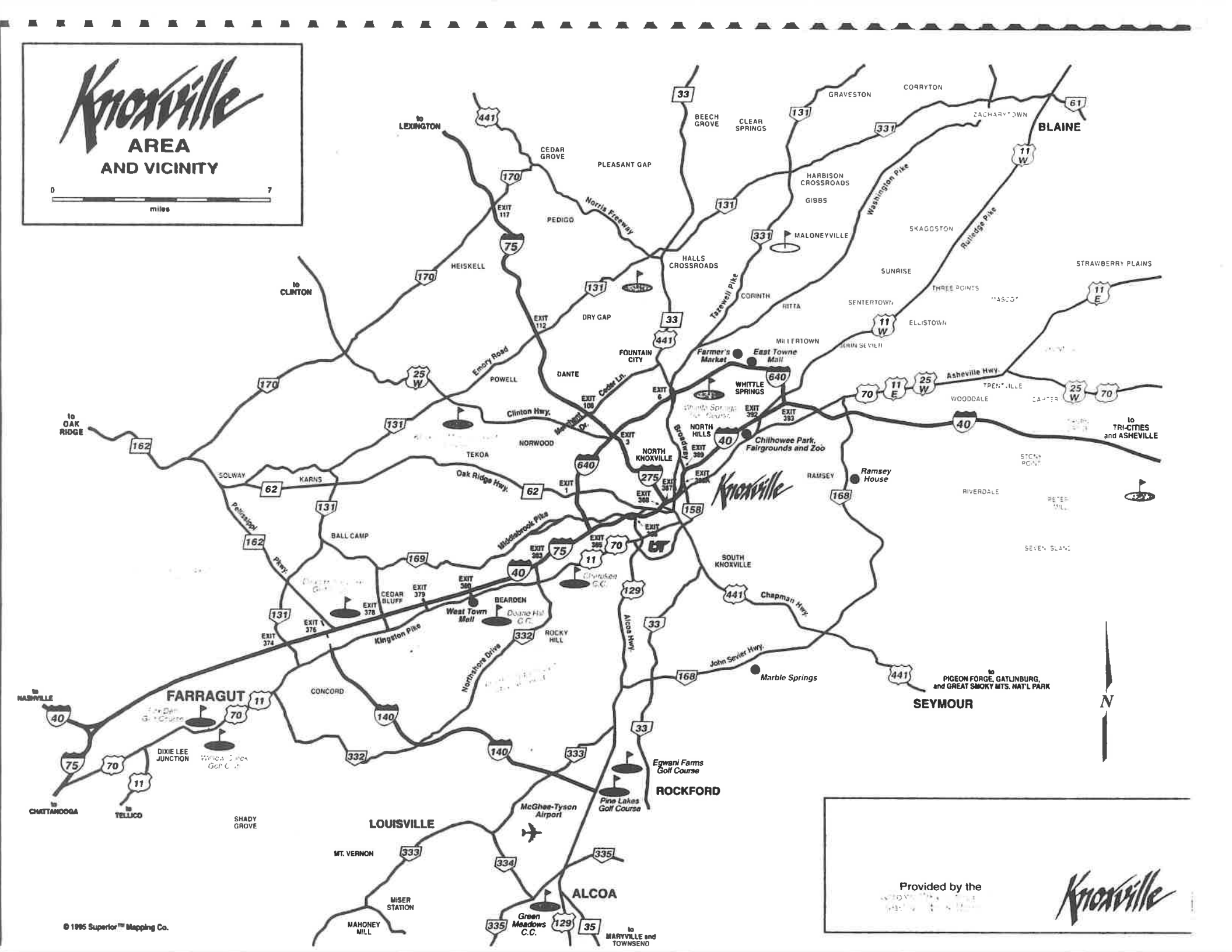


Knoxville Zoo 423/637-5331
Chilhowee Park PO Box 6040, Knoxville, TN 37914
With more than 1,000 exotic animals including gorillas, red pandas, and rhinos, the Knoxville Zoo is full of family fun, adventure, and learning.

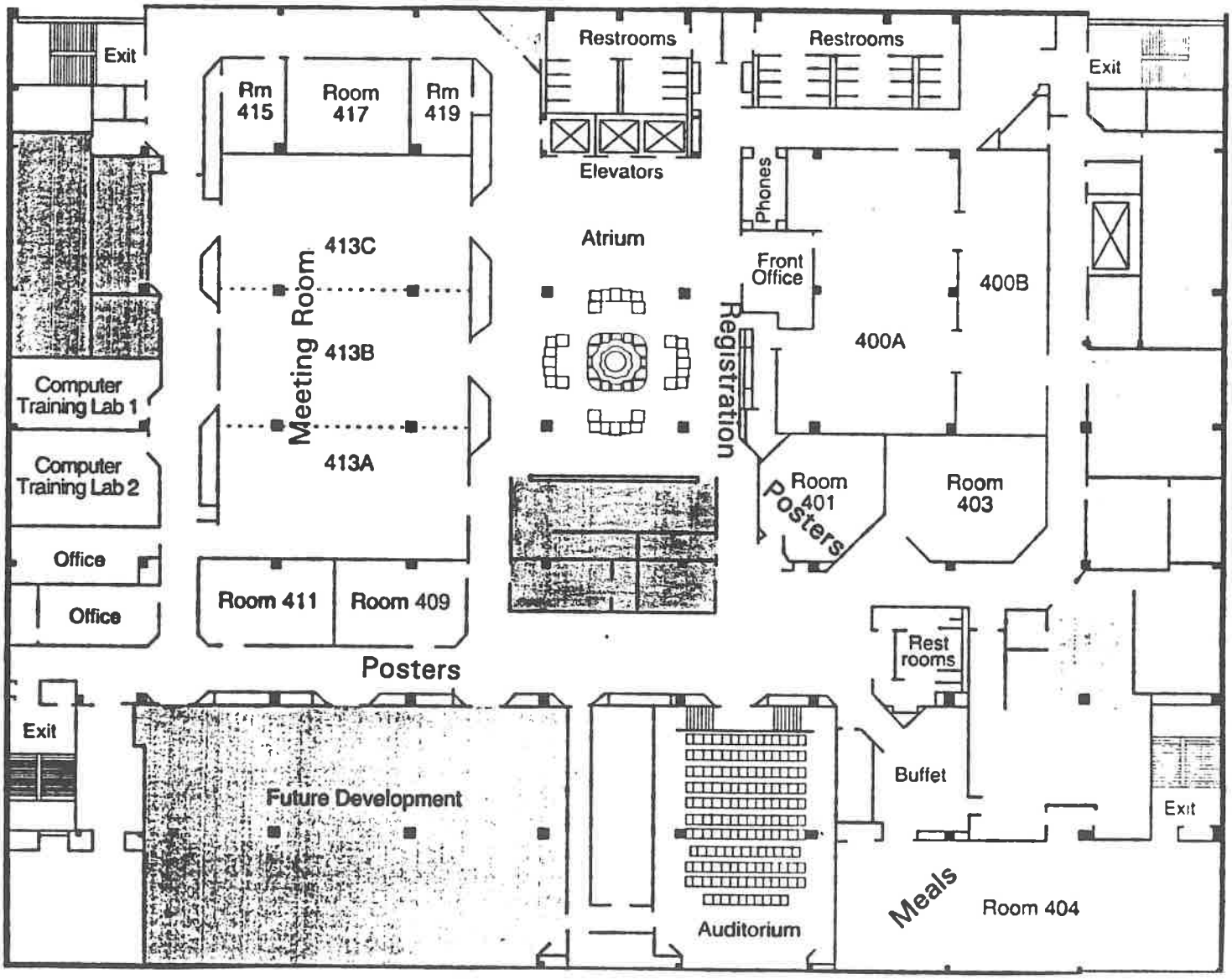
West Town Mall 423/693-0292
I-40 Exit 380. Located in the center of west Knoxville. West Town Mall has a variety of department and specialty stores.

Knoxville Center (Mall) 423/544-1500
I-640 Exit 8. Newly remodeled mall featuring a life size map of the University of Tennessee.

Great Smoky Mountains National Park
Approximately 40 miles southeast of downtown, straddling the border of Tennessee and North Carolina . 520,000 acres encompass over 800 miles of trails for hiking and horseback riding; 735 miles of streams for fishing; many developed and backcountry campsites; spectacular scenery and views from peaks over 6,000 feet; several visitors centers with interpretative exhibits and artifacts. The most visited National Park in the U.S. No charge for park access.

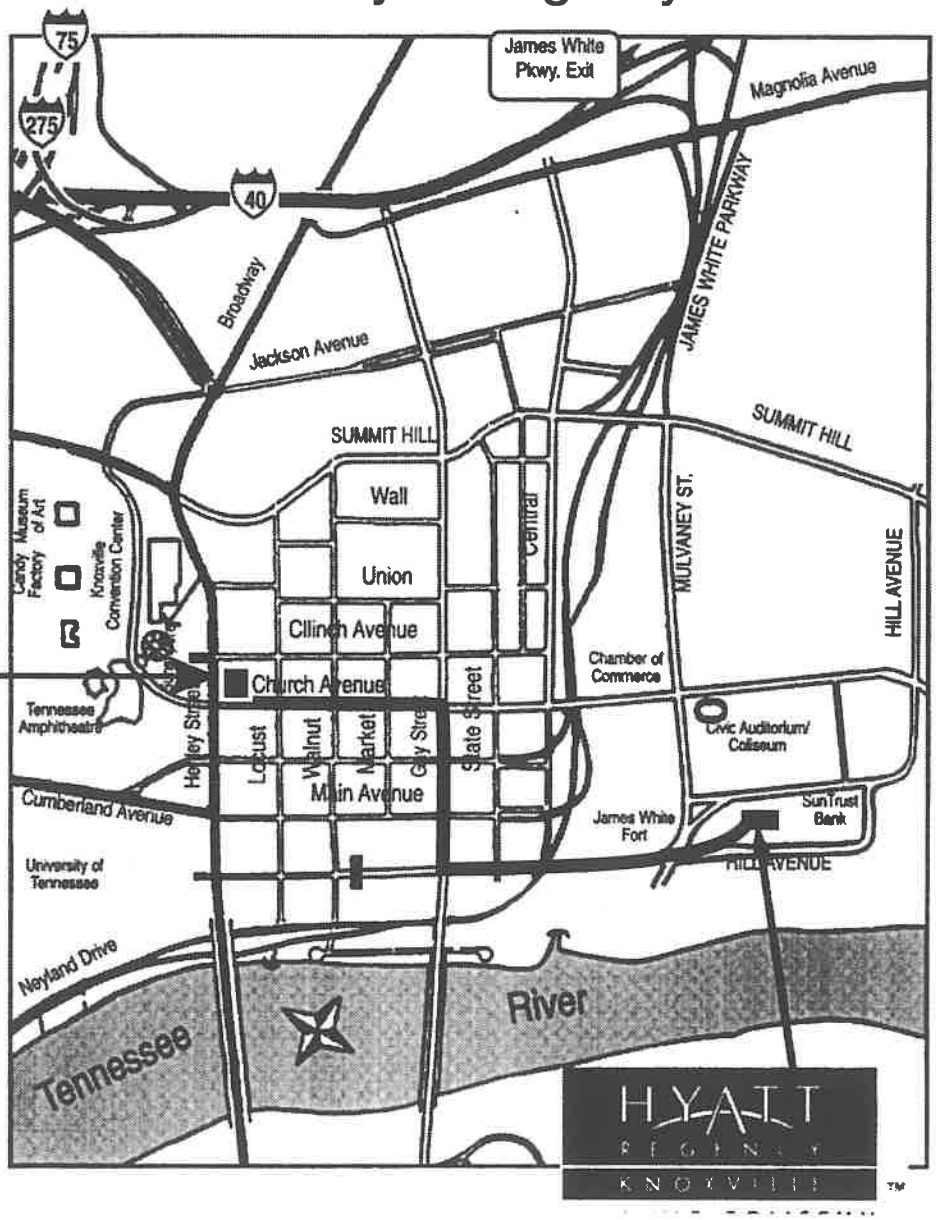


The University of Tennessee
Conference Center



Monday Dinner
Poolside
6:30 p.m. - 9:00 p.m.

Directions to Hyatt Regency Knoxville



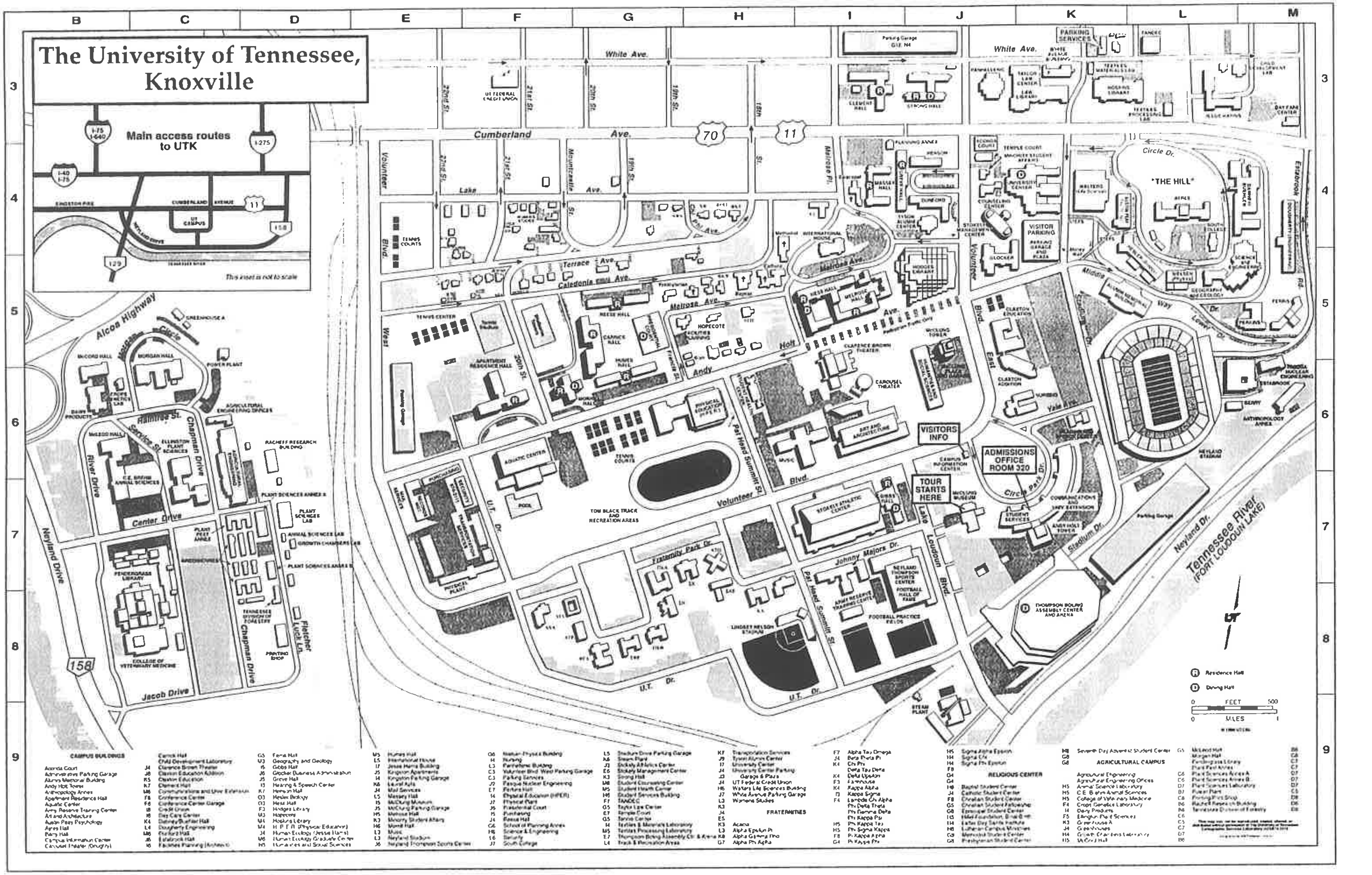
UT Conference Center

To Hyatt from Conference Center

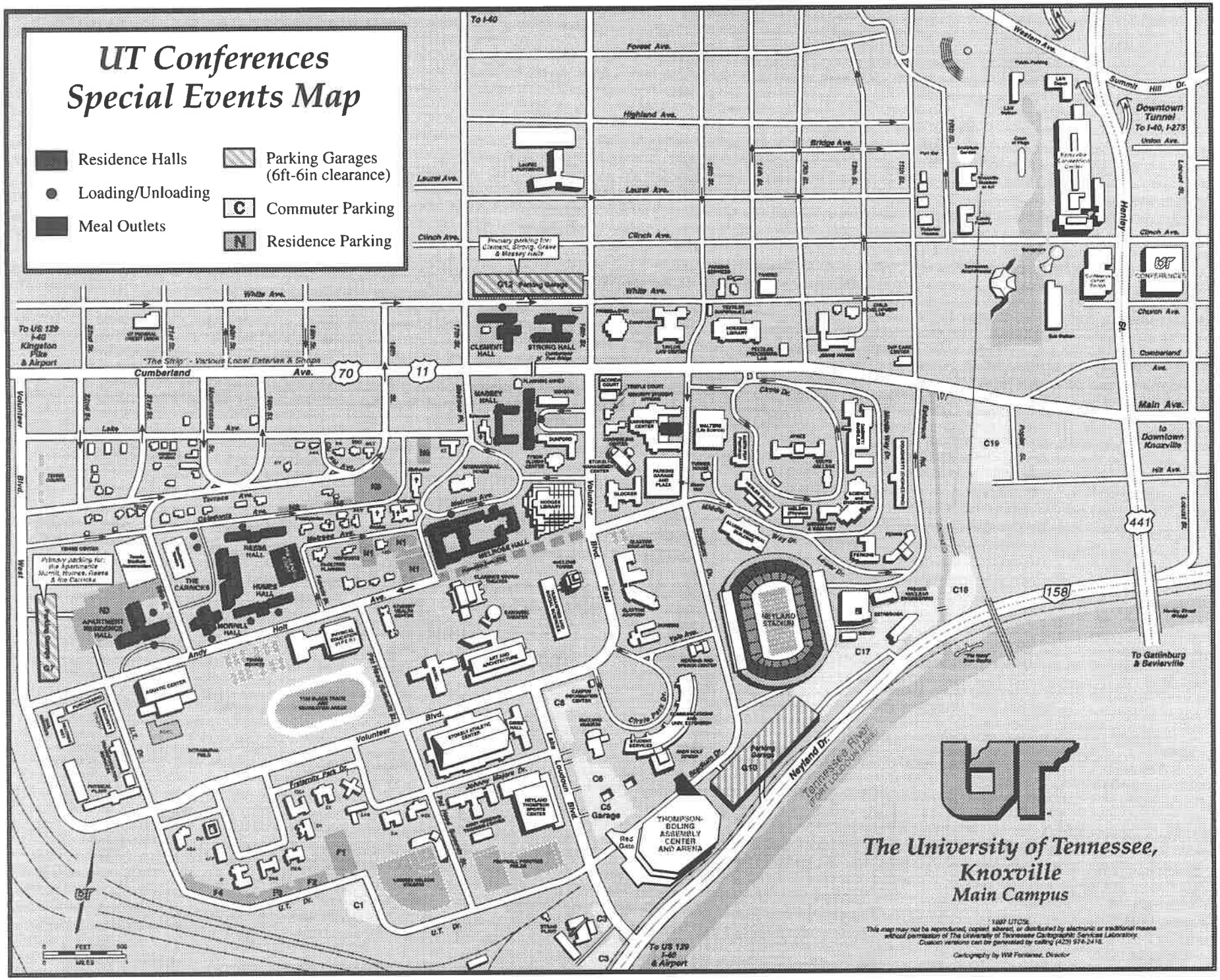
East on Church Avenue

Right on Gay Street

Left on Hill Avenue



The University of Tennessee, Knoxville
Main access routes to UTK
This inset is not to scale
White Ave.
Cumberland Ave.
Volunteer Blvd.
Circle Dr.
"THE HILL"
VISITOR PARKING
PARKING SERVICES
VISITORS INFO
ADMISSIONS OFFICE ROOM 320
TOUR STARTS HERE
Alcoa Highway
Neyland Drive
River Drive
Center Drive
Chapman Drive
Jacob Drive
Fletcher Luck Ln.
Lake Loudoun Blvd.
Johnny Majors Dr.
Pat Head Summitt St.
Fraternity Park Dr.
U.T. Dr.
Stadium Dr.
Neyland Dr.
Tennessee River (FORT LOUDOUN LAKE)
Melrose Ave.
Terrace Ave.
Caledonia Ave.
Andy Holt Ave.
Lake Ave.
Mountcastle St.
Estabrook Rd.
Circle Park Dr.
Yale Ave.
Middle Way
Lower Dr.
TOM BLACK TRACK AND RECREATION AREAS
Residence Hall
Dining Hall
FEET
MILES


UT Conferences
Special Events Map
Residence Halls
Loading/Unloading
Meal Outlets
Parking Garages (6ft-6in clearance)
C Commuter Parking
N Residence Parking
The University of Tennessee, Knoxville
Main Campus

Author's Abstract Reference Numbers

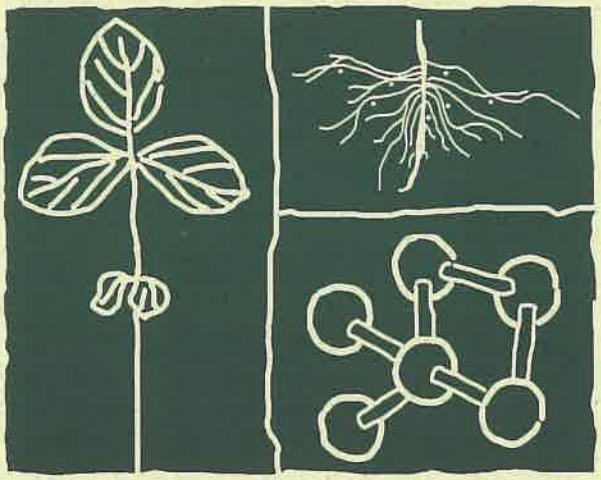
Author's Abstract Reference

Author's Name	Abstract Reference Number
Cook, Douglas	H-4
Coryell, Virginia H.	A-7
Cregan, P.B.	A-2, A-6, B-2, B-14
Dan, Yinghui	C-15
Danesh, Dariush	B-2, B-6
Day, Brad	G-16
Day, David	D-8
Dazzo, Frank B.	H-3
de Bruijn, Frans J.	H-3
Demirbas, Azize	A-5
Denny, Roxanne	B-6
Desfeux, Christine	C-6
Diers, B.W.	B-3
Dinkins, Randy D.	C-1, C-7, C-9
Domier, L.L.	G-6
Donaldson, P.	F-4
Donovan, Christopher M.	F-2
Droste, A.	C-5, C-12
Erickson, Larry	C-8
Espinosa, Bonnie G.	C-10
Fasoula, V.A.	F-3
Finer, J.J.	C-4, E-10
Foster-Hartnett, Dawn	B-6
Frazier, R.E.	D-5
Fredrik Marek, Laura	A-8
Garvey, G.	A-6
Gijzen, Mark	D-1, D-3, D-4
Good, Laura L.	E-5
Grabau, Elizabeth A.	E-3, E-5, E-6
Graef, George L.	A-5, D-5
Graham, Michelle	A-8
Gresshoff, Peter M.	G-5, H-1
Grimm, D.R.	B-2
Guo, P.	B-13
Hanlon, Regina W.	E-3, E-6
Harper, J.E.	G-4, G-6
Heer, Jennifer	G-11
Hegeman, Carla E.	E-3, E-5
Heinz, Ruth A.	D-6
Henk, Adam	B-9
Higgins, Thomas J.	F-2

Author's Name	Abstract Reference Number
Mansur, Levi	F-7
Marek, Laura	B-9
Matthews, Benjamin F.	D-6
Maughan, Peter J.	C-6, C-19
McAllister, Kay	C-1
McDougald, Lynette	C-15
Meksem, K.	A-6
Men, Artem E.	G-5
Meurer, Curtis A.	C-1, C-7
Mian, M.A. Rouf	B-1, B-7, B-10
Miki, Brian L.A.	D-1, D-2, D-3, D-4
Miller, S.Shea	D-1, D-2, D-3, D-4
Morey, Kevin	D-7
Mudge, Joanne	B-2, B-6
Muller, Peter	G-1
Munsanje, E.M.	G-14
Nam, Young-Woo	H-4
Nelson, R.L.	B-14, G-6
Njiti, V.N.	F-5
Okada, Mitsuo	G-16
Oliveira, L.C.	C-13
Olsen, Heather L.	A-5
Opperman, Charles H.	G-10, G-11, G-12
Orf, James H.	B-6, B-14, F-7
Ortega, Jose Luis	D-7
Pantazopoulos, P.	A-6
Park, Hong-Jae	C-17
Parrott, W.A.	B-11, C-2, C-16, F-1
Pasquali, G.	C-5, C-12
Penmetsa, R. Varma	H-4
Penuela, Silvia	B-6
Phillip, R.	C-19
Phillips, D.V.	B-10
Ponappa, T.	E-10
Ponsamuel, J.	C-14
Portis, Jr., Archie R.	C-18
Poysa, Vaino	B-8
Prabhu, R.R.	F-5
Pueppke, Steven G.	G-7
Qiu, J.	B-15, G-2
Qiu, L.J.	B-13

Author's Name	Abstract Reference Number
Vallejos, Eduardo	A-9
VanHouten, Wim	A-9
VanToai, T.T.	B-12
Vodkin, Lila	C-6, C-19, E-1, E-4, E-8, E-9
Vuong, T.D.	G-6
Walker, D.R.	C-2, C-16, F-1
Wang, T.	B-10
Weaver, D.B.	B-15, G-2
Webb, David	A-7
Wei, Guo	G-9
Werner, Dietrich	G-1
Widholm, Jack M.	C-3, C-14, C-17, C-18, C-19
Winzer, Till	G-1
Witzig, Stephen B.	G-13
Wopereis, Judith	H-3
Xing, Aiqiu	E-2
Xu, S.J.	A-1
Yan, Bo	C-8
Young, Nevin Dale	B-2, B-6
Yu, Kangfu	B-8
Yue, Pin	B-5
Zhang, H.	A-6
Zhang, Jian	C-10, C-11
Zhang, Xing-Hai	C-18
Zhang, Zhanyuan	E-2, F-2
Zheng, Xiangyang	G-15

Soybean Genome Advances



THE DEVELOPMENT OF THE SOYBEAN PHYSICAL MAP.

