

Registration of the Essex × Forrest Recombinant Inbred Line Mapping Population

A genetic map of soybean [*Glycine max* (L.) Merr.] constructed with a recombinant inbred line (RIL) mapping population (Reg. no. MP-2, NSL 431663 MAP) from the cross 'Essex MAP' (PI 636326 MAP) by 'Forrest MAP' (PI 636325 MAP) has been used extensively worldwide. Essex was registered by Smith and Camper (1973) and Forest by Hartwig and Epps (1973). Since most morphological traits do not vary greatly, the RIL population has been used extensively worldwide to map genes underlying biochemical and physiological traits (Table 1). The genetic marker data encompass thousands of polymorphic markers and tens of thousands of sequence-tagged site (STS) that were collected at SIUC by Dr. Lightfoot's group (Table 2). Several genetic maps of ExF94 have been constructed (Chang et al., 1997; Iqbal et al., 2001; Kassem et al., 2004b) and will continue to be developed.

The population is used to identify quantitative trait loci (QTL; Table 3) including those underlying biochemical and physiological traits that include resistance to soybean sudden death syndrome (SDS) [caused by *Fusarium solani* (Mart.) Sacc. f. sp. *glycines* (Fsg)]; soybean cyst nematode (SCN), *Heterodera glycine* Ichinohe (Hnetkovsky et al., 1996; Chang et al., 1996, 1997; Iqbal et al., 2001); seed yield (Njiti et al., 1997b; Yuan et al., 2002); seed quality traits (Njiti et al., 1999; Meksem et al., 2001b, 2004); water deficit (Cho et al., 2002); and manganese toxicity (Kassem et al., 2004b). Soybean genome analysis is underpinned by the population (Meksem et al., 2000, 2001a, 2001c, 2001d; Shultz et al., 2001; Wu et al., 2004a, 2004b). The map and RILs were used to anchor a physical map of soybean (Wu et al., 2004a, 2004b). The map and RILs were used for positional cloning of *nts1*, *GmNARK*, (Searle et al., 2003), *Rpg5* (Ashfield et al., 2003), *Rhg1*, *Rhg4*, and *Rfs2* (Lightfoot and Meksem, 1999). The population was used to develop an assay for marker-assisted selection for SDS resistance in the greenhouse (Njiti et al., 2001). Near-isogenic line populations have been created from each RIL for fine mapping and verification of QTL detected in the RIL population (Table 2; Njiti et al., 1998; Meksem et al., 1999; Triwitayakorn et al., 2005).

Forrest has been used as a source of DNA for three bacterial artificial chromosome (BAC) libraries (Meksem et al., 2000; Wu et al., 2004a). The set of materials has been used by many additional collaborators (unpublished to date). The population is very important for the analysis of yield QTL and other agronomic traits because it does not segregate for maturity and growth habit. The registration of this population allows public access to the population and data generated from it. Joint efforts in combating many agronomic problems in the future are expected.

Parents

Forrest is an F₅-derived line from the cross 'Dyer' × 'Bragg' and was developed by USDA-ARS and Tennessee Agricultural Experiment Stations (Hartwig and Epps, 1973). It is late group V in maturity and is characterized by determinate growth habit, white flowers, tawny pubescent, tan pods, yellow seed coats, and black hila. It was released for resistance to soybean cyst nematode (HG type 0; races 3, *Heterodera glycines* Ichinohe), root-knot nematode [*Meloidogyne incognita* (Kofoid & White) Chitwood], bacterial pustule [caused by *Xanthomonas axonopodis* pv. *glycines* (Nakano 1919) Vauterin, Hoste, Kersters & Swings 1995], wildfire [caused by *Pseudomonas syringae* pv. *tabaci* (Wolf & Foster 1917) Young, Dye & Wilkie 1978], target spot [caused by *Corynespora cassii-*

cola (Berk. & M.A. Curtis) C.T. Wei], and moderate resistance to Phytophthora root rot (caused by *Phytophthora sojae* Kaufmann & Gerdemann). Forrest was subsequently found to be resistant (disease index, DX < 1%) to foliar SDS (Hnetkovsky et al., 1996), root infection (infection severity, IS > 20%) by *F. solani* f. sp. *glycines* (Njiti et al., 1997a), and *F. virguliforme* and *F. tucumaniae* (Aoki et al., 2003). Forrest has excellent pod-shatter resistance (Hartwig and Epps, 1973).