T. Hymowitz*, S.J. Xu, R.J. Singh, and K.P. Kollipara, University of Illinois, Urbana, IL 61801.

The objectives of this study were to produce all 20 possible primary trisomics ($2n = 2x + 1 = 41$) having a uniform genetic background and to associate 1-2 genetic markers with each specific chromosome. The sources of the trisomic lines initially were various meiotic mutants (asynaptic, desynaptic, and male sterile) of diverse genetic backgrounds. All 20 primary trisomics were identified based on pachytene chromosome analysis and crossing among the primary trisomics. The soybean cultivar 'Clark 63' was selected as the recurrent parent. All the 20 primary trisomics were backcrossed to the BC_4 generation. Thus far, the *f*, *eu*1, and *lx*1 genes have been mapped to soybean chromosome 3, 5, and 13, respectively. The overall aim of the project is to create a universal map of the soybean.

An Integrated Genetic Linkage Map of the Soybean

P. B. Cregan[1*], T. Jarvik[2], A. L. Bush[3], R. C. Shoemaker[3], K. G. Lark[2], A. L. Kahler[3], and J. E. Specht[5]

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[5] Dept. of Agronomy, Univ. Nebraska, Lincoln, NE 68583-0915.

The purpose of this report is to summarize the current status of the integrated SSR/RFLP/classical marker linkage map of the soybean and to solicit information regarding the map position or putative map position of classical genetic loci i.e., loci controlling color, pigmentation, morphological traits, disease resistances, male sterility, etc. In December of 1997 a total of 600+ SSR loci were publicly released. These consist mainly of loci with $(ATT)_n$, trinucleotide core motifs. Each of these loci was mapped in at least one of three mapping populations: USDA/Iowa State *G. max* x *G. soja* F_2 population; the Univ. of Utah Minsoy x Noir 1 recombinant inbred population; and the Univ. of Nebraska Clark x Harosoy F_2 population. Each SSR mapped to a single locus in the genome, with a map order that was essentially identical in each population. Many SSR loci were segregating in two or all three populations. Thus, it was relatively simple to align the 20+ linkage groups derived from each of the three populations into a consensus set of 20 homologous linkage groups which were presumed to correspond to the 20 pairs of soybean chromosomes. A total of more than 500 RFLP loci are also included in the three maps. The integrated map contains a number of intervals of greater than 20 cM in which no SSR loci are positioned. Targeted marker development is proceeding in an effort to place SSR markers in these intervals. Based on *in situ* segregation or linkage reports in the literature, all but one of the classical linkage groups can now be assigned to a corresponding molecular linkage group. An important objective of this work is the positioning of approximately 200 additional classical genetic loci on the integrated soybean map. This large undertaking is still ongoing. Anyone wishing to assist in this effort with information regarding the position of classical loci should contact Dr. J.E. Specht.

THE AUTHORS WISH TO GRATEFULLY ACKNOWLEDGE THE FINANCIAL SUPPORT OF THE UNITED SOBYEAN BOARD.

FROM GENES TO CHROMOSOMES

Halina T. Knap*, Department of Crop & Soil Environmental Science, Clemson University, Clemson, SC 29634-0359

We have isolated and characterized the expression of more than 50 genes from apical meristematic mutant and elongation zone of the soybean root. Thirty of these genes have been mapped on the soybean linkage map. Currently, these genes are used for isolation of BACs, and their respective chromosomal assignments are in progress. As soybean research makes fast strides toward a functional map, human chromosomes as a model will be discussed.

TRANSPOSABLE ELEMENTS IN SOYBEAN: *MARINER* TAKES A VOYAGE TO THE PLANT KINGDOM

Tyler Jarvik* and K. G. Lark
University of Utah, Deptartment of Biology, 257 S 1400 East, Salt Lake City, UT 84112

A number of soybean DNA probes used for RFLP mapping have characteristics that suggested that they represented mobile genetic elements. We have sequenced four such probes and identified what type of element each represents. The sequence of one (K011) was similar to retrotransposons described from other organisms, and the other three (G214, L050, and BL053) contained inverted terminal repeats (ITRs), suggesting that they represented DNA-mediated transposable elements. All four were distinct from previously described soybean transposons such as *Tgm1*. Using a PCR-based approach, we identified a larger element containing the G214 ITR sequence. This element was also found to contain a 1275 bp open reading frame, encoding an amino acid sequence that was very similar to the transposase found in *mariner* elements. *Mariner* elements are a well-studied group of DNA-mediated transposable elements that have been found in many animals from several phyla but never before in plants. Although the element corresponding to the G214 probe, which we have named *Soymar1*, has sequence features that place it in the *mariner* family, it also has novel features that could be used in the search for similar elements. It is possible that some of these novel features render the transposase inactive; however, the *Soymar1* transposase gene is remarkable among *mariners* for containing neither stop codons nor frameshift mutations. Furthermore, the extreme diversity of G214 banding patterns across various soybean cultivars is consistent with recent activity of the element. The possibility that an active copy of *Soymar1* exists, therefore, cannot be ruled out, and such an element (or a reconstruction of it, based on a consensus of sequences in different soybeans and *G. soja*) might prove to be a useful tool for genetic research and manipulation.

CLASSICAL MARKER POSITIONS ON THE SOYBEAN MOLECULAR MARKER MAP - A PROGRESS REPORT

Brian G. Rector, Azize Demirbas, Mike J. Livingston, Heather L. Olsen, Renee A. Ritchie, George L. Graef, and James E. Specht. Department of Agronomy, University of Nebraska, Lincoln, NE 68583.

Simple-Sequence-Repeats (SSRs), which are *single-locus*, co-dominant multiple-allele markers, were recently used to coalesce the linkage groups of three separate soybean mapping populations into one map that has the expected n=20 linkage groups (Cregan et al., 1998). To increase the information content of this map, we are attempting locate the positions of 200+ known classical markers (i.e., disease reaction, pigmentation, morphology, developmental ontogeny, etc.), by using an approach we have termed homozygosity mapping. An F2 population was derived for each of the 200+ classical genes by mating their source parents with a common (hub) parent. Overt phenotypes were scored in the F2, or F2.3 generation, depending upon the difficulty of scoring. Collaborators (see list below) performed the phenotypic scoring for some traits. Leaf material was collected from the two parents and from homozygous recessive (HR) F2 plants, or from 20 F2:3 progeny plants per HR F2 plant. For each classical gene, the hub parent DNA, source parent DNA, and a *balanced bulk of DNA* from the HR F2 were subjected to an initial SSR screening (by Biogenetics Services) to detect amplicon bimorphism. When the bulked HR DNA displayed only (or discernibly more of) the source parent amplicon *versus* none (or discernibly less) of the hub parent amplicon, putative linkage of the SSR marker to classical gene was inferred. Putative SSR linkages were confirmed (or refuted) by analysis of the DNA collected from ca. 30-50 *individual* HR F2 plants in the given population. Recombination values (and their standard errors) for SSR marker - classical gene linkages were estimated via the maximum likelihood method (i.e., see equation #16 of Allard, 1956). Our objective is to identify at least two SSR marker (flanking or otherwise) linkages for each classical gene. As of this writing, the parental and bulk HR screening is near completion. Of the putative SSR - classical gene linkages identified to date, most have now been confirmed and linkage intensity estimated.

Allard, R.W. 1956. Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia 24 (10):235-278.

Cregan, P.B., et al. 1998. An integrated genetic linkage map of the soybean. Crop Sci (accepted for publication).

Collaborator Acknowledgements: P.Cregan (USDA) - SSR primers & *Rj*; R.Shoemaker (USDA-ISU) - linkage map & *Fe*; G.Lark (Utah) - linkage map; A.Kahler (Biogenetics) - initial SSR screens; D.Lohnes (USDA-OSU) - *Rps*; G.Buss (VPI) - *Rsv*; J.Wilcox (USDA-Purdue) - *Lx* & *Ep*; R.Palmer (USDA-ISU) - *Fr*; Ted Hymowitz (IL) - *Ti*.

DEVELOPMENT OF GENOME INTEGRATED PHYSICAL MAP; POSITIONAL CLONING OF THE CYST NEMATODE RESISTANCE LOCI IN SOYBEAN.

K. Meksem*[1], P. Pantazopoulos[1], K. Chancharoenchai[1], E. Ruben[1], G. Garvey[1],H. Zhang[2],R. Arelli[4],P. Cregan[3], D.A. Lightfoot[1].

1.Department of Plant Soil and General Agriculture, Southern Illinois University at Carbondale, Carbondale, IL 62901-4415 2.Department of Soil and Crop Sciences, Crop Biotechnology Center, Texas A & M University, College Station, TX 77843-2123 3.Soybean and Alfalfa Research Laboratory, USDA-ARS, Bldg. 006, Rm. 100 BARC-West, Beltsville, MD 20705-2350

Inheritance of field resistance in soybean [Glycine max (L.) Merr. In "Forrest" cultivar (Peking source) to soybean cyst nematode (SCN) race 3 (*Heterodera glycines i.*) is conditioned by two loci: *rhg1* on linkage group G and *Rhg4* on linkage group A2. Bulked segregant analysis and AFLP's were used to select molecular markers closely linked to *rhg1* and *Rhg4*. We have screened 1024 (*EcoRI/MseI*) primer combinations against two pools of DNA (a resistant and a susceptible pool). This revealed about 10000 AFLP polymorphic bands, 20 of which map in coupling with rhg1 to G and 7 of which map in coupling with *Rhg4* to A2. Two AFLP markers place *rhg1* within a 1 cM interval and three AFLP markers place *Rhg4* within 0.5 cM. A new type of vector system will accelerate the cloning of *Rhg4*, *rhg1* and other agronomically important genes that underlie QTL. This vector is able to accept large DNA fragments and to transform plants directly. (Bibac, Hamilton et al., 1996; V41 Zhang et al. submitted). We have constructed a Forrest Bacterial Artificial Chromosome (BAC) library in the V41 vector. That will provide clones for physical mapping of the soybean genome and for chromosome walking or landing. Candidate clones containing target genes can be directly used to transform plants for genetic complementation tests via Agrobacterium-mediated methods. Five BAC clones were identified in the 1 cM interval carrying *rhg1* that were shown to overlap by fingerprinting. One clone, A109-4 (insert size of 127 Kb) contains the two AFLP markers flanking either side of *rhg1* in Forrest. Sequencing of subclones of A109-4 has identified candidate resistance genes. Transformation with candidate genes from the A109-4 clone is in process. BAC fingerprinting and contig assembly has provided powerful tools to rapidly generate molecular physical maps. A physical map integrated with the developed DNA marker genetic maps will provide a new strategy to clone genes known only by their phenotypes by gene golfing (Zhang and Wing 1997). Using the integrated physical map, DNA molecular markers up to 10 cM away from a gene of interest can be used to clone the target gene. Genes in regions where repeated sequences are rich can also be cloned with the integrated physical map, whereas it is difficult, if not impossible, to clone the genes in such regions by the currently used map-based cloning strategy. The development of an integrated physical map will provide a "highway" for isolation of large number of genes and for many other genetic and biological studies of plant and animal genomes. Currently, the integrated physical map of the soybean is under development by a multi-disciplinary group using the BAC fingerprinting and contigs assembly technologies.

ALLELE-SPECIFIC HYBRIDIZATION MARKERS FOR SOYBEAN. Virginia H. Coryell[1]*, Holly Jessen[2], James M. Schupp[1], David Webb[2], Paul Keim[1]. [1]Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011-5640. [2]Soybean Research Department, Pioneer Hi-Bred International, Inc., Johnston, IA 50131

Soybean is one of the world's most important crop plants due to extensive genetic improvements using traditional breeding approaches. Recently, marker-assisted selection has enhanced the ability of traditional breeding programs to improve soybeans. Most methods of assessing molecular markers involve electrophoretic techniques that constrain the ability to perform high-throughput analyses on breeding populations and germplasm. In order to develop a high capacity system, we have developed allele-specific hybridization (ASH) markers for soybean. As one example, RFLP locus A519-1 (linkage group B) was converted into an ASH marker by (1) sequencing the pA519 cloned insert, (2) designing locus-specific PCR amplification primers, (3) comparative sequencing of A519-1 amplicons from important soybean ancestors, and (4) design of allele-specific oligonucleotide probes around single nucleotide polymorphisms (SNPs) among soybean genotypes. Two SNPs were identified in ca. 400 bp of sequence. Allele-specific probes generated 100-fold greater signal to target amplicons than to targets that differ by only a single nucleotide. The A519-1 ASH marker is shown to cosegregate with A519-1 RFLP locus. In order to determine ASH usefulness, 570 soybean lines from the Pioneer HiBred soybean improvement were genotyped using both A519-1 SNPs. Combined haplotype diversity (D) was 0.43 in this adapted germplasm set. These results demonstrate that ASH markers can allow for high-throughput screening of germplasm and breeding populations, greatly enhancing breeders' capabilities to do marker-assisted selection.

EXPRESSION AND ORGANIZATION OF RESISTANCE GENE ANALOGS.

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Sequence analyses of cloned plant disease resistance genes have shown that they contain a number of conserved domains with known functions as well as regions of conserved sequence for which no function has yet been identified. Researchers have used the conserved domains to clone and analyze analogous sequences from soybean (Kanazin et al. 1996; Yu, et al. 1996). Many of the soybean Resistance Gene Analogs (RGAs) exist in clusters and map in proximity to known disease resistance genes. One cluster of class 1 RGAs mapped to a cluster of disease resistance genes on linkage group J. Class 1 specific RGA oligonucleotide primers identified a group of BACs in the USDA/ISU 'Williams 82' BAC library which were arranged into a contig spanning 700 kb. Markers developed from BAC-ends in the contig map into the disease resistance cluster on linkage group J. To determine if any of the soybean RGAs were transcribed, two cDNA libraries (epicotyl and root) were screened by hybridization with RGA class-specific probes. Differential expression of RGAs was observed between libraries and between classes of RGAs. All cDNAs that we have analyzed were members of the Toll/Interleukin-1 receptor, nucleotide binding domain, leucine rich repeat family of disease resistance genes. To investigate the genomic organization of these RGA sequences, primers were designed for the 3' end of individual cDNA clones and used to screen the BAC library. Two different cDNAs amplified from a single BAC (34P7) which is part of the 700 kb RGA class 1 BAC contig on linkage group J. Primers designed to distinguish the different cDNA sequences resolved three forms of RGA sequence on BAC34P7 which differed by the presence or absence of a 200 bp duplication (present in one or two copies). We are sub-cloning BAC 34P7 to obtain additional direct genomic sequence information in this region. Similar duplications/deletions may exist in sequences associated with other RGA clusters on BACs not part of the 700 kb contig on linkage group J. These observations suggest the involvement of recombination mechanisms which have been hypothesized to generate diversity among clusters of disease resistance genes.

References:

Kanazin, V., Marek, L.F., and Shoemaker, R. (1996) Proc. Natl. Acad. Sci. USA 93:11746-11750.

Yu, Y.G., Buss, G., and Saghai Maroof, M. A. (1966) Proc. Natl. Acad. Sci. USA 93:11751-11756.

GENETIC ANALYSIS OF HOMOEOLOGOUS REGIONS IN *GLYCINE MAX* AND THE HOMOLOGOUS REGION IN *PHASEOLUS VULGARIS.*

Jennifer M. Lee[1]*, Arla L. Bush[1], James E. Specht[2], Wim VanHouten[3], Eduardo Vallejos[4], Clay M. Baldwin[1,5], Sally A. Mackenzie[3], and Randy C. Shoemaker[1,5]

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[5]USDA-ARS, Corn Insect and Crop Genetics Research Group, Iowa State University, Ames, Iowa 50011

The analysis of duplicate genetic factors within the soybean genome have enabled the detection of homoeologous regions derived from an ancient polyploidization event (Shoemaker et al. 1996). We have mapped two pair of duplicated functional genes to such regions (*Pa1/Pa2*(Lee et al. in review) and *H3-hs28-1/H3-hs28-2*(Kanazin et al. 1996)). Three linkage groups are involved in this homoeologous relationship (LG-B1/S, LG-F, and LG-H). LG-B1/S and LG-F each have significant homology to LG-H. Interestingly LG-B1/S is only homologous to the top portion of LG-H, while LG-F is homologous only to the bottom portion of LG-H. We are now further characterizing these regions to gain insight into the origins of homoeology within soybean and possible mechanisms of diploidization in polyploids. In order to best elucidate the evolutionary history of the soybean genome we are also comparing duplicate regions within the genome to their homologue in the related legume *Phaseolus vulgaris*.

Kanazin, V., T. Blake, and R.C. Shoemaker. 1996. Organization of the histone H3 genes in soybean, barley and wheat. *Molecular and General Genetics*. **250**:137-147

Lee, J.M., A.L. Bush, J.E. Specht, and R.C. Shoemaker. 1998. Mapping of duplicate genes in Soybean. *Genome*. in review

Shoemaker, R.C., Polzin, K., Labate, J., Specht, J., Brummer, E.C., Olson, T., Young, N., Concibido, V., Wilcox, J., Tamulonis, J.P., Kochert, G., and Boerma, H.R. 1996. Genome Duplication in Soybean (*Glycine* subgenus *soja*). *Genetics*, **144**:329-338.

THE PHYLOGENY OF *GLYCINE CANESCENS* F. J. HERM. AND *G. TOMENTELLA* HAYATA OF WESTERN AUSTRALIA.

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Study of the intergenomic relationships among various species and the diversity within each species of the genus *Glycine* has been the focus of a series of investigations in our laboratory for the past several years. In this study, we report the genomic diversity within *G. canescens* and *G. tomentella* accessions and their phylogenetic relationships with the other diploid species of the genus *Glycine* using cytogenetic and molecular methods. Cytogenetics revealed that *G. canescens* accessions (PIs 583944, 583946, 583953, 591575) from the Western Australia were genomically similar. However, they were differentiated by a paracentric inversion from the standard *G. canescens* (PI 440932) collected from South Australia. By contrast, *G. tomentella* ($2n = 40$) accessions from the Western Australia were highly diverse. Cytogenetics and sequence comparisons of the internal transcribed spacer (ITS) region of the rDNA separated the diploid *G. tomentella* accessions in Australia into four distinct groups. The genome symbols DD (isozyme group D3; PI 505222), D_1 D_1 (isozyme group D5; PI 505301), D_2 D_2 (isozyme group D5: PI 505203), and D_3 D_3 (isozyme group D4; PI 441000) are being assigned to these four groups. The D_1 and D_2 genomic group accessions are distributed in Western Australia. The D_3 group of *G. tomentella* accessions are morphologically similar to neither A-genome species nor D, D_1, D_2 groups. However, the D_3 group was phylogenetically grouped with the A-genome species while D, D_1, D_2 showed a close relationship with E, H, and I genome species. This study demonstrates that diploid *G. tomentella* of the Western Australia is a complex species and from an evolutionary viewpoint, it is actively radiating out into several genomic variants.

USE OF MOLECULAR MARKERS FOR IDENTIFICATION OF SOYBEAN VARIETIES

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Cultivated soybean (Glycine max l Merr) is a self-pollinating crop of limited genetic variability, thus impairing cultivar identification exclusively on the basis of morphological traits. This work shows the feasibility of using 15 known basic morphologic, physiologic and phenologic traits and different molecular markers such as ìRandom Amplified Polymorphic DNAî (RAPD), ìAmplified Length Polymorphismsî (AFLP) and Microsatellite or Simple-Sequence-Repeat (SSR) to differentiate a set of 100 varieties registered in the National Register of Cultivars of the National Institute of Seeds of Argentina (INASE). The aim of this study is to characterize a limited set of markers to be used as consensus descriptors to register soybean cultivars. RAPD was assessed by assaying 67 primers, each of them generating 1-3 bands (resulting in 31 different genomic loci scored). Twenty-two primers generated useful polymorphisms. To assess SSR markers, 16 loci were selected because they evidenced high polymorphic indexes (PIC 0.45-0.75). AFLP was assessed with just 3 primer combination of MseI and EcoRI resulting aproximatly in 40-50 bands, 23 of them polymorphic.
The results were grouped by cluster analysis and validated with pedigree data of the varieties. From the analysis, it becomes clear that morphologic data are insufficient to separate all classified genotypes. The molecular markers complement very well the classification based on classical morphological traits and help to further differentiate what becomes difficult with classical criteria. Results shown here indicate that molecular markers constitute a useful tool to unequivocally fingerprint Argentine soybean varieties.

DEVELOPMENT OF AN ASA MARKER FOR THE Kti3- ALLELE IN SOYBEAN.

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The Kunitz tripsin inhibitor type 3 (Kti3) has been proposed as an antinutritional compound in soybean, particularly for birds and pigs. Orf and Horowitz (1979) reported Kti3 null line (Kti3-). Its introduction into locally adapted germplasm will allow for the increase of raw soybean meal in non-rumiant feeding, therefore reducing breding costs.

Kti3 activity evaluation is operator sensitive and not accurate neither reproducible under 20% inhibitory level, (Bouzas y Bertoni, 1980). As a consequence, an ASA marker for the Kti3- allele, in order to utilize it in a marker assisted breeding program in the near future.

The Kti3- marker was developed based on the knowledge of the sequence for the normal and null alleles for Kti3 (Jofuku et al., 1989). Primers were designed with the aid of the LasergeneTM package, and modified to tag specifically the mutation site. Polymerase chain reaction, and subsequent agarose electrophoresis and ethidium bromide staining produced the predicted 525 bp amplicon for genotype PI 157440 (Kti3-), and two spurious bands for Willams 82, which we utilized as the normal (Kti3+) control. Kti3- amplicon is not linked, but constitutes an integral part of the allele to be introgressed during the breeding program. Experimental errors associated with recombination events are therefore prevented, increasing heritability for the trait to a value of virtually 1. The application of this marker into germplasm improvement will allow for plant selection based on genotype at any stage of growth, at any generation and independent of environment. This technique has usefulness in making early generation selections, thereby saving valuable time and resources. The breeder can select for Kti3- genotypes prior to incurring the expense of field work, and breeding decisions can be made during the winter, saving valuable growing time in the spring. Furthermore, several generations may be advanced under greenhouse every year, thereby significantly reducing the time involved in cultivar development.

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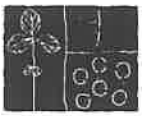
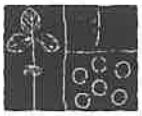
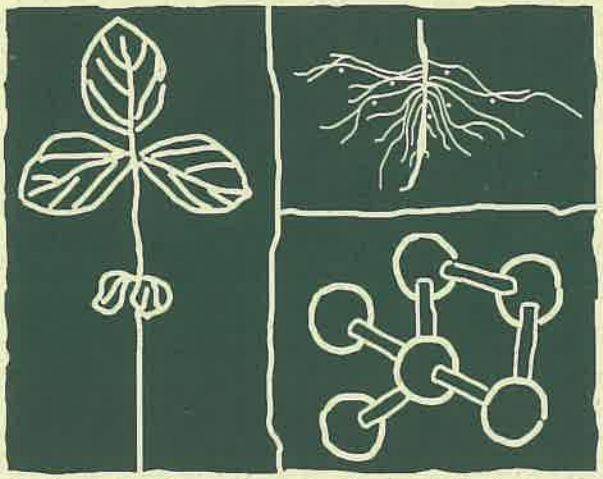




Soybean Gene Discovery (Marker Technology)



APPLICATION OF DNA MARKERS TO SOYBEAN BREEDING.

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In the past decade, researchers have developed public RFLP, AFLP, and SSR genetic linkage maps of soybean. These maps have allowed extensive mapping of genes conditioning qualitative traits, such as single-gene pest resistance, and many quantitative traits (QTL) conditioning agronomic performance and seed composition. The current status of soybean QTL discovery will be reviewed. DNA markers are currently being applied in soybean breeding programs for parental selection, selection for pest resistance, rapid recovery of recurrent parent, and improvement of intractable traits. Examples of these breeding applications will be discussed. Many studies have found QTL to be population specific. This may be caused by limited marker polymorphism within any single population and allele fixation in the specific parents. Presently, there are few examples in the literature of successful application of QTL discovery in marker assisted selection or confirmation of QTL discovery in a population that was independently derived from the original mapping population. The various DNA marker systems differ in their utility for the application in soybean breeding. They differ in level of polymorphism, cost/data point, freedom to commercially operate, potential for automation, and user safety. The current limitations of DNA marker technology in soybean breeding and the potential of new technology to overcome these limitations will be discussed.

ASSESSMENT OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) IN SOYBEAN

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There is now a growing interest in single nucleotide polymorphisms (SNPs), primarily as a result of the efforts of the Human Genome Project. SNPs, which are point mutations, have been shown to be the most abundant type of DNA polymorphism in a number of species. Their average frequency in humans is 2 to 3 SNPs/kbp. SNPs occur throughout the genome in both coding and noncoding regions, the majority being found in noncoding regions. As a result of their abundance, SNPs have become an important source of molecular markers that contain a wealth of genetic information. One attractive feature of SNPs is the possibility of detection using non-gel based systems. As a result of technologies developing from the Human Genome Project, high-throughput, automated systems have already been developed that allow the parallel assay of SNPs at very high rates. In contrast to the large amount of information available in humans and other mammalian species, very limited data are available on SNPs in plants. Thus, we initiated studies to determine if there are sufficient SNPs in soybean to be useful for genetic map development and applications in plant improvement. Three approaches were used to assay the level of SNPs in soybean. First, we are determining SNP frequencies associated with 16 soybean genes (exons, introns, 5' UTR and 3' UTR) chosen from GenBank. Second, SNPs are being assayed in the flanking regions of Simple Sequence Repeat (SSR)-containing sequences. For this study, we have taken advantage of the 650 SSR markers already placed on the soybean linkage map. Thirdly, we are assaying the level of SNPs in random (non-SSR containing) subclones of BACs from a number of unlinked positions in the soybean genome. Our initial efforts have focused on determining the frequencies of SNPs in one region of the genome. We targeted an area of the genome that is important for soybean production and improvement, the Soybean Cyst Nematode (SCN) resistance locus (*rhg1*) in linkage group G. We chose 10 SSR loci surrounding this locus at the top of linkage group G to search for the presence of SNPs. These 10 SSRs span an area of approximately 25cM. In comparison, we searched for SNPs in non-SSR containing sequences subcloned from a 150kb BAC insert that includes the BARC-Satt309 locus (closely linked to *rhg1)*. The 18 soybean cultivars sequenced for SNP discovery were chosen on the basis of their contribution to North American soybean germplasm as determined by Gizlice *et al.* (1994). Initial results from this work suggest that SNPs in the soybean genome occur at a frequency of 3 to 4/kbp, higher than that in humans. The frequency of SNPs in soybean is also approximately equal in SSR flanking regions and in non-SSR containing regions. A high level of linkage disequilibrium was observed among SNPs from the same BAC clone.

NEW SOURCES OF RESISTANCE TO SOYBEAN CYST NEMATODES. B. W. Diers*, P. Arelli, and S. R. Cianzio. Univ. of Illinois, Urbana, IL; Univ. of Missouri, Columbia, MO; Iowa State Univ., Ames, IA.

Soybean cyst nematodes (SCN) (*Heterodera glycines* Ichinohe) are the most important soybean pathogen in North America. Although SCN resistant cultivars have been developed, their resistance was derived from only a few plant introductions (PIs). Many other SCN resistant PIs have been identified which could be used as sources for broadening the diversity of resistance genes in cultivars. The objectives of this project were to evaluate genetic diversity among SCN resistant PIs and to determine whether these PIs have different resistance genes compared to previously used resistance sources. Genetic diversity among the PIs was evaluated by testing 38 resistant lines with 201 restriction fragment length polymorphism (RFLP) markers. The clustering of the PIs based on the markers was consistent with the resistance responses of the lines. Some PIs were identified which were genetically distant from previously used sources of resistance. However, these distant PIs had only moderate levels of resistance. Whether PIs had the same resistance genes as previously used resistance sources was determined by evaluating populations with markers linked to mapped SCN resistance genes. Four populations were developed which had four new PIs as parents. These populations were tested for both the genetic markers and SCN resistance. In all populations, SCN resistance was mapped to the top of linkage group G, the location where other researchers have identified resistance. Resistance also was mapped to other locations where SCN resistance had been previously identified such as linkage groups D, J , A, and C2. These results are consistent with other reports that linkage group G harbors a major SCN resistance gene in most resistance sources.

EVALUATING POLYMORPHIC DIFFERENCES IN SOYBEAN GERMPLASM USING AFLP MARKERS FOR SCN RESISTANCE

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Soybean Cyst Nematode (SCN) *Heterodera glycines* is one of the most destructive pests of soybean (*Glycine max* [L]. Merr) in the United States. Soybean breeders have developed cultivars with genetic resistance derived from primitive plant introductions (PIs), to combat this disease. SCN resistance has been investigated over the past few years through the use of polymerase chain reaction-based techniques which has advanced the amplification of DNA markers to genome mapping. The amplified fragment length polymorphism (AFLP) is an efficient DNA marker technology, which is characterized by the separation of large number of polymorphic restriction fragments on a polyacrylamide gel. AFLP has the capacity to reveal more polymorphic bands in one lane than any other marker technique.

Six germplasm lines were evaluated. These include four PIs; Peking, which is resistant to SCN races 1, 3 and 5; PI88788, which is resistant to SCN races 3 and 14; PI90763, which is resitant to SCN races 1, 2, 3 and 5 and PI437654 which is resistant to SCN races 1, 2, 3, 5, 6, 9 and 14. Germplasm line J87-233 was derived from a complex genetic background that inherited broad based resistance from three resistant sources; Peking, PI88788 and PI90763. Germplasm J87-233 was crossed with susceptible cultivar Hutcheson, which is a cultivar with good yield potential. Our objective was to characterize specific chromosomal regions associated with SCN resistance in germplasm J87-233 using AFLP markers.

Ninety six primer combinations from EcoRI and MseI primers have been tested for polymorphism against the six soybean genotypes. In all, there were fifty two polymorphic primer combinations across the six genotypes. The average polymorphic loci per primer combination were about four. With the use of some anchored markers, the markers obtained will identify specific chromosomal regions that are associated with SCN resistance in the J87-233 genotype. Markers significant ($P<0.05$) to the various SCN races were analyzed by one-way ANOVA. These results will be presented.

RFLP MARKERS RELATED TO SOYBEAN CYST NEMATODE RESISTANCE IN SOYBEAN PI438489B. Pin Yue*, Prakash R. Arelli, and David A. Sleper, University of Missouri, Columbia, MO 65211

Soybean PI438489B has been identified as resistant to all known SCN races (Arelli and Wilcox, 1996). Objectives of this study were to use restriction fragment length polymorphism (RFLP) markers to identify loci conferring resistance to soybean cyst nematode (*Heterodera glycines* Ichinohe, SCN) Races 1, 2, 3, 5, and 14. A total of 184 $F_{2:3}$ lines from a cross of PI438489B and susceptible cultivar Hamilton were tested. Two hundred sixty-four genomic probes were used to screen two parents, 61 were found polymorphic within these populations. Among these probes, seven markers were found highly related with SCN resistance. Three markers were found related with Race 1 resistance, two for Race 3, three for Race 5, and one for Race 14. No probes were found associated with Race 2 resistance. Probe A381 was found related to Races 1, 3, and 5 resistance in this population. The relationship among different Race resistance loci will be e discussed.

REFERENCE

Arelli and Wilcox. 1996. Soybean Genet. Newsl. 23:186-191.

GENOMIC ANALYSIS IN THE REGION OF *RHG1*, A MAJOR SOYBEAN CYST NEMATODE RESISTANCE GENE

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The genomic region near the top of soybean molecular linkage group (MLG) 'G' is important because a major gene for cyst nematode (SCN; *Heterodera glycines*) resistance is located there. This gene, *rhg1*, is found in most sources of SCN resistance and controls up to 70% of total variation in cyst index. We have analyzed this genomic region in detail by a combination of high resolution mapping, positional cloning, DNA sequencing, quantitative trait locus (QTL) mapping, and marker-assisted selection. The locus has been pinpointed to a genomic interval less than 1 cM in length using two RIL populations with more than 900 meiosis. We have identified several bacterial artificial chromosome (BAC) clones located in and around this interval, with inserts ranging from 30 to more than 120 kbp. These BAC clones group into two contigs spanning 300 to 400 kbp. Using single-pass sequencing of end- and sub-clones derived from these BACs, we sampled this genomic region for open reading frames. Of special interest is a cluster of *Pto*-like genes located less than 1 cM from *rhg1*. One of these open reading frames shows 52% amino acid sequence similarity (34% amino acid identify) to *Pto*. This gene cluster is contiguous with another cluster composed of phospholipase C genes and pseudogenes. While identifying BAC clones, we uncovered homoelogous (duplicated) BAC contigs that are presumably located elsewhere in the soybean genome. In one case, the region of homoeology between contigs was quite large — a contiguous stretch of more than 40 kbp.

In the past, SCN resistance has been associated with yield depression, possibly due to linkage drag. We have tested this hypothesis by carrying out QTL mapping with a subset of lines from the two RIL populations described above, as well as marker-selected lines derived from these populations. Field trials replicated over two locations and up to three years indicate a yield depression locus coming from PI 209332 (one of the common sources of SCN resistance) mapping in the region immediately distal to *rhg1* on MLG-G. Nearly isogenic lines (F7:9) that carry contrasting genomic segments in this region further pinpoint the yield depression locus to an interval roughly 8 cM in length.

MARKER ASSISTED SELECTION FOR SOUTHERN ROOT KNOT NEMATODE RESISTANCE IN SOYBEAN. L.R. Jakkula[1]*, M.A.R. Mian[1], J.P. Tamulonis[2], and H.R. Boerma[1], [1] Univ. of Georgia, [2] Asgrow Seed Company.

Southern root-knot nematode [*Meloidogyne incognita* (Kofoid and White)] is a major pest of soybean that causes severe yield loss in the southeastern USA. PI96354, a resistant source identified within the USDA Soybean Germplasm Collection, was shown to contain two genes for resistance, one at a major locus, *Rmi*1, and a second at a minor locus. The previously identified RFLP markers associated with these loci found on the linkage group (LG) O and G of the public RFLP genetic map explained 39% of the total variation for the galling in an $F_{2:3}$ population of PI96354 x 'Bossier'. In our current study, we used newly developed SSR markers on LG-O and -G to identify markers more closely linked to the resistant loci. Two polymorphic markers, Satt358 and Satt445, on LG-O were mapped in the progeny of the original PI96354 x Bossier cross. These markers flanked a major QTL that explained 61% of the total variation in gall number. When Satt012 and Satt288 were mapped on LG-G, a QTL was identified that explained 20% of the variation. Utilizing the flanking SSR markers on both linkage groups, we performed the marker assisted selection in an independently derived $F_{2:3}$ population of PI96354 x Bossier. We tested 96 $F_{2:3}$ lines with the four SSR markers. Lines that were homozygous within the intervals for PI96354 or Bossier alleles were screened for gall number on LG-O, LG-G, or both. The results of this marker assisted selection will be presented.

MOLECULAR MAPPING OF QUANTITATIVE TRAIT LOCI (QTL) UNDERLYING TOLERANCE OF SOYBEAN TO PHYTOPHTHORA ROOT ROT

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Phytophthora root rot (PRR), incited by the fungus *Phytophthora sojae*, is one of the most destructive soybean diseases in North America. Although resistance to a specific race of this pathogen is under the control of a single gene that fits the gene-for-gene model, field tolerance is a quantitative trait. By using bulk segregant analysis with SSR markers, three QTL associated with field tolerance to PRR were identified in a recombinant inbred population, derived from a cross between Conrad and OX760-6, which segregated for PRR tolerance. In total, the three QTL explained about 50% of the phenotypic variation in the population. Epistatic interactions between genomic regions for tolerance were identified as well. Currently, a larger population segregating for PRR tolerance is under development. This population will be tested in different locations for multiple years to confirm the QTL identified.

PROGRESS TOWARDS THE POSITIONAL CLONING OF *RPG1*, A RESISTANCE GENE EFFECTIVE AGAINST BACTERIAL BLIGHT.

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Alleles, or tightly linked genes, at the soybean *Rpg1* locus confer resistance to strains of *Pseudomonas syringae* (the causative agent of bacterial blight) that express the avirulence genes *avrB* or *avrRpm1*[1]. We have genetically mapped the *Rpg1* locus to a cluster of previously identified resistance genes (R-genes) in molecular linkage group-F (RFLP:USDA-ARS:RCS map)[2]. *Rpg1* is flanked by the RFLP markers K644 and B212, and cosegregates with the marker R45. *Rpg1* also cosegregates with nbs61, a clone sharing sequence motifs with previously characterized NBS-LRR type R-genes[2]. *Rpg1* is particularly interesting as a functionally analogous (i.e. has the same specificity) R-gene, *RPM1*, has been cloned from the cruciferous plant *Arabidopsis thaliana*. A comparison of the sequences of *RPM1* and *Rpg1* may provide insight into R-gene specificity and evolution.

A positional approach is being taken to clone *Rpg1*. We have increased our genetic resolution by prescreening for informative recombinants in a population of approximately 900 Flyer x Hartwig (FxH) recombinant inbred lines (kindly provided by David Lightfoot). BACs have been isolated with *Rpg1*-flanking markers which delimit a genetic interval of 0.3cM. The BACs have been oriented relative to *Rpg1* using the FxH informative recombinants. Interestingly, several of the BACs contain sequences displaying homology to previously isolated NBS-LRR type R-genes. Our current efforts are directed towards establishing a BAC contig encompassing *Rpg1*. Progress will be reported.

[1]T. Ashfield, N. Keen, R. Buzzell, R. Innes (1995) Soybean resistance genes specific for different *Pseudomonas syringae* avirulence genes are allelic, or closely linked, at the *Rpg1* locus. Genetics 141: 1597-1604
[2]T. Ashfield, J. Danzer, D. Held, K. Clayton, P. Keim, M.A. Saghai Maroof, D. Webb, R. Innes (1998) *Rpg1*, a soybean gene effective against races of bacterial blight, maps to a cluster of previously identified disease resistance genes. Theor Appl Genet 96 (in press)

MAPPING *Rcs*$_3$ GENE CONFERRING RESISTANCE TO FROGEYE LEAF SPOT IN SOYBEAN. T. Wang*, M.A.R. Mian, J. Alvernaz, D.V. Phillips, and H.R. Boerma, Univ. of Georgia.

Frogeye leaf spot (FLS) (caused by *Cercospora sojina* Hara) is a foliar disease of soybean [*Glycine max* (L.) Merr.] that causes significant seed yield losses in warm-humid environments of southeastern USA. The *Rcs*$_3$ gene in soybean has been reported to condition resistance to all known races of *C. sojina*. Molecular mapping of the *Rcs*$_3$ gene will be helpful in breeding soybean for resistance to FLS. The objective of this study was to map the *Rcs*$_3$ gene. Using bulk segregant analysis and resistant/ susceptible near isolines, we have identified several SSR and RFLP markers linked to the *Rcs*$_3$ gene on a 25 cM section of USDA linkage group J. The FLS resistant and FLS susceptible bulks were created by pooling the DNA of 15 resistant and 15 susceptible F_2 plants from the cross of 'Blackhawk' (susceptible) x 'Davis' (resistant and original source of the *Rcs*$_3$ gene). The Wright-*Rcs*$_3$ near isoline was created using Davis as the donor parent. Phenotypic data were collected by inoculating plants with race 5 of *C. sojina* and scoring them in the greenhouse for FLS reaction. The DNA from 96 F_2 plants segregating for the *Rcs*$_3$ gene are being scored with molecular markers on linkage group J. The results on mapping the gene will be presented.

GENETIC MAPPING OF ANTIBIOSIS AND ANTIXENOSIS RESISTANCE MECHANISMS IN SOYBEAN

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Genetic maps were constructed using restriction fragment length polymorphism (RFLP) markers in F_2 populations of crosses between the insect-susceptible soybean (*Glycine max* (L.) Merr.) cultivar 'Cobb' and three insect-resistant plant introductions (PIs): PI171451; PI227687; and PI229358. Marker data were correlated with insect bioassay results from the same crosses to identify quantitative trait loci (QTLs) for insect resistance. Bioassays were designed to separate antibiosis and antixenosis mechanisms of soybean resistance to defoliating insects. The insect species used in the bioassays was the corn earworm, *Helicoverpa zea* (Boddie). Separate QTLs for antibiosis and antixenosis were detected.

A major QTL for both antibiosis and antixenosis was associated with the PI allele near marker A584V on linkage group (LG) 'M' in the crosses Cobb x PI171451 and Cobb x PI229358. The same marker was tested in Cobb x PI227687 and was significantly associated with neither antibiosis nor antixenosis. Marker A584V on LG 'M' was the only marker which was associated with both antibiosis and antixenosis.

Markers A083I on LG 'F' and A343V-2 on LG 'P'were associated with antibiosis QTLs in Cobb x PI227687. The QTL on LG 'P' was conditioned by the PI227687 allele, while the QTL on LG 'F' was associated with the Cobb allele at that locus. In Cobb x PI229358, antibiosis QTLs were found linked to marker L002H, on LG 'G', and marker K401H, on LG 'J'. The QTL on LG 'G' was associated with the PI229358 allele, whereas the resistance allele on LG 'J' was provided by Cobb.

A minor antixenosis QTL was associated with the resistant parent allele of marker R249T on LG 'H' in all three crosses. Minor antixenosis QTLs were associated with the resistant parent alleles at A132T on LG 'C2' and Bng047D on LG 'D1' in Cobb x PI227687 and Cobb x PI229358, respectively. An antixenosis QTL was associated with the Cobb allele of marker B212V-1 on LG 'F' in Cobb x PI171451. This marker has been previously associated with QTLs for resistance to a variety of soybean pathogens including viral, fungal, bacterial, and nematode infections. Marker B212V-1 was monomorphic in Cobb x PI227687 and Cobb x PI229358 and it is possible that PI227687 and PI229358 both also possess this resistance allele. The antibiosis QTL on LG 'F' detected in Cobb x PI227687 was not linked to the antixenosis QTL found on LG 'F' in Cobb x PI171451.

MAPPING OF QTL FOR TOLERANCE TO SOIL WATERLOGGING IN SOYBEAN. T. T. VanToai[1]*, G. Boru[2], K. G. Lark[3], and S. K. St.Martin[2]. [1]USDA- ARS, Soil Drainage Research, [2]The Ohio State University and [3]University of Utah.

Soil waterlogging is a major environmental stress that suppresses soybean growth and productivity. We have identified a putative QTL associated with soybean tolerance to soil waterlogging. One hundred twenty-two recombinant inbred lines (RIL) of the Archer x Minsoy and 86 RIL of the Archer x Noir populations were grown in Columbus, OH at 40 plants per 5-ft row. The plants were subjected to soil waterlogging for two weeks at the R1 stage. Soil waterlogging was imposed by subirrigation to raise the water table to 5 to 10 cm above the soil surface. Supplemental overhead irrigation was used, as needed, to assure uniform flooding. The control plot was not flooded. Plant height and leaf greenness, determined by the SPAD meter, were taken before and right after flooding. At the end of the season, the seeds were harvested and threshed by hand. No QTL was detected for leaf greenness under flooding. One single QTL, the SAT-064 on linkage group U5, was associated with 50% of the variation in plant growth under flooded conditions. The same QTL was also associated with more than 50% of the variation in seed yields under flooding. This highly significant QTL ($p<0.0001$) is uniquely associated with flooding tolerance and is not associated with maturity, resistance to root rot disease or normal seed yields. The fact that it was found in both Archer x Noir and Archer x Minsoy populations demonstrates that it is not dependent on genetic background nor is it a statistical artifact. This QTL will be used in marker aided selection to improve soybean tolerance to soil waterlogging.

RAPID IDENTIFICATION OF SALT TOLERANCE IN SOYBEAN [*Glycine max* (L.) Merr.] USING RAPD MARKERS

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The major purpose of this study is to develop a simple method for identifying salt tolerance in soybean. The salt tolerant cultivar "Wenfeng7" was crossed with the salt susceptible cultivar "Union" and derived 200 F2 individuals. By salt tolerant identification of F2 population in the filed, we confirmed that the salt tolerance was heritable and controlled by one dominant gene. Except two bulks of Gnomic DNA from 20 F2 salt tolerant plants and 20 F2 salt susceptible plants, the other two bulks of Gnomic DNA from 10 salt tolerant cultivars and 10 F2 salt susceptible cultivars were also used for screening RAPD markers linked with salt tolerance. Only one of 167 random 10-mer Operon primers were regularly amplified two codominant RAPD markers, which was associated with salt tolerant and susceptible gene, respectively. Two codominant markers were tested within 200 F2 individuals of above cross and none recombinant was found. Each of 200 F2 individuals from other two crosses of salt tolerant cultivar and salt susceptible cultivar were identified with the same two dominant markers, only one recombinant was observed in one of the crosses. These two markers can not only identify cultivars for their salt tolerances, but also distinguish homozygous and heterozygous salt tolerant genotypes within segregating populations in soybean. Hence, it is possible to carry out marker assisted selection in developing soybean cultivar with salt tolerance. These two codominant RAPD markers have been using to analyze rice, wheat, barley, and millet for testing their association with salt tolerance.

RFLP MARKER DIVERSITY AMONG MODERN NORTH AMERICAN, CHINESE, AND JAPANESE SOYBEAN CULTIVARS. J. Alvernaz*, and H.R. Boerma, (Univ. of Georgia, Athens GA), P.B. Cregan, (USDA/ARS, Beltsville MD), R.L. Nelson (USDA/ARS, Urbana IL), T.E. Carter (USDA/ARS, Raleigh NC), W.J. Kenworthy (Univ. of Maryland, College Park MD), and J.H. Orf (Univ. of Minnesota, St. Paul, MN).

The objectives of this research were to characterize the genetic diversity of selected North American, Chinese, and Japanese cultivars and identify specific pairs of cultivars that are the most diverse based on RFLP data. We used 121 RFLP marker loci selected for uniform coverage of the soybean genome to characterize 35 North American ancestors, 66 North American modern cultivars (MG 000-VIII), 59 modern Chinese cultivars (MG 000-IV), and 30 modern Japanese cultivars (MG 0-VII). Soybean breeding in China and Japan predates North American breeding and most of these Asian cultivars have no North American cultivars as parents. RFLP loci were scored by using the same probe/enzyme/band sizes as for the RFLP marker on the USDA/Iowa State University map. Genetic distances were calculated for all 17,955 pairs of genotypes. The average GD were 0.357, 0.312, 0.323, and 0.255 for North American ancestors, North American, Chinese, and Japanese cultivars, respectively. Our data support the concept of a narrowing genetic base among modern North American cultivars when compared with their ancestors. There is less genetic diversity among Japanese cultivars than among either of the other two groups. The average GD for all possible pairings of the 66 North American cultivars with all Chinese cultivars or all Japanese cultivars were 0.348 (range 0.182 - 0.498) and 0.365 (range 0.250 - 0.493), respectively. These results show that mating a randomly selected North American cultivar with a randomly selected Japanese or Chinese cultivar should produce progeny more genetically diverse than mating any two randomly selected North American ancestors. The most diverse cross between a North American cultivar and a modern Asian cultivar would have a GD of 0.498. Combining selection for genetic diversity with selection for agronomic performance within these Asian cultivars could be a successful strategy for increasing the genetic diversity and yield of North American cultivars.

GENOMIC ANALYSIS OF *GLYCINE MAX-CERCOSPORA SOJINA* INTERACTIONS USING CONVENTIONAL DNA MARKER TECHNIQUES AND AN AUTOMATED INFRARED DNA ANALYSIS SYSTEM.

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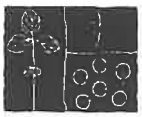
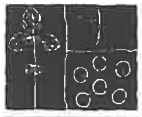
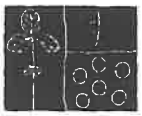
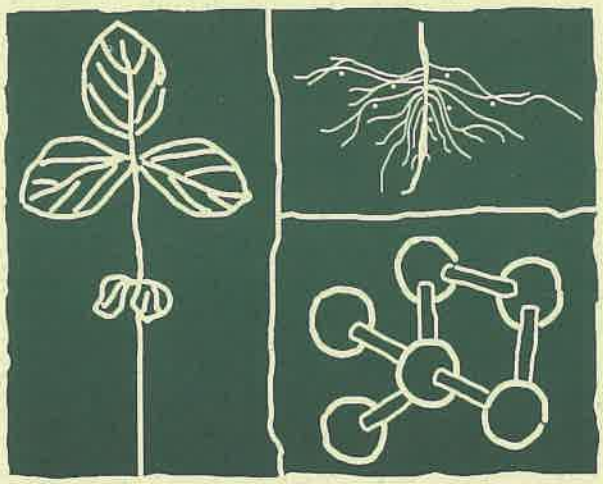
Genomic characterizations of plants and their interactive microbes including pathogens have been carried out extensively, aimed at cloning resistance genes from a target host and virulence genes from a given pathogen. Concurrent with soybean breeding programs, we are also interested in characterizing defense mechanisms of frogeye leaf spot (FLS) disease resistance, which represents an incompatible *Glycine max-Cercospora sojina* interaction in soybean. To accomplish this, our first two objectives have been to: 1) identify molecular markers tightly linked to resistance genes or loci in soybean utilizing well-defined genetic populations, and 2) determine genetic relationships among different isolates or races of the pathogen. We have developed near-isogenic lines (NILs), susceptible (S) or resistant (R) to *C. sojina* from crosses between a FLS-R cultivar 'Stonewall' and a FLS-S cultivar 'Coker 6738'. Moreover, segregating F_2 populations have been developed from a cross between a pair of R- and S-NILs. In addition, we have collected 12 single-spore isolates of the pathogen from seven locations in Alabama, Arkansas, Tennessee, and Florida; and obtained a single-leision isolate of race 5 from Georgia. Initially, conventional PCR-based RAPD, DAF, AFLP in conjunction with silver staining techniques were used to identify polymorphic DNA markers differentiating the R and the S NILs and to assess genetic relationships among the isolates. The major problem we have encountered with the conventional techniques is the time-consuming generation of data and the lack of reproducibility of the data, mainly associated with the silver staining process. Recently, an automated infrared DNA analysis system was used to perform AFLP analysis of the same soybean materials. Several putative DNA markers linked to the FLS resistance have been identified and confirmed. The automatic DNA system, in comparison with the conventional techniques, generates more rapid and reliable high-through-out data and enables eliminations of multiple data-entry steps. Comparative results, especially the AFLP data generated from both the conventional and the preferred automatic DNA system will be presented and discussed.







Soybean Cell Culture and Gene Transfer Technology



IDENTIFICATION OF CRITICAL COMPONENTS FOR SOYBEAN TRANSFORMATION

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Soybean has continued to be a recalcitrant crop to transformation due to low efficiency of gene introduction, low efficiency in selection for transformed tissues and regeneration of the transformed sectors into fertile soybean plants. The Soybean Tissue Culture and Genetic Engineering Center was initiated in 1994, with funding from the United Soybean Board to improve and utilize soybean transformation technology with a focus to: 1) Improve tissue culture methods thereby increasing the efficiency and speed of regeneration of soybean. 2) Develop methods for introduction of foreign DNA for stable gene expression in fertile regenerated soybean plants. This regional Center brought together three publicly supported university laboratories that encompass the majority of the soybean maturity groups in the United States located at The University of Georgia (PI, W.A. Parrott), University of Kentucky, and The Ohio State University (PI, J.J. Finer). The goals of The Center include evaluation, implementation and improvements in the currently available soybean regeneration and transformation systems, as well as development of new systems. The Center locations have shared information on protocols and improvements in the embryogenic suspension tissue culture systems that have led to increases in the reproducibility and shortened time in plant regeneration. Additionally, a new *Agrobacterium tumefaciens* strain, KYRT1, the use of a novel method to introduce *Agrobacterium* by the use of sonication assisted *Agrobacterium*-mediated (SAAT) transformation and improved methods for the use of the biolistic gene delivery system have aided in improved methods for transgene introduction into soybean tissues. Improvements continue to be made in the organogenic methods for soybean transformation and regeneration in addition to the established embryogenic suspension system of The Center. Evaluation of soybean genotypes, *Agrobacterium* strains and SAAT have been evaluated with the cotyledonary node and meristematic axis regeneration protocols. The latest information and protocols will be described, and can also be found at our web site: http://www.cropsoil.uga.edu/homesoybean

CURRENT PROGRESS TOWARDS AN EFFICIENT REGENERATION AND TRANSFORMATION PROTOCOL FOR SOYBEAN

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The development of regeneration systems based on repetitive somatic embryogenesis created one way to transform soybean by microprojectile bombardment. However, maintenance of embryogenic cultures was tedious, and resulting transgenic plants were frequently sterile. Collaborative work conducted by The University of Georgia, the University of Kentucky and The Ohio State University under the collective umbrella of the USB-funded Soybean Genetic Engineering Center has had the objective of increasing the efficiency of soybean transformation while keeping the technology in the public domain to the greatest extent possible. This work has resulted in substantial enhancements in the ability to recover transgenic soybean plants. Initial work characterized the growth dynamics of the somatic embryos, and correlated growth rates with transformation ability. This work also identified transformable embryos as being small and tightly packed together, much like a small raspberry. Additional results showed that embryogenic cultures growing on solidified medium with 20 mg/L 2,4-D were particularly amenable to transformation, although selection for transgenic cell lines is not overly rapid on solidified medium. Additional work has shown that embryogenic cultures could be more rapidly selected and proliferated if they were transferred to a liquid medium after bombardment. The best medium for this purpose is Finer & Nagasawa (FN) medium modified by reducing the total nitrogen level to 35 mM and sucrose to 1%. We have termed this medium Finer & Nagasawa Lite (FNL) medium. A further modification of FNL obtained by increasing the sucrose back to 3% while eliminating the 2,4-D permits the rapid recovery of desiccation- tolerant, cotyledonary-stage embryos in as little as three weeks. Furthermore, the use of liquid medium for histodifferentiation increases the number of embryos recovered per mg of tissue by sixteen-fold. This has eliminated the need for a tissue proliferation stage between the recovery of transgenic tissue and the beginning of the plant recovery process. These two factors combine to decrease the time necessary for transgenic plant recovery by as much as two months. An unexpected but added advantage is that plants thus recovered have a much lower incidence of sterility. The disadvantage to the use of liquid medium for the recovery of cotyledonary-stage embryos is that the percentage of embryos which convert into plants can be greatly lowered in some genotypes. Recent work in which the liquid medium is supplemented with 1.5-3% sorbitol is helping alleviate the problem. Current work by the three laboratories is centering on determining the range of soybean genotypes amenable to transformation with this protocol. The Soybean Genetic Engineering Center maintains a web site which details the embryogenic protocol and information pertinent to soybean tissue culture and genetic transformation. The site may be found at *http://www.cropsoil.uga.edu/homesoybean*.

TRANSFORMATION OF SOYBEAN USING ALS AND BAR GENES AS SELECTABLE MARKERS

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A soybean cotyledonary node transformation protocol has been tested using a mutant acetolactate synthase (ALS) gene and bar gene as selectable markers. Soybean genotypes 'Jack' and P9341 were used for transformation. Cotyledonary nodes from 5-6d old germinated seedlings were infected by *Agrobacterium tumefaciens* for 1h, co-cultivated in co-cultivation medium containing acetosyringone for 3d, and transferred to selection I medium containing 2 mg/l BA for 14d. The concentration of the selective agents were 10-15 mg/l Arsenal or Cadre for the ALS selectable marker and 10 mg/l glufosinate for the bar selectable marker. Explants were then transferred to selection II medium for further selection. At this time, the concentration of the herbicides were half that of selection I medium. After selection in this medium for 2-3 times at intervals of 14d, the explants were transferred to shoot elongation medium amended with herbicides. The elongated shoots were transferred to rooting medium. Two glufosinate-resistant events and five imdazolinone-resistant events has been transferred to the greenhouse. Resistance was supported by callus growth on glufosinate-containing medium for the bar selectable marker and leaf painting assay with Cadre in the case of the ALS gene. Southern blot and progeny analysis are under way to confirm these transformation events.

RECENT PROGRESS IN PARTICLE GUN-MEDIATED TRANSFORMATION OF EMBRYOGENIC CULTURES J.J. Finer*, Department of Horticulture & Crop Science, Ohio State University/OARDC, Wooster, OH 44691

Although soybean can be successfully transformed using a number of different methodologies, many laboratories have adopted the cotyledonary node system and transformation of proliferative embryogenic cultures. Unfortunately, even these more "reliable" transformation systems for soybean suffer from low efficiency, slow clone recovery time, sterility of regenerated plants and cultivar specificity. This laboratory originally developed and, with laboratories at the University of Kentucky and The University of Georgia, has made significant progress in refinement of particle gun-mediated transformation of proliferative embryogenic cultures. Embryogenic suspension cultures of soybean can be very efficiently transformed but suspension cultures have typically been very difficult to establish. In addition, problems of cultivar specificity, sterility of regenerated plants and a time requirement for establishment of transformation competency have made this system quite problematic. Over the past year, we have refined particle bombardment-mediated transformation of proliferative embryogenic cultures in an attempt to completely avoid the use of the liquid suspension cultures. The target tissue for this transformation system is proliferative embryogenic cultures maintained on a medium containing 20 mg/l 2,4-D (D20). D20 tissues are bombarded and placed on solid medium containing hygromycin for selection. Recovery of transgenic clones is efficient, possibly as a result of the slow selection process. Selected D20 tissues have been recovered from a variety different cultivars and with a number of different transgenes (see also Brzozowski *et al.*, this meeting). Regenerated plants, grown under greenhouse conditions, are fertile.

TRANSGENIC FERTILE PLANTS OF BRAZILIAN SOYBEAN CULTIVARS OBTAINED FROM BOMBARDED EMBRYOGENIC TISSUE. A. Droste[1,2], G. Pasquali[3] and M.H. Bodanese-Zanettini[1*]. [1]Depto. de Genética, [3]Centro de Biotecnologia do Estado do Rio Grande do Sul e Depto. de Biotecnologia, IB-UFRGS, Caixa Postal 15053, CEP 91501-970, Porto Alegre; [2]Setor de Botânica, CCS, Universidade do Vale do Rio dos Sinos, Caixa Postal 275, 93022-000, São Leopoldo, RS, Brazil.

A faster method for obtaining transgenic fertile plants of Brazilian soybean cultivars based on bombardment of embryogenic clumps by a low helium pressure gun was described. Proliferating embryogenic tissues developed from cotyledon halves of immature seeds from cultivars BRAGG and IAS5 on induction medium were subcultured every 14 days on proliferation medium. Two independent experiments were conducted 3 and 8 months after culture initiation. Tissue clumps containing embryos at globular stage were bombarded with tungsten particles coated with plasmid pGusHyg, containing the *gusA* gene as a reporter and the *hpt* (hygromycin phosphotransferase) gene as a selectable marker. Bombarded embryogenic clumps were mantained on proliferation medium for 10 days. After this period, the tissues were transferred to proliferation medium containing hygromycin. Subcultures were performed every 14 days. Three months after bombardment, peaces of green tissue were selected and subcultivated on hygromycin-proliferation medium. Transient GUS expression was evaluated. Hight frequencies of blue foci were obtained for the two cultivars. At the second experiment, we found a decrease of 61% for cv. BRAGG, possible caused by the long time of culture. Many stable transgenic clones were obtained and all proliferating transformed tissue and histodifferentiated embryos displayed consistent GUS activity. Morphological classes and percentages of these embryos were the same as those found for *in vitro* culture without transformation. All the recovered plants expressed GUS activity at their roots, flowers and seeds. Southern blots are being performed.

Financial Support: UNISINOS/FUNDEPE, FAPERGS, FINEP, CNPq, CAPES.

TOWARD APPLICATION OF THE *AGROBACTERIUM*-MEDIATED *ARABIDOPSIS* GERM-LINE TRANSFORMATION METHOD TO SOYBEAN.

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We are attempting to develop a new method for soybean transformation that uses the *Agrobacterium*-mediated 'germ-line transformation' approach (also known as the 'floral dip,' or 'vacuum infiltration' method). This method has been a tremendous success with *Arabidopsis thaliana*, allowing genetic transformation of that plant species with minimal expense, labor or expertise and without use of tissue culture. At present, the germ-line transformation procedure has only been reported to work with *Arabidopsis*. We are attempting to apply the method to soybean and are simultaneously examining the basis of the successful method in *Arabidopsis*. For soybean, we have inoculated several thousand mature flowering plants with *Agrobacterium* containing either the selectable marker BAR (resistance to Liberty herbicide) or the screenable marker GUS (blue staining). To date, we have harvested and tested more than 100,000 seed but have yet to recover any confirmed transformants. In our work with *Arabidopsis*, we have tested specific components of the transformation method and have significantly streamlined the procedure. Transformation rates of greater than 2% of all progeny seed tested can be obtained by simple dipping of *Arabidopsis* flowers into *Agrobacterium* solution. Transformation apparently occurs after developmental divergence of male and female gametophytic tissues in the flower, and we have used both microscopy and targeted inoculations to determine that transformation occurs in female floral tissues. Additional work on the physical site of transformation and the genetic target of transformation will be presented.

GENOTYPIC FACTORS AFFECTING RESPONSE TO *AGROBACTERIUM* -MEDIATED TRANSFORMATION OF SOYBEAN.

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Soybean transformation efficiency was evaluated using a sonication assisted *Agrobacterium*-mediated transformation (SAAT) protocol, three dissimilar *A. tumefaciens* strains, and explants derived from 28 diverse cultivars and/or genotypes of soybean [*Glycine max* (L.) Merr.] in cotyledonary node and proliferative embryogenic culture protocols. Cotyledonary node explants were evaluated at 10 and 45 days after co-cultivation for transformation with a binary vector containing both a GUS-intron gene and an NPTII selectable marker. The *A. tumefaciens* strain KYRT1 was determined to be superior to strains EHA 105 and LBA 4404 for stable transformation of soybean cotyledonary node explants, as measured at the terminal evaluation point . Strain effects within the embryogenic culture system were less significant. SAAT did not significantly increase stable transformation at 45 days post-transformation in cotyledonary nodes. SAAT was determined to significantly decrease cotyledonary node shoot proliferation of some genotypes but it is unclear what effect that this may have on the overall recovery of transformed shoots. Significant differences were also detected between genotypes for transformation and shoot proliferation frequency.

FACTORS INFLUENCING TRANSIENT EXPRESSION OF THE GUS GENE IN IMMATURE COTYLEDONS OF SOYBEAN(*Glycine max* L.)

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Factors affecting transient expression of the GUS gene in immature cotyledons of soybean were investigated using the *Agrobacterium*-mediated transformation system. The results showed that cotyledons isolated from larger embryos (6-7mm in length) exhibited the highest level of GUS gene expression. Bisected cotyledons expressed higher levels of GUS activity than whole cotyledons. A 4 day co-cultivation period increased GUS expression 2.5 fold in cotyledon halves over that seen for 3 days of co-cultivation. Significant differences in levels of transient GUS expression in cotyledons were found among 5 diverse soybean genotypes with the soybean line OAC-93-05 showing the highest GUS expression. Significant differences in the levels of GUS expression were also found in immature cotyledons infected with different *A. tumefaciens* strains and vector combinations. Strain EHA101/pIG121-Hm gave the highest level of GUS expression in immature cotyledons of all soybean genotypes tested. In addition, significant interactions between genotypes and *A. tumefaciens* strains were also found, with soybean line OAC-93-05 and *A. tumefaciens* strain EHA105/pIG121-Hm being the best combination for genetic transformation using immature cotyledons as the target tissue.

OPTIMIZATION OF GENETIC TRANSFORMATION OF SOYBEAN BY PARTICLE BOMBARDMENT OF EMBRYONIC AXES.

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A shoot regeneration system using proliferating embryonic axes without intermediate callus was evaluated for soybean transformation by particle bombardment. Particle bombardment was carried out using 1.0 or 1.6 µm gold particles at 1350 psi pressure. Ten diverse cultivars and/or genotypes of soybean [Glycine max (L.) Merr.] were evaluated for their ability to produce shoots on three different B5 media containing 5 µM, 15 µM and 50 µM 6-Benzylaminopurine (BAP), respectively. Two other media containing 1 µM indole-3-butyric acid (IBA) with 5 µM or 50 µM BAP were also used. The medium containing 15 µM BAP yielded the most shoots with reduced number of shoots on 50 µM and 5 µM BAP, respectively. Among the ten genotypes evaluated, Stonewall and Williams performed the best in producing a greater number of shoots. Two constructs containing the zein storage protein and bean pod mottle virus coat protein genes were used for particle bombardment of the embryonic axes. Several plants were generated after particle bombardment and these are presently being evaluated for the presence of the transgenes.

JELLY FISH (*AEQUOREA VICTORIA*) GREEN-FLUORESCENT PROTEIN (GFP) IN DEVELOPING AN *IN SITU* COMPLEMENTATION SYSTEM IN SOYBEAN.

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We are interested in studying the recognition and signal transduction processes in the soybean-*Phytophthora sojae* interaction. As a first step towards understanding these processes we are in the process of cloning the *Rps1*-k gene that confers race-specific resistance of soybean against the fungal pathogen *P. sojae*. Map based cloning approach has been applied in isolating this gene. Last step in a map-based cloning approach is the gene identification process. The BAC contig construction process for the *Rps1* region is nearly completed. We may find many ORFs in the BAC contig carrying the *Rps1*-k gene. To reduce the amount of work at the stable transformation step, it is necessary to evaluate these ORFs in an *in situ* complementation system. The putative cDNA/ORF showing complementation in an *in situ* system can then be further characterized by a stable transformation procedure.

Here we report the use of jelly fish (*Aequorea victoria*) green-fluorescent protein (GFP) in developing an *in situ* complementation system. The main advantage in using this reporter gene in tagging transformed cells is that one can identify transformed cells just by visualizing alive cells under an epiflurescence microscope (Haseloff and Amos 1995). We have transformed etiolated hypocotyls of both resistant and susceptible cultivars by bombarding the plasmid pRTL2-GFP carrying the *gfp* gene under cauliflower mosaic virus 35S promoter with a PDS 1000/He particle gun (Bio-Rad). In the following day these hypocotyls expressing GFP were inoculated with *Phytophthora sojae* zoospores, and infected tissues were examined for hypersensitive cell death. Presumably in the resistant cultivar there will be more cell death than that in the susceptible cultivar following infection. Once the parameters for this *in situ* complementation system is optimized, the candidate cDNA/ORF and pRTL2-GFP could be co-transformed into hypocotyls of a susceptible cultivar. The candidate gene that produces the most hypersensitive cell death among the GFP positive cells should be the candidate *Rps1*-k gene, and can be further evaluated in a stable transformation system.

IMPROVED REGENERATION FROM COTYLEDONOUS NODES OF SOYBEAN (*GLYCINE MAX* L.) USING THIDIAZURON OR COCONUT WATER.

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An efficient system for soybean regeneration from cotyledonary nodes of cv. PI 9341 was established. Thiadiazuron (TDZ) induced adventitious shoot/bud structures more efficiently than N^6-benzylaminopurine (BAP). The optimal TDZ concentration for shoot organogenesis from cotyledonary nodes of cv. PI 9341 was 0.5 mg/l. Coconut water was also found to be useful in stimulating shoot organogenesis efficiently from cotyledonary nodes. Effect of other factors including surfactant (Pluronic F-68), casein hydrolysis, the reduced nitrogen nutrient in culture medium, other hormone combinations on regeneration from cotyledonary nodes were also investigated. However, these factors failed to enhance regeneration from cotyledonary nodes. The regeneration protocol using thiadiazuron or coconut water can thus be applied in the production of transgenic soybean plants.

INTEGRATED BOMBARDMENT & *AGROBACTERIUM* SYSTEM: AN ALTERNATIVE METHOD TO OBTAIN TRANSGENIC SOYBEAN.

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In an attempt to establish a soybean transformation method that couple the advantages of microparticle bombardment and the *Agrobacterium* system, we transformed proliferating embryogenic tissue from Brazilian cultivars BRAGG and IAS5. Somatic embryos obtained from cotyledon halves of immature seeds on induction medium were mantained on proliferating medium to allow proliferation of embryogenic clumps, with subcultures every 14 days. Two independent experiments were conducted 3 and 8 months after culture initiation. Prior to *Agrobacterium* inoculation, dishes containing 10 embryogenic clumps were bombarded with tungsten particles without DNA to wound the embryos. The tissues were co-cultivated with the disarmed *Agrobacterium* strain LBA4404:pTOK233, which contain the *gusA-intron* as reporter gene and the *hpt* gene as selectable marker. Treated embryogenic clumps were mantained for 10 days on proliferation medium containing cefotaxime and were than transferred to proliferation medium containing hygromycin and cefotaxime, with subcultures every 14 days. Evaluation of transient GUS expression was performed 48 h after co-cultivation showing a high frequency of blue foci. Differences among the two cultivars were found, BRAGG showing about 70% and 90% more foci than IAS5 in the two experiments, respectively. In the second experiment, we found a decrease of about 35-45% in the number of blue foci in both cultivars. After 2-3 months on selection, green peaces of proliferating tissue were isolated and individually cultured. These clumps were GUS positive and Southern blots are nowadays being performed.

Financial Support: UNISINOS/FUNDEPE, FAPERGS, FINEP, CNPq, CAPES.

MICROSPORE-DERIVED EMBRYO DIFFERENTIATION IN SOYBEAN: A STUDY OF COLCHICINE EFFECTS. E. Kaltchuk-Santos*, L. C. Oliveira and M.H.Bodanese-Zanettini. Depto. de Genética, IB-UFRGS, Caixa Postal 15053, CEP 91501-970, Porto Alegre, RS, Brasil.

Although anther culture is a technique currently used in breeding program of different species there are still some recalcitrant crops, like soybean, that exhibite barriers to induce embryogenesis. Studies have showed that the microtubule inhibitor colchicine promoted embryogenesis from cultured anthers, both with regard to the number of symmetrically dividing microspores and the number of embryos induced. The present experiments were performed to determine the effect of colchicine on androgenetic pathway of soybean pollen and try to improve the efficiency of embryo induction. In the experiment of 1997, soybean anthers were incubated from 24 to 72 hours in induction media containing 4 different colchicine concentrations (colchicine free, 0.5%, 1.0% and 2.0%). The experiment of 1998 year had the same design but used the following colchicine concentrations, 0%, 2.5% , 5.0% and 10.0%. Some anthers were fixed in Carnoy solution at 0, 5, 10 and 15 days of culture for cytological analysis. The binucleated symmetrical pollen and multinucleated pollen were stained and counted. The scoring of callus and embryos formed *in vitro* was done 30 and 60 days after inoculation, respectivelly. The results of both tests didn't show any clear tendency for the colchicine treatments to be significantly better than their controls. Concerning to the number of microspores entering symmetrical division when different levels of colchicine were supplied to cultured anthers, the increase was not so evident as expected; although, the highest frequency (25%) was found at the colchicine treatment of 2% for 72 h and the lowest one was at 0.5% for 72h (0%). In regard of to embryo induction, the best results were obtained in 1998 and the two highest frequencies of embryos were observed with 5% and 0% of colchicine during 24h (8.23% and 7.95%, respectively). Embryos obtained from induction medium showed histodifferentiation after transfer to maturation medium. The histodifferentiated embryos resemble the somatic ones obtained from cotyledons of soybean at the same stage. The androgenetic embryos were classified into morphological classes according to Santos et al. (1997; Plant Cell Rep. 16:859-864) and transfered to germination medium. Embryos that reached this stage were maily that induced in colchicine media. Just two embryos came from the control medium. This result suggests that colchicine might be a promoter of soybean embryogenesis.

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ENHANCED SHOOT PRODUCTION OF PRIMARY-LEAF-NODE CAULOGENIC SYSTEM AND OPTIMIZATION FOR *AGROBACTERIUM* AND BIOLISTICS MEDIATED GENE TRANSFER USING TRANSIENT GUS EXPRESSION ASSAY.

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The Primary-Leaf-Node (PLN) organogenic system of soybean is optimized for enhanced shoot production. Of the several growth regulators and combinations investigated using MS medium on this system, Thidiazuron (TDZ) 0.04 mg. L^{-1} produced 67.9 $\pm$ 1.6, and 53.8 $\pm$ 4.2 shoots in the Jack, and Peking, cultivars which is 72.8% and 82.5% more than the shoots produced using Kim's medium ([1]Kim et al., 1994) respectively. Dwight and Peking cultivars produced 65.4 $\pm$ 1.5 and 50.9 $\pm$ 4.9 shoots in the combination treatments in MS medium with TDZ 0.02 mg. L^{-1} + Kinetin (KIN) 1 mg. L^{-1} + indolebutyric acid (IBA) 0.2 mg. L^{-1} which is 39.1% and 89.2% higher than the shoots produced in Kim's medium. Although Proline at 1 g. L^{-1} increased shoot production in independent treatments, it does not promote shoot growth in combination treatments. Adding allantoin (1g. L^{-1}) does not control the excessive production of non regenerable callus produced from the cut end of the PLN explants. This caulogenic system provides a continuous regeneration system that could be maintained over long term without losing the regenerability. Shoots are harvested when they are ~2.5 cm tall, rooted in MS hormonal free medium and transferred to greenhouse after acclimation. The plants set flowers and regular pods with normal seeds. Using the shoot regenerating clumps derived from PLN the DuPont/ Biorad (PDS1000/He) gun parameters for the optimum transient *uidA* gene expression were established. The highest transient *uidA* expression was observed in clumps placed on Petri plates with ~2mm thick 0.7% agar, 1100 psi pressure, 7 mm flying distance and 9 cm target distance with 1 µm dia. gold particles. Freshly isolated PLN from 7 d old axenic seedlings with a 3 d rather than 5d co-cultivation period appear to be more ideal for *Agrobacterium* infections. Attempts are in progress to transform the PLN caulogenic system using both biolistic and *Agrobacterium* mediated gene delivery.

[1]J. Kim, E. Hack and C. E LaMotte (1994). Synergistic effects of proline and inorganic micronutrients and effects of individual micronutrients on Soybean (Glycine max) shoot regeneration in vitro. J. Plant Physiol. 144. 726-734.

BIOLISTIC-MEDIATED TRANSFORMATION COUPLED WITH ORGANOGENIC REGENERATION OF SOYBEAN. Nancy A. Reichert*, Yinghui Dan[$], and Lynette L. McDougald. Mississippi State University, Department of Plant and Soil Sciences, Box 9555, Mississippi State, MS 39762. [$]Current address: Monsanto Company, 700 Chesterfield Parkway North, St. Louis, MO 63198. Email: <nreichert@onyx.msstate.edu>

A unique, genotype-independent organogenic regeneration protocol was developed for soybean using hypocotyl sections excised from seven day old seedlings (Dan and Reichert, 1998). In all 13 genotypes tested, adventitious shoots arose from the acropetal end of the hypocotyl sections after culture on a medium containing 5.0 μM 6-benzyladenine (BA). Explant responses ranged from 72 - 95%. Shoots were elongated on a medium containing 0.36 μM BA with excised shoots rooted on media containing 12.5 - 29.2 μM indole-3-butyric acid. The regeneration protocol took an average of 12 - 13 weeks. No aberrant phenotypes were noted among regenerants or their progeny. The regeneration protocol was combined with an optimized biolistic-mediated transformation protocol using the PDS-1000/He apparatus. Hypocotyl explants were bombarded twice with plasmid pAHC25 DNA [contained chimeric β-glucuronidase (GUS) and *bar* (phosphinothricin resistance) genes] coated onto 1.0 μm gold microprojectiles at a helium pressure of 1350 psi. Post-bombardment, tissues were placed on media described above which also contained 3.0 - 5.0 mg/l Ignite (a phosphinothricin-based herbicide). Transient and stable expression of GUS was determined histochemically. Explant responses for all 13 genotypes in transient GUS assays were 100%. Stable GUS expression was noted in regenerating tissues and leaves excised from developing shoots. Putative transformants will be analyzed for presence of the introduced genes via PCR analyses.

Dan, Y., and N.A. Reichert. 1998. Organogenic regeneration of soybean from hypocotyl explants. In Vitro Cell. Dev. Biol. 34P:14-21.

MANIPULATION OF OSMOTIC POTENTIAL TO IMPROVE GERMINATION AND CONVERSION OF SOYBEAN SOMATIC EMBYROS,

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The utility of somatic embryo-based plant transformation systems ultimately depends on the ability to recover fertile plants. The histodifferentiation and maturation of soybean somatic embryos in suspension cultures expedites the recovery of mature embryos, thus reducing the deleterious effects of prolonged culture on fertility. However, germination and conversion frequencies of these embryos tend to be lower than for their counterparts matured on solid media. Failure of embryos matured in liquid FNL0S3 histodifferentiation/maturation (H/M) medium to convert often appears to be associated with callus development at the radicle and/or at points along the hypocotyl. H/M media must promote the accumulation of storage compounds and the acquisition of desiccation tolerance. We investigated the effects of supplementing FNL0S3 with several levels of both plasmolyzing (mannitol and sorbitol) and non-plasmolyzing (PEG 4000) osmotica in an attempt to improve conversion of embryos to plants following partial desiccation. Supplementation of FNL0S3 with 1.5 or 3% sorbitol, or with 5 or 10% PEG 4000 significantly improved embryo germination and/or conversion frequencies, but did not prevent callus formation on certain embryos. Addition of 3% mannitol failed to improve response, and addition of any of the osmotica generally resulted in a decrease in fresh weight of mature embryos. The failure of embryos to germinate and convert appears to be due primarily to damage incurred before, during, or after desiccation, and we are now trying to optimize both osmotic conditions and procedural modifications to the standard maturation and germination protocols to reduce the incidence of callus formation and accompanying embryo disintegration. We have also found some evidence for genotypic differences in response.

***Agrobacterium-Mediated* Genetic Transformation of soybean with phenylpropanoid metabolism genes.**

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Genetic modification of Soybean plant was performed in order to alter the cell wall phenolic composition aimed at understanding the biological importance of wall phenolics and enhancing resistance to fungal pathogens and the soybean cyst nematode (*Heterodera glycines*). Since many phenolic compounds have anti-pathogen activity, the goal of this study is to transform soybean with several genes that will modify cell wall phenolics and estimate how it will affect soybean root resistance to pathogen infection. Various binary vectors containing phenylalanine ammonia-lyase (*PAL*) or cinnamyl alcohol dehydrogenase(*CAD*) genes under control of the CaMV 35S as well as *npt*II gene encoding kanamycin resistance under control of nopaline synthase promoter have been constructed. In preliminary experiments we have developed *Agrobacterium rhizogenes* strain K599- mediated soybean transformation. Four cultivated soybean genotypes, Peking, Macon, Williams 82 and Savoy, were used for transformation in this experiment. Kanamycin selection was found to be very efficient in our system of hairy roots transformed with *A. rhizogenes* strain K599 containing pBI121 and pBIN m-*gfp*5 ER GFP5 binary vectors which confer kanamycin resistance encoded by *npt*II gene. Kanamycin resistant soybean roots were screened by PCR for the presence of foreign genes. *PAL* and *CAD* activities were analyzed in transformed and untransformed soybean roots. It was found that the PAL activity level varied in a considerable range in transgenic material, being a t the level of control plants in many samples tested. The PAL activity data of other samples will be discussed in connection with cell wall phenolic alterations in transgenic roots.

SOYBEAN CHLOROPLAST TRANSFORMATION WITH CHLAMYDOMONAS RUBISCO GENES

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Ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) is the key enzyme that determines the rate of photosynthesis. Compared with some other species, soybean Rubisco has a low efficiency of net photosynthesis under current atmospheric CO_2 conditions, and the relative efficiency will be even lower in the increased CO_2 levels projected in near future. One way to improve the photosynthetic efficiency and thus increase the production of soybean is to replace the soybean Rubisco proteins with a "better" enzyme from another species. We are trying to genetically modify soybean by chloroplast transformation. We attempt to transfer Rubisco-coding genes for both large and small subunits (rbcL and rbcS) from the alga, *Chlamydomonas reinhardtii,* into the soybean plastid genome, so that the soybean Rubisco enzyme would be replaced by *Chlamydomonas*. The successful transformation of the soybean chloroplasts with the *Chlamydomonas* Rubisco could potentially increase the photosynthesis rate by 7~21%, and result in a significant increase in soybean yield. The manipulation of plastid genome may also provide a powerful tool for investigation of plastid gene regulation and interaction of nuclear and organelle genomes. We have completed several transgene constructs for soybean chloroplast transformation. The coding sequences of a selectable marker gene, bacterial aminoglycoside 3"-adenyltransferase (conferring resistance to spectinomycin and streptomycin), *Chlamydomonas* rbcL and rbcS without transit peptide sequence were fused with soybean rbcL promoter and terminator. Soybean plastid atpβ/ε gene and rbcL 3'-noncoding region were used as flanking sequences for gene targeting. Different lengths of the soybean rbcL 5'-noncoding regions were used as promoters for *Chlamydomonas* rbcS gene in these constructs, in order to study the functionality of the rbcL promoter. Soybean (*Glycine max* L., cv. Jack) embryogenic tissues from suspension culture were bombarded with gold particles coated with the DNA of these transgene constructs using the Biolistic PDS 1000/He System. These tissues were cultivated in a suspension medium containing spectinomycin, and resistant embryos were selected. Preliminary PCR analysis showed the presence of *Chlamydomonas* rbcL and rbcS coding sequences in the putative transformed soybean embryos. We are now continuing the selection and multiplication of transformed embryos and attempting regeneration of the putative transformants for further analysis.

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BIOLISTIC TRANSFORMATION, EXPRESSION, AND INHERITANCE OF BOVINE β-CASEIN IN SOYBEAN

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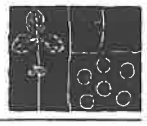
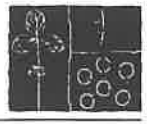
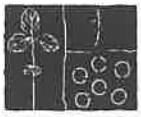
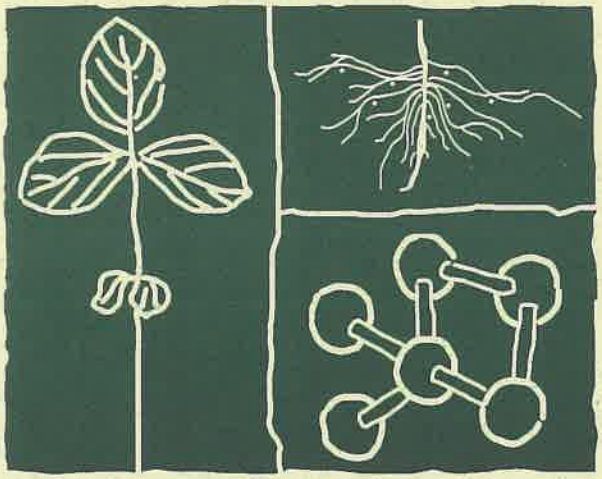
A 630 bp fragment encoding a 28 kD bovine milk protein β-casein was cloned into a seed specific lectin promoter expression cassette and introduced into soybean somatic embryos via particle bombardment. Four hygromycin resistant embryogenic cell lines were selected. All four lines were PCR and Southern blot positive for the β-casein gene, however, only one line did not show extensive rearrangement of the b-casein gene and/or lectin promoter. The presence of the β-casein gene in the genome of the four plants regenerated from this culture was confirmed via Southern blot hybridization analysis. Gene copy experiments and progeny inheritance analysis indicate that the plants likely contain four to eight tandem copies of the β-casein gene and that the insertion occurred at a single genetic locus. Bovine β-casein mRNA was highly expressed in developing cotyledons. A very low level of β-casein mRNA was found in leaf tissues although endogenous lectin transcripts are not found in the same samples. Bovine β-casein protein was highly expressed in cotyledons of transformed plants, but was not detectable in leaf tissues of transformed plants by Western blot analysis. Bovine β-casein produced in transgenic plants migrated as multiple bands very similar to pure bovine β-casein, with a main band at approximately 28 kD. This experiment represents the first report of the expression of a milk protein in soybean and opens the way for the general improvement of protein quality in soybean directed by seed specific promoters.







Soybean Biochemistry



THE SOYBEAN SEED COAT
1. EARLY DEVELOPMENT OF THE SEED COAT

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During the first three weeks of development, the soybean seed coat undergoes a dramatic increase in complexity, as various tissues that are not evident in the mature seed coat appear and subsequently disappear or are modified. Immediately after anthesis, the seed coat is a relatively simple organ, consisting of a cuboidal epidermis, several layers of undifferentiated parenchyma, and a cuboidal endothelium. Starch granules are present throughout the undifferentiated parenchyma, but not in the epidermis or endothelium. No lipid or protein bodies are detectable in the seed coat at this time. In fact, at no stage in development were protein bodies detected in the seed coat. By three days post anthesis (dpa), the simple parenchyma layer has differentiated into an outer integument of thin-walled cells, containing vascular tissue in the sub-hilar region, and an inner integument, consisting of up to 6 layers of deeply-staining thick-walled cells. Starch is present in both the inner and outer integument, and small amounts in the endothelium, but not in the epidermis. There is still no lipid present in the seed coat proper, but a few small droplets are detectable in the funiculus, adjacent to the as yet undifferentiated hilum. By 12 dpa, the hourglass cells are starting to differentiate from the hypodermis near the top, or hilum region, of the seed; development is not as far advanced at the bottom of the seed. The outer integument has differentiated into two layers: an outer layer of thin-walled parenchyma containing a prominent vascular region, and an inner layer of thick-walled parenchyma. The inner integument is becoming compressed and crushed. Starch is found throughout the thin- and thick-walled parenchyma, but not in the vascular region. Small amounts of starch are now visible in the palisade layers of the hilum, in the hypodermal layer that is starting to differentiate into hourglass cells, and some very tiny granules in the epidermis around the periphery of the seed coat. In the hilum region, the counter-palisade is rich in lipid, as is the endothelium on the inside of the seed coat; lesser amounts are seen in the thick walled parenchyma. By 18 dpa, differentiation of the macrosclereids (palisade layer) and the osteosclereids (hourglass cells) is complete. Small starch granules are abundant in both of these layers. The outer, thin-walled parenchyma is starting to take on the characteristics of aerenchyma, and there are small starch granules throughout this layer, but not in the thick-walled inner layer. The inner integument has become completely stretched and crushed, leaving a single, deeply staining wall layer directly above the endothelium. The hilum region contains a well-developed counter-palisade, and a tracheid bar. There is virtually no lipid left in the seed coat, except in the endothelium. By 45 dpa, although the seed itself is not yet mature, the seed coat has reached maturity, with the palisade and hourglass cells being prominent. A small amount of aerenchyma remains, and the endothelium is also still distinct.

THE SOYBEAN SEED COAT
2. DIFFERENTIAL EXPRESSION OF SEED COAT-SPECIFIC GENES DURING SEED DEVELOPMENT

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Despite the importance of the seed coat, there is very little information in the literature concerning genes expressed in the seed coat layers and their impact on seed development. Our lab has isolated a number of cDNA clones from a seed coat library by differential screening. Northern blot analysis of various soybean plant organs showed that two of these cDNA clones SC4 and SC20 were expressed in seed coat tissue and not in embryo, leaf, stem, root, pod or flower tissues. This organ-specific expression was confirmed by *in situ* hybridization of soybean pod and seed tissues. In addition, *in situ* hybridization analysis was carried out on various developmental stages of soybean seed to demonstrate the spatial and temporal gene expression patterns of the seed coat clones. SC4 was expressed within the seed coat from 3 days post anthesis (dpa) throughout the inner integument, however, from 6 dpa to 18 dpa the gene expression was demonstrated to have moved to the outer integument layer of the seed coat. SC20 was determined to be expressed from 12 dpa to 18 dpa and the expression was localized to the thick-walled parenchyma layer of the seed coat. Another of our cDNA clones, SC21, which does not show organ-specific expression, has also been shown to have tissue-specific expression within the seed coat. *In situ* hybridization has localized gene expression to only the thin-walled parenchyma of the outer integument of the seed coat from 3 to 24 dpa, which is as far as we have investigated developmentally. Southern blot analysis of different plant species has shown that both SC4 and SC20 are specific to soybean. It was also determined that SC4 is a single gene and that SC20 is a member of a small gene family. Sequence analysis of the two cDNA clones has revealed that the putative protein encoded by SC4 exhibits considerable similarity to RD22, an *Arabidopsis thaliana* drought-induced protein, and the putative protein encoded by SC20 has similarity to subtilisin-like proteases, containing the conserved catalytic serine residue. The corresponding genomic clones for SC4 and SC20 cDNA clone have been isolated and are currently being characterized.

THE SOYBEAN SEED COAT
3. HYDROPHOBIC PROTEIN SYNTHESIZED IN THE POD ENDOCARP ADHERES TO THE SEED SURFACE

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Hydrophobic protein from soybean (HPS) is an abundant seed constituent and a potentially hazardous allergen. Reoccurring community-wide outbreaks of asthma in Barcelona in the 1980's were caused by the release of soybean dust through the unloading of seed from container vessels. These epidemics affected hundreds of individuals and resulted in several deaths, and HPS was identified as the main allergen causing the asthmatic reactions. We have isolated the *HPS* gene and studied the expression and localization of the protein and mRNA transcript. Analysis of protein extracts by SDS-PAGE shows that HPS is localized to the seed surface and is a component of the powdery bloom occurring on the surface of many soybean varieties. Isolation of the cDNA and genomic clones indicates that the *HPS* gene consists of a single open reading frame without introns and that the HPS protein is translated with a long leader sequence. The *HPS* gene is specifically expressed in the membranous endocarp of the inner ovary wall, as determined by *in situ* hybridization and by RNA blot analysis. Portions of the membranous endocarp may adhere to the seed during the course of development. The deposition of this material alters the physical properties and the composition of the seed surface. Soybean varieties with 'dull' seed coats accumulate large amounts of HPS on the seed surface when compared to 'shiny' types. Furthermore, DNA blot analysis shows that the *HPS* locus is polymorphic between 'dull' and 'shiny' varieties.

THE SOYBEAN SEED COAT
4. LOCALIZATION OF SEED COAT PEROXIDASE mRNA DURING SEED DEVELOPMENT BY *IN SITU* HYBRIDIZATION

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The soybean *Ep* gene encodes an anionic peroxidase enzyme that accumulates in large amounts in the seed coat tissues. Histochemical localization of peroxidase in mature, fully developed seed coats has shown that most activity is concentrated in the hourglass cells of the subepidermis. Also, analysis of transcript abundance by RNA blot hybridization has shown that high expression of this peroxidase is restricted to seed coat tissues. To fully characterize *Ep* gene expression, the corresponding mRNA was localized by *in situ* hybridization to seed coat sections from seeds at various stages of development. Although enzyme histochemistry has localized the *Ep* peroxidase in the hourglass cells in the mature seed coat, the gene is expressed well before these cells have formed. Expression is first detected at 6 days post anthesis (dpa), in a small area of the thin-walled parenchyma of the outer integument, adjacent to the thick-walled parenchyma just beneath the recurrent vascular bundles of the sub-hilum region. By 9 dpa, a thin band of expression extends around the seed coat, at the junction of the thin and thick-walled parenchyma. Expression shifts to the hourglass cells as they start to differentiate, at 12 dpa. In addition to the *Ep* peroxidase, expression of another peroxidase gene, *Prx2*, was also studied. Sequence analysis of the *Prx2* cDNA indicates that this transcript encodes a cationic peroxidase isozyme. The *Prx2* gene was isolated from a seed coat library by differential screening, and is highly expressed in this tissue. However, in contrast to the specific expression observed for the *Ep* peroxidase, expression of *Prx2* occurs in most tissues throughout development, as determined by RNA blot analysis. Localization of *Prx2* mRNA by *in situ* hybridization detected expression throughout the seed coat, but not the pod, from 3 dpa to 24 dpa.

GENOTYPE DIFFERENCES IN SULFUR-INDUCED SEED PROTEIN QUALITY VARIATION . D.J. Lee, R.E. Frazier, C.A. Caha and G.L.Graef. Department of Agronomy, University of Nebraska, Lincoln, NE 68583.

Seed protein quality can be improved in soybean by increasing methionine content. We were interested in determining if genetic variation existed among soybean lines in their ability to maintain protein quality under low levels of sulfur. Seed protein quality was quantified by measuring relative levels of glycinin and conglycinin protein using gel electrophoresis and scanning. Methionine content of seed meal was shown to correlate with this gel based system. Five soybean lines that were parents of high protein x high yield populations were assessed for their protein quality in both low and normal sulfur conditions in a greenhouse study. All lines had a reduction in protein quality under low sulfur but the magnitude of the reduction varied among some parents. This variation may be important in maximizing the ability of genetically modified soybean to meet increased sulfur demands in the developing seed.

DIFFERENTIALLY EXPRESSED GENES INDUCED IN RESISTANT SOYBEAN PLANTS IN RESPONSE TO SOYBEAN CYST NEMATODE, *HETERODERA GLYCINES*, INVASION

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The soybean cyst nematode (SCN), *Heterodera glycines* Ichinoe is a devastating pest of the soybean, *Glycine max* L. Merr, causing chlorosis, root necrosis, and suppressing shoot growth. We used differential display to identify genes expressed in a resistant soybean cultivar (Peking) compared to susceptible (Kent) and uninoculated plants. Gene expression was monitored 6, 12, 24, 48, 96 and 144 hours after inoculation with SCN. We identified over 50 unique DNA fragments corresponding to genes specifically induced in leaves or roots of resistant inoculated plants. We compared the DNA sequences against nucleotide and protein databases to identify putative gene function. We identified genes induced at an early stage of the infection process, such as transcription factors, nucleotide binding proteins, protein kinases and leucine-rich repeats. These genes are induced during nematode penetration and migration through the root. At a later stage, genes involved in general defense response were induced, coinciding with degeneration of the nematode feeding site. This information is useful to molecular biologists interested in designing new resistance to SCN.

THE GLUTAMINE SYNTHETASE GENE FAMILY IN SOYBEAN – REGULATION OF EXPRESSION OF THE DIFFERENT GENE MEMBERS

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Glutamine synthetase (GS) is the key enzyme in nitrogen metabolism and it catalyzes the biosynthesis of glutamine from glutamate, ATP and NH_4^+. In plants, GS is an octamer and has a native molecular weight of 320 – 380 kD. There are two major isoforms of GS, a cytosolic form (GS_1) and a chloroplastic form (GS_2). In leaves GS_2 functions to assimilate primary ammonia reduced from nitrate and also to reassimilate ammonia produced by symbiotic N_2-fixation in the nodules and by catabolism of proteins in germinating seeds. GS_1 has also been implicated in the transport of nitrogen.

Both GS_1 and GS_2 are encoded by small multi-gene families in the soybean. Based on sequence divergence in the 3 UTR, three distinct classes if the GS_1 have been identified in soybean (α, β, γ) and each class appears to have two distinct members, each member presumably having been derived from the two different ancestral plants from which the allotetraploid soybean is derived. Genomic Southern analysis suggests that the three classes of OS_1 genes are linked and may have been derived by gene duplication. The α and the β forms exhibit a more constitutive expression pattern while the γ form shows nodule-specific/enhanced expression pattern. The β forms are ammonia inducible and along with the γ GS_1 subunits assemble to form the nodule specific GS isozymes. It is interesting to note that the two members of each class of GS_1 genes show subtle differences in the expression pattern. The $\gamma 1$ gene is strictly nodule specific while the $\gamma 2$ gene member, though nodule enhanced is also expressed in the cotyledons and the flowers. Analysis of the promoter regions of the $\gamma 1$ and $\gamma 2$ genes show complete divergence and only the $\gamma 1$ gene shows conservation of cis-elements with the promoter region of the leghemoglobin gene and the *gl*$\eta\gamma$ gene of *Phaseolus vulgaris.* This would suggest divergent evolution of the promoters. Besides regulation at the transcriptional level, our data also suggests that there is regulation at the level of GS holoenzyme turnover in soybean roots and nodules. As with bacterial GS, there appears to be a two step turn over mechanism – oxidative modification of a histidine residue at the active site followed by disassembly and proteolytic turnover of the subunits. The active sight is protected from oxidative modification by the presence of the substrate at the active site.

While the major site of GS_2 expression are the green tissues, GS_2 genes are also expressed in the nodules suggesting that other triggering signals besides light might be involved in the induction of GS_2 genes. Promoter analysis of one of the GS_2 genes from the soybean shows high degree of sequence conservation with the promoter regions of GS_2 genes of other legumes.

DEVELOPMENTAL AND ENVIRONMENTAL REGULATION OF ALTERNATIVE OXIDASE EXPRESSION IN SOYBEAN

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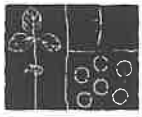
The alternative oxidase (AOX) is a non-energy conserving quinol oxidase located on the inner membrane of mitochondria of plants (and other organisms). One of its functions in plants may be the prevention of over-reduction of respiratory chain components, thereby minimising generation of reactive oxygen intermediates (ie, it is an anti-oxidant stress protein). It also allows deregulation of respiration in processes such as thermogenesis in flowers and the ripening climacteric of fruits. Soybean is unusual in that it possesses relatively large quantities of AOX which contributes substantially to the respiration of the plant (as much as 50% of total oxygen uptake). AOX expression and operation in soybean provides a model system for the study of nuclear-mitochondrion interactions and coordination of carbon metabolism in plants.

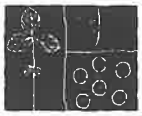
Three isoforms of AOX are encoded by three separate genes in soybean. All three isoforms have targeting presequences and are actively imported and processed in isolated mitochondria. Expression of the *AOX* gene family is regulated in a tissue-specific manner. In roots, AOX3 is the only isoform expressed. It is present as early as 4 days after imbibition and its content on a mitochondrial protein basis does not change appreciably over the first 3 weeks of growth. AOX does not contribute to oxygen consumption by very young roots but becomes progressively more active over the next 20 days. At 21 days after planting, more than 50% of total root respiration occurs via AOX. AOX2 is only expressed in shoot tissues. In cotyledons, little AOX protein is detectable 3-4 days after planting; soon after, AOX2 is synthesised and its activity can be detected in intact organs by mass spectrometry. AOX3 appears at about 7 days after planting and becomes the predominant form during cotyledon senescence. At 20 days after planting, almost 70% of the respiration of the cotyledon is via AOX, partly because of the increase in AOX protein amount but also because of a decrease in activity and content of cytochrome path components. These changes in AOX protein amounts are mirrored by changes in transcript abundance. In mitochondria from nitrogen fixing root nodules only about 10% of AOX protein is detected and this is mainly due to AOX3. Suspension cells of soybean express AOX3 strongly throughout their growth, but treatment with inhibitors of the cytochrome chain, or exposure to cold temperature or salicylic acid, induce synthesis of substantial quantities of AOX1 protein and mRNA. Taken together these results suggest that AOX3 is constitutively expressed in soybean, AOX2 is found only in photosynthetic tissues, and AOX1 appears to be stress induced.

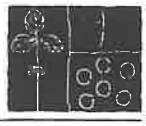
In addition to the tissue-specific expression studies, we have used mitochondria from soybean and tobacco to identify novel post-translational regulatory factors which coordinate the synthesis and activity of AOX in response to the carbon status of the tissue. We are currently attempting to place these regulatory mechanisms in a physiological context.

Reference

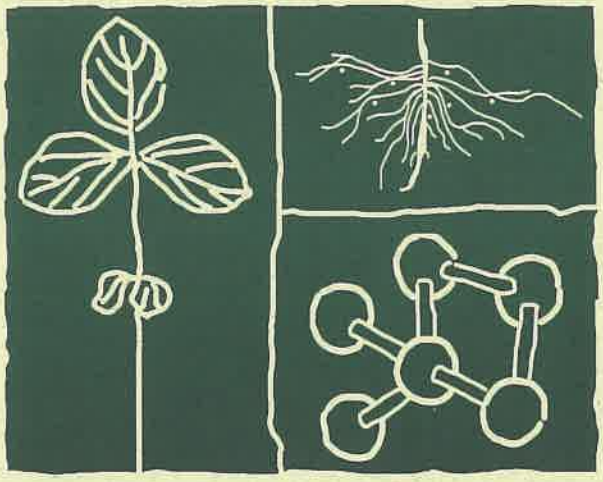
Finnegan, P. M., Whelan J, Millar, A. H. , Zhang, Q., Smith, M. K., Wiskich, J. T. and Day, D. A. 1997. Differential expression of the multi-gene family encoding the soybean mitochondrial alternative oxidase. Plant Physiology 114: 455 - 466







Soybean Molecular Physiology



NATURALLY OCCURRING DUPLICATIONS AND DELETIONS INVOLVED IN TISSUE SPECIFIC SILENCING OF A CHALCONE SYNTHASE GENE FAMILY

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The biochemical and molecular basis for the soybean *I* (inhibitor) locus that prevents pigmentation of soybean seed coats has been shown to be a cluster of at least three genes that encode chalcone synthase (CHS), a key enzyme in regulating the production of flavonoids and anthocyanins in plants[1]. This genomic region displays a series of naturally-occurring gene duplications that suppress, rather than increase, expression of the mRNAs encoded by the CHS genes in the duplications. Paradoxically, deletions of one member of the gene cluster leads to increased expression of the other members. The behavior of the duplicated CHS genes appears to be a naturally occurring example of cosuppression or homology-dependent gene silencing phenomena that have been found in transgenic plants. Methylation patterns of the CHS cluster appear constant in leaves and seed coats of the mutant lines indicating that the silencing effect likely does not involve methylation. Analysis of expression patterns in seed coats, pods, cotyledons, leaves, stems, and roots of isogenic lines shows that the silencing effect is tissue specific and occurs only in the seed coats[2]. The tissue specific silencing effect is quite unusual especially considering that multiple CHS genes are expressed in many tissues including the seed coats.

1. Todd, J.J. and L.O. Vodkin (1996) Duplications that suppress and deletions that restore expression expression from a chalcone synthase multigene family. Plant Cell 8: 687-699.

2. Chan, W-C. and L.O. Vodkin. Tissue specific gene silencing and DNA methylation patterns of a chalcone synthase gene cluster in soybean. Molecular and Cellular Biology of the Soybean, 1998 abstract, this volume.

DOWN REGULATION OF SOYBEAN VEGETATIVE STORAGE PROTEINS VIA RNA ANTISENSE TECHNOLOGY IN TRANSGENIC SOYBEANS [*GLYCINE MAX* (L.) MERR.].

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The use of RNA antisense technology to ascertain gene function has been widely applied in plant systems. Soybean vegetative storage proteins have been postulated to play a role in plant nitrogen allocation and seed protein composition. We report here the characterization of soybean lines expressing the antisense RNA of the *vspA* gene in transgenic soybeans. A construct containing a partial sequence of the *vspA* open reading frame, in antisense orientation, under the control of the 35s promoter was introduced into soybean. A total of 14 independent transgenic plants have been derived from *Agrobacterium*-mediated transformation utilizing the *bar* gene as a selectable marker. Southern blot analysis confirmed the integration of the *vspA* antisense cassette into the soybean genome in the 8 events characterized to date. Events displayed different degrees of *vspA* gene expression, as detected by Northern blot analysis. Western blot analysis was reflective of the Northern blot results. Progeny analysis has been performed on 1 event. Segregating progeny were initially screened using a leaf paint assay with a 100 mg/L solution of Liberty® and then confirmed by Southern blot analysis. Southern positive progeny displayed various levels of suppression of both *vspA* RNA and protein, while Southern negative progeny had wild type expression pattern at both RNA and protein level. Studies are underway to evaluate variations in phenotype within the soybean lines displaying *vspA* down regulation.

STRATEGIES FOR MODIFYING PHYTIC ACID CONTENT IN SOYBEAN SEEDS

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Phytic acid, the storage form of phosphorus found in plant seeds, is largely unavailable to monogastric animals and has potent antinutrient properties. The reduction of phytic acid levels in soybean meal should improve phosphorus and other nutrient availability in animal feed. With recent outbreaks of *Pfiesteria* on the East Coast, there have been heightened concerns about phosphorus run-off into critical watersheds. The availability of low phytic acid corn and soybeans should provide poultry and swine producers with additional nutrient and waste management strategies. Our laboratory is exploring two approaches to altering phytic acid content. The first project involves the degradation of phytic acid through the introduction of specific phytase genes. A phytase gene (*phy*A) from *Aspergillus niger* has been tested in soybean for correct expression and subcellular localization. Transgenic soybeans expressing the fungal phytase have been used in poultry feeding studies and showed improvement of phosphorus availability when used as a dietary supplement, similar to results with the commercially available phytase enzyme supplements (1). However, the unmodified phytase is secreted from soybean cells rather than being targeted to the site of phytic acid accumulation. To directly lower phytic acid in soybean seeds, the fungal phytase gene has been modified to include a vacuolar targeting sequence (VTS) at either the N- or C-terminus. These constructs are being used to bombard regenerable soybean cultures. A second project involves altering the biosynthesis of phytic acid. The first committed step in biosynthesis of *myo*-inositol, a phytic acid precursor, is the conversion of glucose-6-phosphate to *myo*-inositol-3-phosphate by the enzyme *myo*-inositol-3-phosphate synthase (MIPS). We are utilizing an antisense strategy to reduce the expression of the MIPS gene. We have cloned the MIPS cDNA by RT-PCR using primers designed from MIPS sequences from other organisms. We have examined MIPS expression during seed development and have constructed transformation vectors containing the MIPS sequence in both sense and antisense orientations.

References:

1. Denbow, D.M., Grabau, E.A., Lacy, G.H., Kornegay, E.T., Russell, D.R. and Umbeck, P. F. 1998. Soybeans transformed with a fungal phytase gene improve phosphorus availability for broilers. Poultry Science in press.

SAGE ANALYSIS OF IMMATURE COTYLEDON GENE EXPRESSION. James Schupp[1], Lila Vodkin[2] and Paul Keim[1]*.
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Immature cotyledons are an important stage in seed development where much of the seed composition is determined. Characterizing both the qualitative and quantitative aspects of gene expression in this organ will lead to a greater understanding of seed development and eventually to the manipulation of seed composition. We are using Serial Analysis of Gene Expression (SAGE) as a quantitative and qualitative approach for characterizing developing seed mRNA populations. Short nine nucleotide "tags" were "captured" from immature cotyledon mRNA molecules, ligated into concatamers, cloned into plasmid vectors and then sequenced. SAGE tags contain sufficient information to unambiguously identify most genes from expressed sequence tags (ESTs) type databases or they can be used to amplify cDNAs from libraries. In addition, the frequency of a specific tag in SAGE analysis is indicative of the frequency of its expression level in the total mRNA of the organ. From the cotyledon mRNA 1,512 SAGE tags identified, 849 were unique. Most of these tags were observed only once (730) but 119 were observed multiple times. Tags observed only once represent moderate or low level expression. Tags observed multiple times indicate higher levels of gene expression. A full 30% of the total mRNA from immature cotyledons was due to 30 tags. Most of these highly expressed genes could be identified (23/30) from the current Genbank database. Kunitz trypsin inhibitor-A was the most highly expressed (4.9%) gene in the cotyledon tissue. Some of the other highly expressed genes included: glycinin GY1 (3.2%), 7S-like seed storage protein (2.5%), lipoxygenase-3 (2.9%), 2S albumin (2.0%), glycinin G3 (1.8%), beta-conglycinin (1.5%), sucrose binding protein (1.3%), 7s seed storage protein (1.3%), and lectin (1%). Even though this may be the most molecularly characterized tissue in soybean, several highly expressed mRNAs (e.g. the second most frequent tag at 3.4%) were not identified by Genbank searches. We are in the process of isolating these clones by rapid amplification of cDNA ends (RACE) analysis of cDNA libraries. The discovery of novel genes is one advantage of SAGE over other global approaches to characterizing gene expression.

ISOLATION OF A SOYBEAN *MYO*-INOSITOL-3-PHOSPHATE SYNTHASE cDNA CLONE

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Phytate (*myo*-inositol hexakisphosphate), the major storage form of phosphorus in soybean seeds, accumulates in protein bodies during seed development. Upon maturity and harvest of seeds, phytate levels are at their highest. Non-ruminant animals such as poultry and swine are unable to efficiently digest and utilize the abundant phytate phosphorus in soy-based feed. When manure containing excreted phytate is applied to the land as fertilizer, it can contribute to increased soil phosphorus levels, leading to environmental phosphorus pollution. Our goal is to generate low-phytate soybeans for use as a nutritious and environmentally sound feed source. The specific objective of this project is to decrease seed phytate levels by downregulating the expression of a gene involved in phytate biosynthesis. The conversion of glucose-6-phosphate to *myo*-inositol-3-phosphate is catalyzed by *myo*-inositol-3-phosphate synthase (MIPS). The MIPS gene has been isolated from several plant sources, including *Phaseolus vulgaris, Lemna gibba, Brassica napus, and Arabidopsis thaliana.* Oligonucleotide primers were designed based on conserved regions between the plant MIPS gene sequences. RT-PCR was used to amplify a MIPS product from cDNA isolated from developing soybean seeds. RACE (Random amplification of cDNA ends) technology was used to amplify the 5' and 3' ends of the soybean MIPS sequence. The full length cDNA was amplified by high-fidelity PCR and cloned into soybean transformation plasmids in both the sense and antisense orientation. These constructs will introduced into soybean via particle bombardment. Expression of the introduced MIPS gene will be analyzed in transformed cells.

EXPRESSION AND LOCALIZATION OF FUNGAL PHYTASE IN TRANSGENIC SOYBEAN CELLS

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Phosphorus is stored in plant seeds as phytate (*myo*-inositol hexakisphosphate). Non-ruminants do not efficiently utilize phytate as a phosphorus source in soybean rich animal diets. Excreted phytate is an environmental concern that has received increased attention in light of the recent *Pfiesteria* outbreaks in the Chesapeake Bay and elsewhere. Phosphorus is released from phytate by the action of the enzyme phytase, which can be added to animal diets. Enzyme supplementation is widely used in European countries where fines are levied for excess phosporus output, but the cost of supplementation is an obstacle to widespread use in the United States. Our goal is to express a fungal phytase gene (*phy A*) from *Aspergillus niger* in the soybean seed prior to harvest to lower phytate levels. This would eliminate phosphorus or phytase supplementation and reduce the excretion of phytate into the environment. The fungal *phy A* gene, under control of the constitutive dual-enhanced 35S CaMV promoter, has been tested for expression in soybean suspension cells and resulted in secretion of the enzyme from these cells. *Phy A* has also been cloned behind the soybean ß-conglycinin seed specific promoter. In order to release phosphorus stored in the seed, phytase must be targeted to vacuoles or protein bodies where phytate is stored. To direct the fungal phytase to the vacuole, the *phy A* sequence has been further modified to contain a C-terminal vacuolar targeting sequence (VTS) from barley lectin. Transgenic suspension cells containing *phy A* with the C-terminal VTS behind the 35S promoter showed phytase activity. Southern analysis confirmed the presence of the *phy A* gene in these cells. Western analyses will be done with both cells and culture media to test for the site of phytase localization. Embryogenic cultures have been bombarded with the *phy A* gene containing the C-terminal VTS behind the seed specific promoter. Embryos from these bombardments are being selected and will be used to regenerate transgenic plants. *Phy A* has also been modified to contain an N-terminal VTS from sweet potato sporamin, under control of the 35S promoter or the seed specific promoter. These constructs will be used to bombard soybean suspension cultures and somatic embryos, respectively.

TEMPERATURE REGULATION OF TISSUE SPECIFIC CHOLINEPHOSPHOTRANSFERASE MRNA LEVELS IN DEVELOPING SEEDS

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Cholinephosphotransferase is responsible for the synthesis of phosphatidylcholine, forward reaction, and polyunsaturated diacylglycerol, reverse reaction, in developing soybean seeds. The level of this enzyme is regulated by environmental temperature during seed development. Enzyme activity is increased by growth at 20° and decreased by growth at 35°C. *In situ* hybridizations show that the levels of mRNA are also modulated in response to these same temperature changes. Developing seeds, late R4 through early R6 stages, were co-hybridized with a fluorescein-labeled antisense-CPT probe and a TMR-labeled antisense-18S-RNA probe. Single color fluorescence intensities were then captured and quantitated with a digital camera system. CPT fluorescence was normalized relative to the 18S-RNA levels in each tissue. Growth at 20°C lead to increased CPT mRNA levels in cotyledons and embryos from all developmental stages, but no clear trend was seen in integument tissues. CPT-mRNA decreased to undetectable levels in all tissues after 10 hours at 35°C. These changes mirror and antedate changes in enzyme activity.

TISSUE-SPECIFIC GENE SILENCING AND DNA METHYLATION PATTERNS OF A CHALCONE SYNTHASE GENE CLUSTER IN SOYBEAN

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The classically defined *I* locus determines seed coat color in *Glycine max* by controlling the presence or absence as well as the spatial distribution of anthocyanin pigments (flavonoid compound) in the soybean seed coat. Chalcone synthase (CHS) which is encoded by seven *CHS* gene members is a key enzyme of the flavonoid biosynthetic pathway. Soybean cultivars with yellow seed coat have either the *I* or i^i alleles at the *I* locus, and spontaneous mutations from these alleles have given rise to pigmented seed coats (*i*) which have been preserved in the form of near isogenic lines. Previous work from our laboratory established that the *I* locus is the 10-kb *CHS* duplication region containing three *CHS* genes. Analysis of spontaneous mutations of the *I* locus showed that duplications of *CHS* genes suppress *CHS* multigene expression in seed coats, while deletions restore their expression. Moreover, the suppressive effect of *I* or i^i on *CHS* expression is *trans*-dominant. This unusual regulation of the *I* locus represents a naturally occurring form of the co-suppression phenomenon, one general type of homology dependent gene silencing. Therefore, a first objective of this study was to determine whether this effect of gene silencing (co-suppression) exhibited by the dominant *I* alleles on *CHS* expression in seed coat is also present in other tissues of the soybean plant. The results of the study, performed by comparing the *CHS* mRNA level in different tissues (seed coats, cotyledons, young pods, old pods, young leaves, old leaves, stems, roots) of isogenic lines having *I* and *i* genotypes, indicate that the gene silencing effect of the dominant *I* allele is indeed seed coat specific. The phenomenon of gene silencing is known to operate either at the transcriptional or post-transcriptional level. In many cases of transcriptional gene silencing and also some instances of post-transcriptional gene silencing, the process of DNA methylation is implicated in the silencing phenomenon. Thus, a second objective of this research was to determine if this seed coat-specific gene silencing effect is correlated with the methylation state of the 10-kb *CHS* gene cluster. Using the technique of PCR-Mediated Methylation Assay (PMMA), we detected and compared the methylation states of the 10-kb cluster residents (*CHS*1, *CHS*3, *CHS*4), in alleles of the *I* locus in leaf and seed coat. The results show that the dominant form (*I* or i^i) and its mutant line (*i*) have the same basic methylation pattern with the three *CHS* genes in both leaf and seed coat. This finding suggests that cytosine methylation is not involved in the seed coat-specific gene silencing event in soybean.

ISOLATION AND TRANSGENIC ANALYSIS OF A SOYBEAN POD-SPECIFIC PROMOTER

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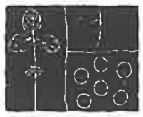
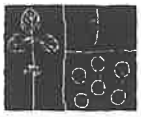
Despite an increasing number of gene sequences reported from soybean in recent years, there are not many well-defined tissue specific promoters other than those representing storage proteins of the developing seed. We are interested in promoters that can be used for disease resistance strategies in various tissues of the plant. We report the isolation of a soybean cDNA that represents a highly abundant mRNA expressed in the developing pods but not in the developing cotyledons, or in most other tissues of the plant. Database searches shows that this gene belongs to a low-copy family of fruit specific genes for which the functions of the gene products are unknown. Southern blots indicated that there are probably only two copies of this or a related gene in soybean. A 16 kb genomic clone was isolated and 4 kb have been sequenced, including the whole gene and 2.8 kb of the 5' upstream promoter region. The coding region of the gene is 459 bp and it is interrupted by an intron of 105 bp. A promoter analysis was performed by cloning 14 different PCR fragments from the 5' promoter region into the multiple cloning site upstream of the *uidA*(GUS) gene in the promoterless, binary vector pBI101. *Agrobacterium tumefaciens* was used to transform *Arabidopsis thaliana* by the vacuum infiltration method. The transgenic plants express GUS in a tissue specific manner under the control of the soybean pod promoter. Different patterns of expression can be seen as a consequence of the different sizes of the promoter. Transient expression of GUS in pods of soybeans and green beans (*Phaseolus vulgaris*) following particle bombardment also demonstrate the functionality of this promoter.

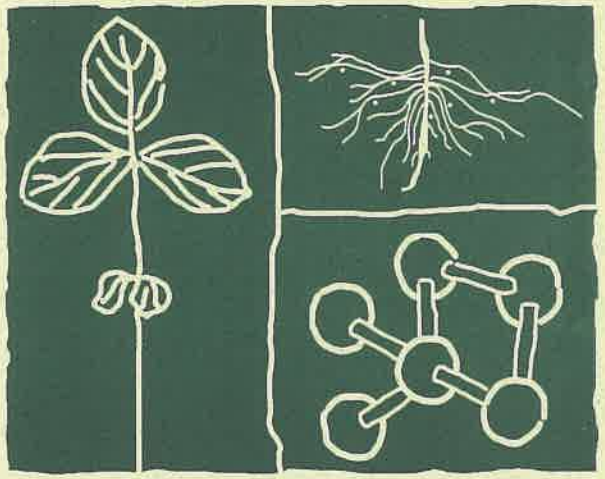
USING GFP AS A REPORTER GENE IN SOYBEAN. A.E. Brzozowski*, T. Ponappa, and J.J. Finer, Department of Horticulture & Crop Science, Ohio State University/OARDC, Wooster, OH 44691

The jellyfish (*Aequorea victoria*) green fluorescent protein (GFP) provides a new tool to monitor gene expression in plants. Following UV or blue light excitation, the presence of GFP can be easily detected by observing bright green fluorescence directly in living tissue. The objectives of this study were to identify the GFP constructions that give high levels of expression in soybean cultures, to evaluate GFP expression in soybean over time, to examine the effect of prolonged blue light exposure on GFP activity, and to use GFP expression coupled with hygromycin selection for rapid evaluation of the timing and level of hygromycin application for optimum stable transformation. Embryogenic suspension cultures of soybean *(Glycine max* [L.] Merrill.) were bombarded (~10 clumps/bombardment) using several gene constructions and expression was monitored under blue light. Transient expression was observed with all DNA constructions tested, but was greatest in quantity and intensity with a soluble modified red-shifted GFP (smRS-GFP). Expression of smRS-GFP was visible as early as 1.5 h following bombardment. Peak expression (> 40 spots/clump of tissue) was seen at approximately 6.5 h and decreased to less than 1.5 spots/clump 7 days after bombardment. Prolonged exposures of soybean tissue to high intensity blue light did not reduce the number of GFP-expressing cells over time. Use of an ER-targeted GFP also gave very high levels of gene expression but peak expression was delayed, possibly as a result of protein secretion followed by maturation. Recovery of stably-transformed GFP-expressing clones was obtained with a variety of different hygromycin selection schemes, indicating some flexibility in the timing and levels of hygromycin application needed for successful transformation of these embryogenic tissues. Molecular evidence for the presence of genes encoding a soluble modified GFP (native chromophore) or smRS-GFP (S65T) in stably transformed cultures was obtained by PCR and/or Southern analysis.





Improved Soybean Lines



DEVELOPMENT AND EVALUATION OF POLYGENIC INSECT RESISTANCE IN SOYBEAN

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Pyramiding resistance genes might (1) delay evolution of insect populations with resistance to toxic proteins produced by *Bacillus thuringiensis* (Bt) transgenes, (2) increase resistance, and (3) broaden the range of insect pests controlled. We are field-testing resistance of 'Jack' soybean transformed with a synthetic *cry1Ac* gene (Jack-Bt), and using Jack-Bt as the recurrent parent in a backcross program to introgress three antixenotic quantitative trait loci (QTLs) from PI 229358 using marker-assisted selection (MAS). Jack-Bt is being compared to untransformed Jack and the resistant breeding line GaTIR81-296, which was derived from PI 229358. After 14 d of feeding, mean defoliation of Jack-Bt and GaTIR81-296 by velvetbean caterpillar (VBC) was 11% and 103%, respectively, of that sustained by Jack. Defoliation of these genotypes by corn earworm (CEW) was 39% and 47%, respectively, of Jack defoliation. The levels of resistance to VBC and CEW conferred by the *cry1Ac* gene suggest that it should provide adequate protection against these pests, though earlier data indicates that control of soybean looper by this transgene is poor. Simple sequence repeats (SSR) from the linkage groups carrying these QTLs are being used both for MAS and to better map the positions of the QTLs using a 'Cobb' × PI 229358 F_2 population. Of 22 SSR markers screened on three linkage groups, 64% were polymorphic between Jack and PI 229358, and 68% were polymorphic between Cobb and PI 229358. Loci flanking the putative locations of the QTLs are being used to detect local crossover events. Backcross progenies carrying various combinations of insect resistant genes will be evaluated for resistance to a variety of lepidopteran pests to determine the value of this gene pyramiding strategy.

GENETIC ENGINEERING A SUNFLOWER SULPHUR-RICH PROTEIN GENE INTO SOYBEAN

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Transgenic soybean plants were obtained from cultivars Asgrow 3237 and Bert using the *Agrobacterium*-mediated cot-node method to deliver a gene construct pBSF16. This plasmid contains the CaMV 35S- *bar* gene as a selectable marker, CaMV 35S-*uid*A (GUS) and a seed-specific sulphur-rich protein gene coding for sunflower seed albumin. Cotyledonary node explants from 5-day-old seedlings were treated with *Agrobacterium* (AGL1) harboring pBSF16, cocultivated 3 days in 1/10th concentration of B_5 minerals containing 200 µM acetosyringone. Explants were transferred to full-strength of B5 medium supplemented with 5 mg/L phosphinothricin (PPT) from the herbicide Liberty (AgrEvo) for four weeks. Explants were then transferred to MS medium supplemented with zeatin, IAA, GA_3 and 2.5 mg/L PPT for subsequent growth of PPT-resistant shoots. More than 30 transgenic shoots were produced. The frequency of transgenic shoots obtained varied between 0.5 - 4.0% of the cot-node explants. Many of the transgenic shoots were lost during the rooting process. Transgenic shoots were rooted in MS medium supplemented with 1 mg/L IBA and transferred to growth chambers. GUS activity was observed in T_0 leaves, stem tissue, pollen and T_1 embryos as determined by histochemical GUS assay. Leaves of a regenerated soybean plant were resistant to PPT application at rates as high as 2 g/L indicating expression of the *bar* gene. Three GUS-positive plants grown in a growth chamber produced T_1 generation seeds. T_1 generation plants of one T_0 plant segregated in a 3:1 Mendelian ratio for GUS expression. Characterization of the other plants and biochemical tests are in progress.

CONFIRMATION OF MARKER-QTL ASSOCIATIONS FOR SEED COMPOSITION AND SEED WEIGHT IN A SOYBEAN POPULATION.

V. A. Fasoula* and H. R. Boerma, Dept. of Crop and Soil Sciences, University of Georgia, Athens, GA 30602.

Molecular markers provide the opportunity to identify marker-quantitative trait locus (QTL) associations for various traits. In this study we used Restriction Fragment Length Polymorphism (RFLP) markers. RFLP-QTL associations for protein, oil, and seed weight have been identified in the soybean population PI97100 x Coker 237. Four markers for protein and four markers for oil explained a total of 40% of the variation for each trait, whereas five markers explained 50% of the variation in seed weight. One hundred and eighty F_2-derived lines from an independently derived population of PI97100 x Coker 237 were evaluated for protein, oil, and seed weight at two locations in 1996. Analysis of the phenotypic data showed there were significant differences among the lines for each trait. The F_2-derived lines were classified for the markers associated with protein, oil, and seed weight. We were able to verify one out of two QTL for protein, one out of three QTL for oil and two out of three QTL for seed weight.

DEVELOPMENT OF WHITE MOULD RESISTANT SOYBEAN

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Sclerotinia sclerotiorum (white mould) has become a major disease of soybean in Ontario and the northern US. A molecular approach has been used to increase resistance to this fungal pathogen as conventional breeding has not been successful in development of resistant varieties. An important component of *S. sclerotiorum* pathogenesis is the secretion of oxalic acid. Using transformation, the wheat germin gene (*gf-2.8*)[a] encoding an oxalate oxidase was introduced into short-season soybean under the control of CaMV 35S promoter, enhancer and 3' termination sequences. The enzyme oxalate oxidase catalyses the oxidation of oxalic acid to hydrogen peroxide and carbon dioxide. Homozygous progeny was identified which express oxalate oxidase activity at high levels. Greenhouse pathogenesis tests have shown that these transgenics have superior resistance to white mould. T2, T3 and T4 progeny are currently being grown in confined field trials in white mould nurseries. Transgenic plants were obtained using both *Agrobacterium*-mediated transformation and particle bombardment. Data will be presented on the patterns of gene integration and oxalate oxidase activity in plants from the two transformation approaches.

SELECTING SOYBEAN CULTIVARS FOR DUAL RESISTANCE TO SOYBEAN CYST NEMATODE AND SUDDEN DEATH SYNDROME USING TWO DNA MARKERS.

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DNA markers for QTL and genes conditioning resistance to soybean root infection by *F. solani* (*Rfs1*); to sudden death syndrome (SDS) and to soybean cyst nematode (SCN; *Rhg4* and *rhg1*) were previously identified in 'Essex' x 'Forrest'. Our objective was to test the effectiveness of those DNA markers to select for disease resistance among recombinant inbred lines (RILs) from 'Flyer' x 'Hartwig'. Fifty recombinant inbred lines (RILs), were evaluated for resistance to SDS by *F.solani* root infection severity (IS,%) at two infested locations and resistance to SCN race 3 by index of parasitism (IP,%) in the greenhouse. Selection with BLT65, a sequence characterized marker, identified 281 among 671 RILs predicted to have *Rhg4* derived resistance to SCN. Selection with Satt038, a microsatellite marker, identified 230 among 613 RILs predicted to have resistance to SDS (*rfs1*) and *rhg1* derived resistance to SCN; providing 93 RILs predicted to be dually resistant to SDS and SCN. Segregation of both markers was not random ($P \leq 0.05$). Among the 50 RILs phenotyped, IS means for genotypes with the Hartwig allele at Satt038 (28-29%) were significantly lower ($P=0.0001$, $R^2=28\%$) than with the Flyer allele (31-42%). Mean SCN index of parasitism was significantly lower ($P \leq 0.05$) only for genotypes carrying the Hartwig allele at both Satt038 and BLT65. Therefore alleles of QTL conferring resistance to SDS and SCN in Essex x Forrest also underlie resistance in other populations.

PLEIOTROPIC QTLs - SOME EXAMPLES AND IMPLICATIONS

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Phenotypes are observed. Genes are inferred. This is the gist of classical genetics. With molecular-marker mapping technologies, we now not only infer genes (i.e., QTLs), we also infer their genomic locations. When multiple traits are observed to be genetically correlated, one must discern between a hypothesis of *pleiotropy* (i.e., a single locus with effects on two or more correlated traits), and a hypothesis of *linkage* (i.e., two or more tightly linked loci, each affecting a different trait). For qualitative (i.e., categorizable) traits, the observation of just one (non-erroneous) recombinant type establishes the validity of a *linkage* hypothesis. If not one recombinant is observed, then one must then accept the null hypothesis of *pleiotropy*. If the *linkage* hypothesis is still preferred (for other reasons), then a maximum recombination value (for the linkage of two presumed loci) must be computed to account for the fact that no recombinant types were found among the *n* observations. Hanson (1959) noted that $P_{RC} = (1 - \sqrt[n]{P})$, where P_{RC} = the numerical probability of observing a recombinant type in a given data set, and P = (the user-selected) probability of failure (usually 0.05 or 0.01). For a complete set of F2 and F3 genotypic data, $P_{RC} = [2p-p^2]$, and one simply solves for the recombination value *p*. While these calculations are straightforward, how does one distinguish between *pleiotropy* and *linkage* when the correlated traits are quantitative instead of qualitative? The statistical methods currently used for mapping QTL (both the simple and composite interval procedures) were designed for the analysis of one trait at a time, and thus do not provide a formal means of identifying the basis of genetic correlations among quantitative traits measured at the same time. Jiang and Zeng (1995), however, have described a composite interval method that provides a mechanism for the "joint" mapping of multiple traits. With their method, specific genomic regions (i.e., QTLs) are tested for their effects on two or more traits. More importantly, the method offers a statistical test of null hypotheses of *pleiotropy* versus the alternative hypothesis of *linkage*. The "joint" mapping procedure was recently incorporated into the computer package QTL CARTOGRAPHER. We are using this program to evaluate nature of major QTLs in two of our mapping populations that seem to simultaneously affect yield, maturity, protein, oil, etc. We will present the results of these tests, and discuss the implications of pleiotropy in plant breeding and genetics research.

Hanson, W.D. 1959. Minimum family sizes for the planning of genetic experiments. Agron. J. 59:711-715

Jiang, Changjian and Zhao-Bang Zeng. 1995. Multiple trait analysis of genetic mapping for quantitative trait loci. Genetics 140:1111-1127.

Results of seven years of QTL mapping using three sets of recombinant inbred lines of soybean (Glycine max L.).

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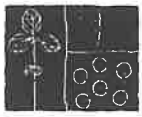
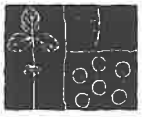
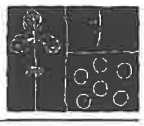
This paper summarizes a series of experiments using three large populations of recombinant inbred (RI) lines of soybean (Glycine max L.) and their respective saturated genetic maps, in an effort to discover the genotype of important agronomic traits under polygenic control. Prior to 1990 when this research was begun, the most common approach to mapping quantitative trait loci was to cross parents of the same genus but of different species and using their F2 and F2-derived families to conduct field experiments. Our research departed from this approach because most traits of interest to breeders such as yield, lodging, plant height, couldn't be meaningfully evaluated in interspecific crosses whose progenies are trailing vines with a great tendency to shatter. Moreover, the impossibility of maintaining the genetic integrity of F2 populations in soybean seriously limited the utility of genetic linkage maps since is not feasible to share seeds of the same genotypes among researchers.

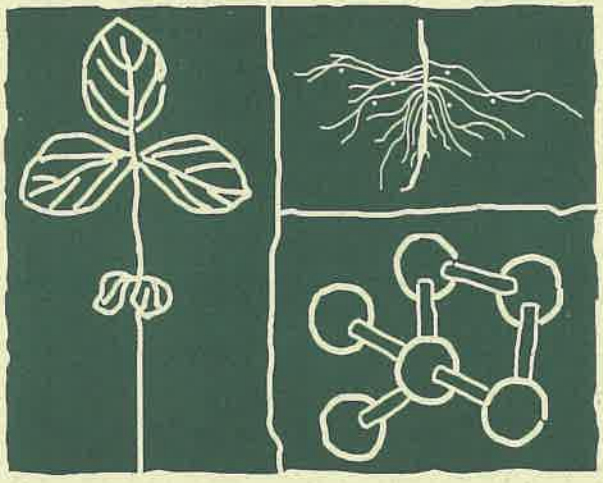
Therefore, in order to enhance collaborative efforts and to perpetuate the mapping populations one of us (L. M.) developed in 1990 four sets of RI lines actually being used in genetic research today. Three of these, namely the cross between Minsoy and Noir 1 (MN, 240 individuals); Minsoy by Archer (MA, 233 individuals); and Noir 1 by Archer (NA, 240 individuals) are the subject of our research reported here. The other population PI 437654 by BSR 101 (300 individuals) has been used by Pioneer Hybrid Int. to map resistance to *Heterodera glycines*. and other traits. Because RI lines are replicable genetic individuals, it has been possible for us to carry out field experiments in the northern (J. Orf, Minnesota, USA) and southern hemispheres (Levi Mansur, Chile), while the maps were developed in Utah by K.G. Lark and collaborators. Our most important results to date are: 1. The RI lines from the MN, MA, and NA crosses all show a high degree of transgressive variation yielding many offspring which surpass either parent for various reproductive, morphological, yield and seed traits. This means that the genotypes which control the traits in each of the parents are not the same. 2. There are single QTL which control large portions of the total variation of polygenic traits (up to 36%) whose effects are stable regardless of environment or genetic background; 3. In all three populations the traits tended to be controlled by a few QTL with large effects (>10-36%) followed by many others with relatively lower effects 4. Epistatic effects between QTL are common and can have profound effects on most traits including seed yield 5. Epistatic interactions can be population and environment specific.







Host-parasite-pathogen-symbiont interactions



THE SYMBIOSOME MEMBRANE OF SOYBEANS UNDER CONTROL OF THE HOST PLANT AND THE MICROSYMBIONT

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The symbiosome membrane (SM) in nodules of soybeans is produced by the host plant via membrane flow from the Golgi and/or directly from the ER. The fatty acid composition of the SM in nodules is more similar to the ER than to the Golgi. In this well-established pathway, evidence will be given that the microsymbiont has a decisive role in formation, stability, protein composition and particle density of this membrane. The SM from soybean nodules infected with the mutant RH 31-Marburg of *Bradyrhizobium japonicum* has in the PF face a particle density of about 1200 particles . μm^{-2} compared to the wild type with about 2200 particles in the PF face. A discrete set of particles in the size range 13-14 nm are missing in the EF face of the mutant-affected SM compared to the wild type.

By mutation analysis *TnphoA* two signal peptidase genes (*sipS* and *sipF*) in *B. japonicum* have been identified, affecting compartmentation and colonization of the infected tissue and soybean nodules. The phenotypes of mutation in the two signal peptidases are different. The genetic structure of the operons of *sipS* and *sipF* will be presented.

The effect on the host plant of the symbiosome membrane was further studied by comparing induced senescence and normal aging in relation to the nodulins on the symbiosome membrane. After induced senescence the symbiosome membrane persists as a structure; however, the symbiosome membrane specific nodulins are extensively degraded, whereas other membrane proteins are more stable.

A nodule specific 53 kDa protein (GmNOD53b) of the symbiosome membrane was isolated, purified and microsequenced. cDNA clones of this novel nodulin, obtained from cDNA library screening with a RT-PCR generated hybridization probe, exhibited no homology to proteins identified so far. The expression of GmNOD53b coincides with the onset of nitrogen fixation. Therefore, it is a late nodulin. Among other changes this GmNOD53b is clearly reduced in nodules infected with the *B. japonicum* mutant 184 on the protein level as well as on the level of mRNA expression, compared to wildtype infected nodules.

Werner, D. et al., Planta 174, 263-279 (1988)
Jacobi, A. et al., J. Plant Physiol. 144, 533-540 (1994)
Müller et al., Planta 197, 163-175 (1995)
Winzer et al. (submitted)

MOLECULAR CHARACTERIZATION OF ROOT-KNOT NEMATODE RESISTANCE IN SOYBEAN. J. Qiu*[1], H. Carroll[1], N. Kokalis-Burelle[2], D.B. Weaver[3], R. Rodriguez-Kabana[1], and S. Tuzun[1]. [1]Dept. Plant Pathology, Auburn University, Auburn, AL 36849; [2]USDA-ARS Horticulture Research Lab, Ft. Pierce, FL 34945; [3]Dept. Agronomy and Soils, Auburn University, Auburn, AL 36849.

Root-knot nematodes (*Meloidogyne incognita*) or RKN are major agricultural pests causing severe losses in many crops including soybean worldwide. The use of resistant cultivars has been the primary means of managing the disease. However, resistance mechanisms of soybean against RKN remain unclear. We recently reported that higher chitinase activity and early induction of specific chitinase isozymes were associated with resistance to RKN in soybean (Qiu et al., 1997[†]). The objectives of this continued study were to: 1) confirm the results obtained previously by utilizing an independent and more defined genetic population, and 2) determine if other defense-related enzymes including peroxidase and catalase are also involved in the host resistance responses. Two recombinant inbred lines (RILs), susceptible (S) or resistant (R) to RKN, along with their parental lines 'Carver' (a resistant cultivar) and 'N90-516' (a susceptible experimental line) were used. Greenhouse experiments with a split-plot design with three replications were conducted. Main plots consisted of the four soybean lines with and without *M. incognita* infestation and subplots consisted of six sampling dates (0, 3, 7, 14, 21, and 35 days after infestation or DAI). Seedlings were infested with 1,300 J2 and 400 eggs at the trifoliolate leaf stage, harvested at the six different dates, and stored at -80 °C. Proteins were extracted from lyopholized roots using 0.1 M Na-acetate buffer (pH 5.2), quantified, and subjected to enzyme activity assays and gel electrophoresis. The resistant cultivar Carver and the R-RIL had significantly higher chitinase activities than the susceptible line N90-516 and the S-RIL upon nematode infestations. Five major chitinase isozymes were detected in all soybean lines prior to and after nematode infestation; however, two acidic isozymes with isoelectric points (pI) of 4.2 and 4.4 accumulated to a greater extent in the resistant lines after challenge. These data are in accordance with our previous results, indicating that plant chitinases are associated with RNK resistance in soybean. No significant differences in total peroxidase or catalase activities were detected among the lines at 0, 3, 7, and 14 DAI; however, the susceptible lines had significantly higher peroxidase activity than the resistant lines at 21 and 35 DAI, probably due to more symptom development in the susceptible lines. At least eleven peroxidase isozymes with pI's ranging from 3.0 to 6.0 were detected in the soybean lines with and without nematode infestation. Among them, two isozymes with pI of 4.4 and 5.8 appeared to be accumulated earlier in the resistant lines and were up-regulated upon challenge. These results suggest that root-knot nematode resistance in soybean is quantitative in nature, in other words, it represents a complex horizontal type of resistance which involves multiple genes or QTL interactions within the host. More research is needed to map the QTL associated with the resistance and to test potential nematicidal properties of target chitinase isozymes.

[†]Qiu, J., J. Hallmann, N. Kokalis-Burelle, R. Rodriguez-Kabana, D.B. Weaver, and S. Tuzun. 1997. Activity and differential induction of chitinase isozymes in soybean resistant or susceptible to root-knot nematodes. J. Nematology. 29:523-530.

GENETIC ANALYSIS OF SCN RESISTANCE IN SOYBEAN INTRODUCTIONS: AN OVERVIEW. Prakash R. Arelli, University of Missouri, Columbia, MO 65211

Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, causes severe damage to soybean [*Glycine max* (L.) Merr] in North America and worldwide. The most efficient way to control the infestation of this pest is to plant SCN resistant cultivars.

Genetic studies have shown that SCN resistance in soybean is complex and is conditioned by multiple genes. Caldwell et al., in 1960 reported that 'Peking' possessed three resistance genes for SCN Race 1 isolate and an additional dominant gene for SCN Race 3 isolate was documented by Matson and Williams (1965). We have verified three resistance genes ($rhg_?$, $rhg_?$, Rhg_4) in Peking (Arelli et al., 1992). Our data also showed that PI88788, a common source of SCN Race 14 resistance, carried two genes $rhg_?$ and Rhg_4 in addition to a new dominant gene Rhg_5 for conditioning resistance to SCN Race 3 isolate. Most other sources of Race 3 resistance soybean lines lack Rhg_5. We reported partial dominance of susceptibility including over dominance and dominance in soybean to SCN Races 3, 5, and 14 (Arelli et al., 1989). Myers et al., (1989) reported that in PI437654 there were three resistance genes for Race 3 isolate which were in common with that in Peking and PI90763. Recently, we have proposed genetic models for inheritance of resistance to SCN Race 3 isolate in PI209332, 438489B, 404166 and 89772 (Arelli, 1992).

Currently molecular markers are used to mapping SCN resistance loci, especially to Race 3 isolate, using different populations. Linkage groups, A and G are found to be strongly associated with SCN resistance loci. (Webb et al., 1995; Concibido et al.,1996). A few other LGs that are associated with SCN resistance include B, H and C (Vierling et al., 1996; Mahilingam and Skorupska, 1995; Qiu et al., 1997; Chang et al., 1997). We found in Peking LGs B, E, H are associated with resistance to SCN Race 1 isolate, B and H for resistance to Race 3 isolate and I, E for Race 5 isolate, respectively. Today, we have 118 sources of SCN resistance available in soybean germplasm collection (Arelli et al. 1997). DNA fingerprinting studies have reported that several of them were not closely related to most frequently used sources of resistance for breeding soybean cultivars (Diers et al., 1997; Arelli and Webb, 1996). These lines may have potentially new genes to allow for broadening the diversity of resistance gene utilization.

SOYBEAN NODULATION CONTROL - GENETICS AND PHYSIOLOGY

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Symbiotic N_2 fixation is an important biological process which provides a portion (ranging from nil under high nitrogen fertilizer application to perhaps 90% plus under sandy soil conditions) of the nitrogen needs of the nodulated legume. Dinitrogen fixation is also important due to contribution of residual nitrogen carry over to other nonleguminous crops being grown in rotation. Increased understanding of the control of symbiotic N_2 fixation thus has implications in terms of maximizing nitrogen-use-efficiency in crop rotations and minimizing environmental concerns associated with nitrogen fertilizer management decisions.

Nonnodulating and partially-nitrate-tolerant hypernodulating soybean mutants have been isolated and have been useful as experimental tools in assessing nodulation control. Two genes (rj5 and rj6) have been shown to control the nonnodulation trait, and one gene (rj7) controls hyper-nodulation/supernodulation mutants of Bragg, Williams, and Enrei. The control of the nonnodulating characteristic is at an early stage of infection and involves lack of root hair curling. Hypernodulation expression appears to be due to loss of the normal autoregulatory control of nodulation expressed in typical soybean cultivars. It is known that a shoot-transmissible signal controls expression of hypernodulation, although the specific signal has not been identified. The signal originates in the leaf, as opposed to the shoot apex, and appears to be a common signal among soybean, mung bean, and dolichos, based on grafting studies. The report that the signal is not common between soybean and green bean (*Phaseolus vulgaris*) (Hamaguchi et al., 1993, Crop Sci. 33:794-797) has not been repeatable in our laboratory. The apparent linkage of hypernodulation expression with depressed root growth has limited progress in selecting hypernodulated lines with good agronomic performance. The depressed root growth is not due solely to excess nodulation in that root growth is inferior on the hypernodulated lines even in the absence of inoculation. One report has indicated that some hypernodulated lines perform as well agronomicaly as the normally nodulated parent (Song, et al., 1995, Soil Biol. Biochem. 27:563-569). Our own results with the four hypernodulating mutant (NOD) lines, including advanced backcross generations, indicates that agronomic yield is inferior to that of the original parent Williams; maximum yield of the best back cross lines are about 90% of the Williams parent. We concur with the conclusion of Song et al (above ref.) that the advantage of hypernodulated mutants may be manifested in subsequent crops grown in rotation with soybean, rather than to any direct benefit to the soybean crop, because most data indicates that the capacity for nitrogen metabolism by soybean is not a primary limitation to soybean yield. Identification of the shoot-derived signal which provides autoregulatory control of nodule number remains a major goal, as well as furthering understanding of the gene at the molecular level which would perhaps enable direct alteration of nodulation control in legume species. One must, however, keep in mind that the symbiotic N_2 fixation system in legumes must remain in close balance with other biochemical/ physiological processes, and cannot be manipulated at will without other secondary impacts on growth and seed yield.

MOLECULAR ANALYSIS OF THE *nts-1* MUTATION CONFERRING SUPERNODULATION IN SOYBEAN (*GLYCINE MAX* L.)

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Nodule initiation is a fundamental plant process, involving interaction of several plant organs (root and shoot), initiation of localized cell division and differential plant gene expression. Several soybean genes that control the early steps of nodulation have been identified by experimental mutagenesis. Mutation at the *NTS-1* locus discovered in EMS treated *G. max* cv. "Bragg", causes increased root nodulation. Different allelic homozygous recessive mutants develop 3 to 40 times more nodules than wild-type plants and nodulation in these plants is not inhibited by nitrate. The *NTS-1* locus governs the systemic autoregulation response that controls nodule number during the earliest stages of nodulation through production of a presumptive inhibitor. The extensive genetic and physiological data that are available for the mutation in soybean, as well as the commercial importance of this crop, make soybean an attractive choice for further investigation of this gene.

The recently identified proximal molecular marker pUTG-132a (0.7 cM to the *nts-1* mutation) is being using as an anchor in the positional cloning and characterization of *NTS-1*. The first goal of our project is to assemble a contiguous region of soybean genome containing the gene. After the screening of the soybean (cv. Williams) Bacterial Artificial Chromosome (BAC) library, ten candidate BAC clones presumably containing pUTG-132a were identified. After PCR analysis and RFLP mapping of the clones only one BAC clone was confirmed to be truly positive. The clone is being subjected to isolation of ends for sequencing and further screening of the BAC library for the contig building.

The distal RFLP marker pA381 (3.5 cM to the *NTS-1*) was sequenced and analyzed for internal polymorphisms between *G. max* and *G. soja* parental genotypes used for the cross and obtaining of F_2 population. Three single-nucleotide substitutions were found and are being used for the PCR mapping of the pA381. The 5' end of the marker shows a high homology (72-74%) to several *Arabidopsis thaliana* amino acid transporter mRNAs. Additionally, the soybean pA381-specific primers amplify strong PCR products from *Arabidopsis* DNA (Columbia and Landsberg ecotypes), giving the possibility of a synteny between soybean and Arabidopsis genomes in the region containing symbiosis *NTS-1* gene. If it is indeed the case i) the *NTS-1* homolog might be presented in the model *Arabidopsis* genome and ii) the *NTS-1* is involved in more general plant developmental processes.

USING SSR, RAPD, AND AFLP TECHNOLOGIES FOR DETECTION OF MOLECULAR MARKERS LINKED TO THE rj_7 MUTANT GENE IN SOYBEAN [*G. max* (L.) Merr.]

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The four hypernodulating mutants derived from mutagenized Williams are genetically controlled by a single recessive gene (rj_7). The objectives of the present study were to identify molecular markers associated with this mutant gene using PCR-based DNA polymorphism analysis, and to construct a genetic linkage map of the chromosomal region around the rj_7 locus. Genomic DNA of two parents, Harosoy 63 and NOD1-3, and 122 F_2 plants derived from this cross were isolated using the CTAB method. PCR amplifications of SSR, RAPD, and AFLP were performed. Initially, the two parents and two contrasting DNA pools were used for bulked segregant analysis to identify markers linked to this gene. Subsequently, segregation analysis was conducted in an F_2 mapping population for the construction of genetic linkage map. Map distances in cM were estimated using MAPMAKER program. The results indicated that no SSR markers were found to be linked to the rj_7 locus, while four RAPD and six AFLP markers were linked to this gene. Of the linked AFLP markers, the E3/M8-a and E5/M5-a were mapped closely to the rj_7 locus at 2.3 and 7.4 cM, respectively. The remaining AFLPs and the RAPDs were relatively distal to the locus. Further examination to confirm the inheritance in F_4 progenies isolated from F_2 hypernodulating individuals and backcrosses (BC1 and BC2) indicated that these linked markers were heritable in descendants. In addition to the NOD1-3 mutant, three other NOD mutants and the En6500 mutant, which was isolated from mutagenized Enrei cultivar, were also tested for the presence of these markers. The results were in agreement with the conclusions of our previous work on the involvement of an allelic gene in the NOD mutants and the En6500. It can be concluded that these AFLP and RAPD markers can be used for a DNA diagnostic approach and for marker-assisted breeding program.

CULTIVAR-SPECIFIC NODULATION OF SOYBEAN BY *SINORHIZOBIUM FREDII* STRAIN USDA257: A ROLE FOR TYPE III PROTEIN SECRETION AND PILI?

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Sinorhizobium fredii strain USDA257 forms nitrogen-fixing nodules on Peking soybean, but it fails to infect the cultivar McCall. This symbiotic specificity is controlled by a complex sym plasmid locus that contains at least 12 genes (*nolXWBTUVhrcNQRSTU*). Insertional inactivation of these genes allows nitrogen-fixing nodules to form on McCall, and thus the locus functions negatively to restrict host range at the level of soybean cultivar. We have sequenced the entire cultivar specificity region of USDA257 and deduced the amino acid sequences of the gene products. Eight of these proteins have significant sequence homologies to proteins that function in Type III protein secretion by Gram-negative plant and animal pathogenic bacteria. These pathogens utilize Type III systems to target proteins for delivery into cells of their eukaryotic hosts.

As is the case in other N-fixing bacteria, expression of the *nod* genes of *S. fredii* is triggered by flavonoid signals from the plant host and under the jurisdiction of *nodD*. The isoflavone genistein induces USDA257 to secrete five proteins that are not produced by *nolXWBTUV*- or *nodD*-negative mutants. We have begun to determine if these molecules, which we term SR proteins, are secreted via a Type III system and if they help condition the cultivar specificity that characterizes the *S. fredii*-soybean system. We report here that some of these proteins are associated with fibrillar extracellular appendages that appear after genistein treatment.

Under normal conditions, the only appendages on cells of USDA257 are flagellae with an average diameter of 10.5 nm. They are inserted into the cell poles and react with antibodies directed against flagellin of *S. meliloti*. Both genistein and a second signal molecule, daidzein, induce profuse production of much finer, pilus-like structures. Immunocytochemistry with negatively-stained cells confirms that three of the SR proteins (SR5, SR3, and SR1, which is equivalent to NolX) are associated with an amorphous material that appears to coat these appendages. We have isolated pili from genistein-treated cells, solubilized them, fractionated the proteins on gels, and then probed Western blots with anti-SR5, anti-SR3, and anti-SR1. These experiments confirmed that all three proteins copurify with pili. We currently are determining if pili are produced by mutants with insertions in the cultivar specificity region. We also want to know if the proteins are structural components of the pili and if they interact with McCall roots. This work was supported by grants from the USDA-NRI.

NODULIN 26 CONFERS AQUAPORIN AND UNCHARGED SOLUTE TRANSPORT PROPERTIES TO THE SOYBEAN SYMBIOSOME MEMBRANE.

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Nodulin 26 is among a subset of nodulin proteins that are produced and targeted to the symbiosome membrane enclosing the rhizobia symbiont (1). This protein is a major constituent of this membrane, representing 10-15% of the total symbiosome membrane protein (2). Nodulin 26 is a member of ancient membrane channel family known as the Major Intrinsic Protein (MIP) family (reviewed in 3), and it has long been predicted that this protein serves a function in symbiosome membrane transport. In support of this, it has been found that nodulin 26 (similar to the MIP protein and gap junctions) forms a large membrane ion channel upon in vitro reconstitution into planar lipid bilayers (4). However, upon examination of its activity in *Xenopus* oocytes we find that nodulin 26 does not transport ions, but rather confers enhanced water permeability to the oocyte membrane suggesting a role as an aquaporin (2). This is further strengthened by the finding that both nodulin 26 proteoliposomes and native symbiosome membrane vesicles have a high, facilitated water transport activity. In addition, unlike other plant aquaporins, nodulin 26 also permits the flux of uncharged carbon solutes such as glycerol, and SM vesicles have an unusually high permeability to this test solute. Thus, the protein appears to be the first plant member of the "aquaglyceroporin" subclass of aquaporin proteins (reviewed in 5) that show multiple transport properties. Both water and glycerol transport through nodulin 26 proteoliposomes as well as SM vesicles shows a high rate, low activation energy, and inhibition by $HgCl_2$, all hall marks of aquaporin protein-facilitated transport. The role of the solute transport function of nodulin 26 is less clear, although glycerol has been proposed as an osmoprotectant that is produced and taken up by certain plant and algal cells. Nodulin 26 is also phosphorylated on serine 262 by an SM-associated calmodulin-like domain protein kinase (CDPK) and this appears to affect the gating properties of the channel. Overall, we suggest that nodulin 26 might play an osmoregulatory role on the SM, that is capable of facilitating rapid water transport, and possibly the transport of uncharged solutes, upon changing osmotic conditions.

1. Fortin, M.G., Morrison, N.A., & Verma, D.P.S. (1987) *Nucleic Acids Res.***15**, 813-24.

2. Rivers, R.L., Dean, R.M., Chandy, G., Hall, J.E., Roberts, D.M., & Zeidel, M.L. (1997) *J. Biol. Chem.* **272**, 16256-61.

3. Reizer, J., Reizer, A., & Saier, M.H. (1993) *Crit. Rev. Biochem. Mol. Biol.* **28**, 235-57.

4. Weaver, C.D., Shomer, N.H., Louis, C.F., & Roberts, D.M. (1994) *J. Biol. Chem.* **269**, 17858-62.

5. Agre, P., Bonhivers, M. & Borgnia, M.J. (1998) *J. Biol. Chem.* **273**, 14659-62.

DESCRIPTION OF A SOYBEAN EARLY NODULIN CDNA. Arthur T. Trese* and Guo Wei, Ohio University, Department of Environmental and Plant Biology, Athens OH 45701.

A novel early nodulin cDNA isolated from soybean will be described. Sequence analysis suggests that the protein is targeted to the extracellular space, and potential functions of the protein will be presented. The homologous transcript is rapidly induced in inoculated soybeans, but is absent in uninoculated roots or roots inoculated with mutant bacteria unable to elicit nodulation.

GENETIC ANALYSIS OF *HETERODERA GLYCINES* PARASITISM

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The soybean cyst nematode (SCN), *Heterodera glycines*, is a sedentary endoparasite that causes substantial economic damage to soybean on a worldwide scale. Although management via the use of resistant soybean cultivars may be achieved, there are numerous genotypes (races) of SCN that may evade host resistance responses. Because SCN maintains an intimate parasitic relationship with its host, it is suspected that numerous nematode genes are involved in parasitic abilities. In order to identify how many nematode genes are involved and to isolate these genes, we have taken a genetic approach. To attain this goal, we have constructed a linkage map of the *H. glycines* genome. Controlled crosses have been performed between several SCN strains strains. Segregation analyses have revealed that inheritance of parasitic ability is Mendelian in nature. The genes controlling parasitic ability on a specific host differential appear to be unlinked loci. The SCN gene controlling ability to parasitize PI 88788 appears to be inherited as a dominant allele, whereas the genes conferring parasitic ability on PI 90763 and Pickett are recessive. We have screened approximately 1,000 RAPD 10mer primers against three SCN inbred parental strains, and 1,600 progeny lines. We have mapped parasitism loci using a RAPD-PCR approach with a modified bulk segregant analysis. Specifically, we have identified 2 DNA markers tightly linked to the PI 88788 parasitism locus. We have tentatively mapped the parasitism genes for PI 88788, PI 90763, and Peking to different linkage groups. Using a SCN transformation protocol developed in our labs, we will clone and characterize the PI 88788 parasitism gene.

A GENETIC MAP OF THE SOYBEAN CYST NEMATODE, *HETERODERA GLYCINES*

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The soybean cyst nematode (SCN) is a sedentary endoparasite of soybean, and the cause of millions of dollars of damage to soybean production in the U.S. Soybean genotypes resistant to SCN have been identified and are commonly used in managing this pest in the field. However, natural isolates of SCN have always existed that are capable of overcoming these soybean resistance genotypes. Through the controlled matings of two highly inbred *H. glycines* strains, a double backcross population of 223 single cyst derived nematode lines has been developed that segregates for the ability to parasitize soybean PI 88788 and PI 90763. The nematode locus for parasitism on PI 88788 is in fact segregating in this population as a dominant locus, and is referred to as *Ror-1* (*R*eproduction *o*n a *r*esistant host). Previously, several RAPD markers were developed that were found linked to *Ror-1* in this population. In an effort to expand the SCN genome map and especially to fine map the *Ror-1* region, an AFLP map has been developed. Preliminary steps have also been taken for the inclusion of RFLP and EST markers, and the eventual development of a physical map. Using these tools, it is our goal to isolate and characterize the parasitism genes in SCN. This data will provide important insight into the mechanism of host-resistance breaking by the soybean cyst nematode, and may suggest novel ways to employ either natural or transgenic resistance.

CLASS II TRANSPOSABLE ELEMENTS IN THE SOYBEAN CYST NEMATODE

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Class II mobile genetic elements transpose via a DNA intermediate and are widely distributed among both prokaryotes and eukaryotes. Class II transposons have been extensively used as tools for gene tagging and mutation in the model nematode *C. elegans*. Soybean cyst nematode (SCN, *Heterodera glycines*) has sequences that hybridize to the Tc1 element of *C. elegans*. Using degenerate oligonucleotide primers several transposase fragments were isolated from SCN which show relationship to the Tc1 family of class II elements. A 1.7 Kb fragment having the inverted terminal repeats characteristic of class II elements was found during fine mapping of a locus responsible for the ability of some SCN strains to reproduce on resistant soybean hosts. Our goal is to study the activity of these elements *in vivo*, determine their role (if any) in overcoming host resistance, and develop them into the mutagenesis, gene-tagging, and transformation tools necessary for the study of host-parasite relationships.

A MICROBIAL SYMBIONT USED TO ALTER THE NUTRITIONAL QUALITY OF SOYBEAN AND OTHER CROP PLANTS.

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PPFMs (pink-pigmented facultative methylotrophs - especially *Methylobacterium spp.*) are bacterial symbionts of plants, shown previously to participate in plant metabolism. One on-going theme of research in our lab is the development of methods for exploiting the relationship between these bacteria and their host plants for plant improvement. We report here on our efforts to alter the amino acid profile and vitamin content of soybean plants by replacing their native PPFMs with selected mutant strains of the bacterium. Using a combination of selective media and auxotrophic mutants of *E.coli* and *Arthrobacter sp.*, we isolated methionine- and vitamin B12-overproducing PPFM mutants and have colonized soybean and lettuce plants with them. We hypothesize that the PPFM overproducers will feed their host plants, resulting in increases of B12 or methionine in host tissues. Analysis of these plants is in progress. The significance of this strategy for improvement of soybeans and other crop plants is discussed.

FOLIAR APPLICATIONS OF A PHYLLOPLANE BACTERIUM USED TO ENHANCE SOYBEAN YIELD.

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Foliar application of small amounts of methanol to crop plants has been reported to increase their growth and yield dramatically, but results are sometimes variable and details of the mechanism by which methanol works is not clear. At the 6th biennial meeting of this conference, we (Munsanje *et al.* 1996) reported that a methylotrophic bacterium that normally inhabits plant leaf surfaces probably acts as intermediary in the process of methanol-stimulated growth since it is known both to consume methanol and to produce cytokinins. Our data showed that methanol is effective at stimulating bacterial growth and that methanol-stimulated plant growth occurs only in the presence of the bacteria. Now, we show that applying the bacteria directly to leaves can substitute for the application of methanol. We conclude that methanol acts by stimulating the growth of the methylotrophic bacteria and that the bacteria are responsible for stimulating plant growth by their production of cytokinins. A model for this interaction is presented.

Reference:

Munsanje, E.M., Joshi, J., Kittel, M., Holland, M.A. 1996. Foliar application of methanol and urea increases soybean growth and yield by stimulating phylloplane bacteria. 6th Biennial Conference on Molecular and Cellular Biology of Soybean (Columbia, MO - 12-14 August 1996).

ISOLATION OF ETHYLENE-INSENSITIVE MUTANTS OF SOYBEAN THAT DISPLAY ALTERED RESPONSES TO SOYBEAN PATHOGENS.

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Ethylene plays an important role in many stages of plant growth, including response to infection by plant pathogens. Previous work with other species has demonstrated that disease tolerance is enhanced in some plant lines that are deficient in their response to ethylene. This suggested that it may be possible to generate plant lines with improved disease tolerance by selection of ethylene-insensitive mutants. To explore this and other hypotheses concerning the role of ethylene in soybean growth and development, we have isolated a number of soybean mutants that display reduced sensitivity to ethylene. Both semi-dominant and recessive mutations have been isolated, and the mutants display varying degrees of reduced ethylene responsiveness. We have studied the response of these mutants to the soybean pathogens *Phytophthora sojae*, *Septoria glycines*, *Rhizoctonia solani*, and *Pseudomonas syringae* pv. *glycinea*. Both favorable and non-favorable alterations in the plant response to infection have been observed. Ethylene sensitivity has also been associated with control of root nodule formation in legumes, but the mutants we have isolated display normal nodulation behavior. We have, however, observed altered root growth phenotypes. In mutants that display strong ethylene-insensitivity, ethylene-stimulated leaf senescence is greatly reduced. However, late-season leaf senescence in the field was unaltered in these mutants. Replicated yield trials are currently in progress with the ethylene-insensitive lines. These mutants are now available as a resource to the community for further characterization of the roles of ethylene in soybean growth and development.

IDENTIFICATION OF A HIGH AFFINITY BINDING SITE FOR *N*-ACETYL-CHITOOLIGOSACCHARIDES IN THE PLASMA MEMBRANE OF SOYBEAN (*GLYCINE MAX*).

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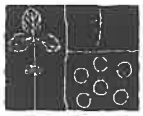
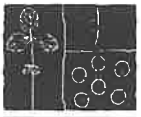
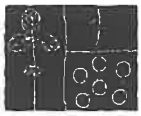
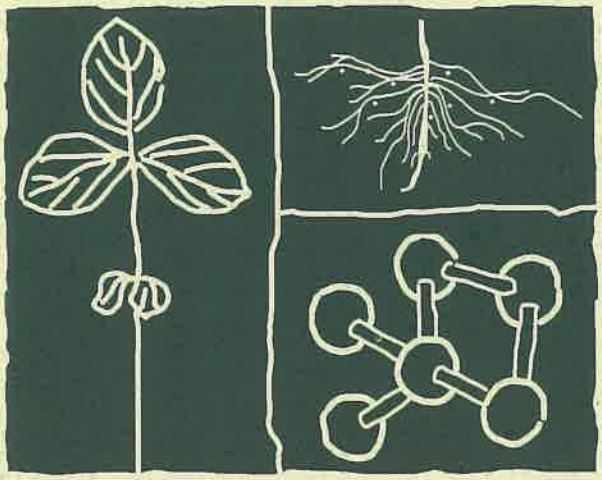
A high affinity binding site for *N*-Acetylchitooligosaccharides has been identified in the plasma membrane of soybean by affinity cross-linking. Using an ^{125}I-labeled 2-(4-aminophenyl) ethylamino conjugate ([^{125}I]-GN_8-APEA) of *N*-Acetylchito-octaose, binding experiments were performed to further characterize chitin binding to the plasma membrane of soybean. Affinity cross-linking and SDS-PAGE identified the presence of a single 90kDa band. Binding of the ^{125}I-$(GN)_8$-APEA conjugate was shown to be saturable, and could be inhibited in a size-dependent manner by the addition of purified *N*-acetyl-chitin oligosaccharides, when using plasma membrane isolated from suspension cultured soybean cells. When using plasma membrane isolated from root tissue, chitin oligomers of a lesser degree of polymerization were the best inhibitors of ligand binding. The addition of de-*N*-acetylated oligosaccharides showed no appreciable inhibition at concentrations as high as 250μM, indicating the specificity of the binding protein for acetylated oligosaccharides. Scatchard analysis indicates an apparent Kd of 30nM, suggesting a high affinity binding site for chitin oligosaccharides.







Model Legumes to Aid Soybean Research



The value of model legumes for soybean research:

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Soybean obviously in a major crop and deserves extensive research attention. Many fascinating biological processes such as nodulation and mycorrhizal associations, also occur in this plant making it the target of researchers involved in plant-microbe interactions. Soybean is a major protein and oil producer and therefore the analysis of biochemical pathways and their regulation is of academic interest, as well as commercial relevance.

However, soybean, being an ancestral tetraploid, with large amounts of repeated DNA, with a genome of over 1000 Mb, a relative long generation time and a large plant and seed size, and comparatively inefficient gene transfer technologies possesses some experimental disadvantages. Additionally, soybean reach is mainly stimulated through commercial (agronomic) interests. This has led to a diversification of genotypes used as experimental subject. This contrasts to the focused work occurring with the model plant Arabidopsis, where resources from around the world are harnessed to advance our understanding of plant biology. It is thus clear that a model legume approach could aid soybean research. As it stands, two plant species, namely *Lotus japonicus* and *Medicago truncatula*, (see Cook, this conference) have emerged as leading candidates (Jiang and Gresshoff, 1997; see Stougaard, this conference). High transformation frequencies (Stiller et al, 1997) have opened their application as material for molecular physiology. Promoter trapping and gene inactivation as a means of gene discovery have been achieved (see Stougaard, this conference). Skeletal maps and valuable mapping populations have been established. Because of the probable chance of synteny among many legumes, as recently demonstrated for soybean, mungbean and *Phaseolus*, it is possible to use comparative genomics to supplement efforts in functional as well as structural genomics.

In this overview I will introduce the model legume *Lotus japonicus*, discuss our results from T-DNA driven promoter trapping, our establishment of a F2 map based mainly on arbitrary DAF markers, our RIL program designed to harbor anchored F2 DAF markers, but mainly containing ESTs (such as those discovered by the MSU group of Frans de Bruijn), and markers from the soybean map known to be close to nodulation related genes such as *enod2* (Ghassemi and Gresshoff, 1998) and *nts-1* (=*rj7* of Jim Harper, see this conference; Kolchinsky et al, 1997; see Men et al, this conference).

Ghassemi, F. and Gresshoff, P.M. (1998). The early *enod2* and the leghemoglobin (*lbc3)* genes segregate independently from other known soybean symbiotic genes. *Molecular Plant Microbe Interactions* **11**: 6.

Jiang, Q. and Gresshoff, P.M. (1997) Classical and molecular genetics of the model legume *Lotus japonicus*. *Mol. Plant Microbe Interactions* 10: 59.

Kolchinsky, A., Landau-Ellis, D. and Gresshoff, P.M. (1997) Map order and linkage distances of molecular markers close to the supernodulation (*nts-1*) locus of soybean. *Mol. Gen. Gen.* 254: 29.

Stiller, J., Martirani, L., Tuppale, S., Chian, R.-J., Chiurazzi, M. and Gresshoff, P.M. (1997) High frequency transformation and regeneration of transgenic plants in the model legume *Lotus japonicus*. *J. exp. Botany* 48:1357.

The model legume *Lotus japonicus*: Insertional mutagenesis and mapping approaches.

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Legume biotechnology is obviously focused on the agriculturally important crop plants such as soybean, pea, peanuts, beans, alfalfa and clovers. The technological possibilities are therefore largely dependent on research within these crops and on the general plant knowledge base. To broaden general plant research and to strengthen the legume knowledge base we suggested *Lotus japonicus* as a model legume for molecular and genetic studies[1,2]. *Lotus japonicus* is diploid, the genome size is small, procedures for production of transgenic plants are available, seed production is ample and the generation time reasonable. A science community using *L. japonicus* has now formed and methods for biochemical, molecular and genetic studies are continuously being developed. At University of Aarhus the focus has been on the plant genetic control of the development of nitrogen fixing root nodules. Results from a mutagenesis program directed towards the isolation of plant mutants affected in root nodule development or root nodule function will be presented. A collection of symbiotic mutants isolated after insertion mutagenesis with either T-DNA or the maize transposable element *Ac* will be described. The phenotypes of the mutants will be presented and for some of the mutants also the molecular analysis. An outline of the future transposon tagging approaches in *L. japonicus* will be given together with a description of attempts to generate an AFLP based map of the *Lotus* genome as the start for map based cloning in *Lotus*. The implications for legume biotechnology will be discussed.

1. Handberg K and Stougaard J. (1992) *Lotus japonicus*. an autogamous, diploid legume species for classical and molecular studies. Plant J, 2, 487-496.
2. Thykjær T, Schauser L, Danielsen D, Finneman J, and Stougaard J (1998) Transgenic plants: *Agrobacterium*-mediated transformation of the diploid legume *Lotus japonicus*. in Cell Biology: A Laboratory Handbook. Second Edition Vol 3, 518-525. Academic Press.

SYMBIOTIC MUTANTS OF THE MODEL LEGUME *Lotus japonicus*.

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We have recently initiated a molecular genetic analysis of the forage legume *Lotus japonicus*, which has been proposed as a model system for studying determinate nodule formation (Handberg, Stougaard, 1992; Jiang, Gresshoff, 1993, Cook et al., 1997). *Lotus japonicus* appears to have outstanding potential as a 'model' legume system since in addition to its relatively small diploid genome (about 400Mb), it has several favorable characteristics, including short generation time, and availability of high frequency transformation and regeneration protocols. To contribute to the development of this 'model' legume system we have initiated both molecular and genetic analyses to clone novel nodule specific genes and to identify novel loci important for symbiotic nitrogen fixation. In the first approach, a range of novel nodule-associated expressed sequence tags (ESTs) of *L. japonicus* was identified (Szczyglowski et al., 1997). The second, complementary approach involved chemical EMS mutagenesis, directed towards the isolation of *L. japonicus* "Gifu" symbiotic variants (Szczyglowski et al., 1998). In this presentation we will focus on our recent progress in the characterization of the *L. japonicus* mutants. The analysis of approximately 3000 M1 plants and their progeny yielded 20 stable *L. japonicus* symbiotic variants, consisting of at least 14 different symbiosis associated loci or complementation groups. The phenotypes of the symbiotic mutants obtained were grouped into six distinct classes ranging from plants that did not display macroscopically visible signs of nodulation (non-nodulating or Nod$^-$ mutants), to variants forming white, mostly ineffective nodule (Nod$^+$Fix$^-$), or excessive numbers of nodules. Of 20 *L. japonicus* root-nodule mutants isolated, at least 7 mutants, representing 5 different complementation groups, were also found to form a defective symbiosis with mycorrhizal fungi. In addition, a mutation affecting *L. japonicus* root development was identified that conferred a hypernodulation response (HNR) when plant of the mutant line LjEMS102, carrying corresponding allele (*har1-1*), were inoculated by rhizobia. The phenotype of the LjEMS102 line was characterized by the presence of nodule structures covering almost the entire root length, and by a concomitant inhibition of both root and stem growth. A mutation in a single nuclear gene was shown to be responsible for both root and symbiotic phenotypes observed in the *L. japonicus* LjEMS102 line. Based on the above observation we postulate that the *har1* mutation modifies the *L. japonicus* root developmental program, which in turn dramatically alters its interaction with symbiotic bacteria. A direct inference of this hypothesis is that the Har1 gene may represent a regulatory locus involved in both root and nodule organogenesis.

Cook J et al (1997) Plant Cell 3, 275-281.
Handberg K, Stougaard J (1992) Plant J. 2, 487-496.
Jiang Q, Gresshoff PM (1993) Curr Top Plant Mol. Biol. 2, 97-110.
Szczyglowski K et al (1997) Plant Physiol. 114, 1335-1346.
Szczyglowski K et al (1998) MPMI 11, (in press)

COMPARITIVE LEGUME BIOLOGY IN *MEDICAGO TRUNCATULA* AND POSSIBLE CONNECTIONS TO *ARABIDOPSIS THALIANA*.

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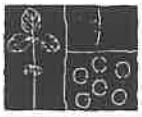
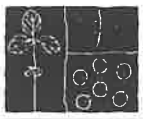
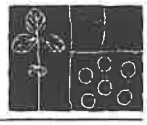
Medicago truncatula is a legume species that has emerged as a model for aspects of plant biology that are unique to, or best studied in a legume system. Model legumes have potential to make important contributions in areas such as human nutrition, root development, symbiotic and pathogenic interactions, carbon, nitrogen and phosphorous metabolism, and natural product biochemistry. The key elements of a tractable molecular-genetic system have been assembled in *M. truncatula*, including large collections of characterized mutants, EST and BAC libraries, efficient methods for transformation and regeneration, DNA marker maps, and extensive germplasm collections.

In our laboratory we study symbiotic nitrogen fixation. We have characterized several *M. truncatula* mutants that are either blocked for early nodule development or that form excessive numbers of nodules. Based on deficiencies in infection and/or formation of nodule primordia, early nodulation mutants can be tentatively assigned to pathways for infection or nodule initiation, respectively. We have also characterized hypernodulation mutations that identify separate loci and differ in spatial organization of the nodulation zone and their responsiveness to plant hormones.

To facilitate cloning of these and other legume genes, we are assembling tools for genome analysis in *M. truncatula*. Our current efforts include (1) development of genome-wide gene-specific PCR markers, (2) mapping of nodulation mutants relative to candidate genes (e.g. ethylene perception loci of *Arabidopsis*), and (3) characterization of BAC physical contigs in selected genome regions. One strategy to accelerate map-based cloning efforts in *M. truncatula* is to exploit the expected syntenic relationship with other legumes and with non-legume species such as *Arabidopsis*. We have used mapping data from the legume species pea and alfalfa to select evenly distributed markers for analysis in *M. truncatula*. The results are consistent with a high degree of macrosynteny between these three genomes and they underscore the feasibility of developing a unified legume map in *M. truncatula*. We have recently expanded this comparative analysis to include sequence-characterized regions of the *Arabidopsis* genome. Briefly, *Arabidopsis* and *M. truncatula* appear to be microsyntenic in several genome regions we have tested.







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