Essex is an F₇-derived line from the cross 'Lee' × S55-7075 and released by the Virginia Experiment Station (Smith and Camper, 1973). It was initially released for high seed yield and seed quality. It is maturity group V and is characterized by a determinate growth habit, purple flower, gray pubescent, yellow seed coat, and buff hila. It is resistant to bacterial pustule, several races of downy mildew [caused by *Peronospora manshurica* (Naumov) Syd. In Gaum.], frogeye leaf spot (caused by *Cercospora sojina* K. Hara), and purple seed strain disease [caused by *Cercospora kikuchii* (Mastsumoto & Tomoyasu) M.W. Gardner]. It is moderately resistant to Phytophthora root rot, as is Forrest. It has been found to be susceptible (DX > 10%) to SDS (Hnetkovsky et al., 1996), root infection (IS > 40%) by *F. solani* f. sp. *glycines* (Njiti et al., 1997a), and *F. tucumanes* (unpublished).

Development of the Population

The cross was made in 1983 at Southern Illinois University at Carbondale using seed obtained from the originating programs. About 4500 F₂ plants were inbred to the F₃ generation by modified single seed descent (Brim, 1966). In 1988, 500 F₃ plants were harvested of which 150 were randomly selected, intentionally excluding a few undesirable extremes. Seeds of each plant were planted in a progeny row and in 1989, 100 recombinant inbred lines were retained for evaluation of morphological and agronomic traits, stress resistance, and 94 were used for construction of genetic map and QTL discovery. The population is now at the F_{5.15} generation. The RILs are identified as ExF1 to ExF100. ExF78 has been released as disease resistant germplasm under the name LS-G96 (Schmidt et al., 1999). Ninety-four of these 100 lines have been officially released by Southern Illinois University at Carbondale and all individuals interested in studying these lines may request seeds from Dr. Lightfoot at the above institution for small samples of seeds from the above institution for the next 5 yr. Large samples are available each biennium as the seed source is refreshed. For security lines are stored at National Center for Genetic Resources Preservation, 1111 S. Mason St. Fort Collins, CO 80521-4500.

Description of the Population

The population has a 7-d spread in maturity (105–112 d after planting). Forty-six RILs produce white flowers, 52 produce purple flowers, and two are heterogeneous for flower color. Fifty-three RILs produce gray pubescence, 44 produce tawny pubescence, and three are heterogeneous for pubescence color. All RILs have a determinate growth habit. Plant height ranges from 71 to 106 cm for full season planting. All RILs produce seeds with yellow seed coat, 46 have black seed hila, 52 have buff seed hila, and two are heterogenic for seed hila color. Seed yield ranges from 2.9 to 3.9 Mg ha⁻¹. The RILs show differential response to the SCN Hgtype 0 (race 3) isolate AP3, with 22 resistant [index of parasitism (IP) 10], 65 susceptible (IP > 10), and 13 heterogenic (based on individual plant IP). The RILs show differential response to SDS, with disease incidence ranging from 5 to 100% and disease index (DX) ranging from 1 to 24%. The population segregates for resis-

Table 1. Means and ranges of Essex Forrest and their F5 derived progeny for important traits.

	Maturity	Seed yield	Leaf SDS	Seed isoflavones
	d	kg/ha	DI	mg/g
Essex	104	3.6	58.9	2.2
Forrest	111	3.3	15.5	3.1
Progeny mean	108	3.4	48.5	2.8
Progeny range	100–114	3.0–3.8	0.2–95	1.9–3.7
No. of QTL	0	6	6	8

tance to frogeye leaf spot, Phytophthora root rot, Mn toxicity, salt tolerance, water deficit tolerance, seed isoflavone (daidzein, genistein, and glycitein) content, and trigonelline content. The population may have the potential to segregate for protein, oil, and carbohydrate content and quality. The population may segregate for phytate and other dietary micronutrients and anti-nutritional factors.

Genetic and Physical Maps

The ExF RIL genetic map currently contains 368 linked markers, but 231 are high quality microsatellite markers that have been scored repeatedly (Kassem et al., 2004b). The average distance between markers is 17 cM, and there are about 23 markers per linkage group and 79 markers unlinked. The polymorphic markers include 90 RAPD markers, 27 RFLP markers, 20 AFLP markers, and 231 microsatellite markers. All are publicly available on the web at Soybase <http://soybase.agron.iastate.edu/> (verified 7 March 2005), on the physical map at <http://bioinformatics.siu.edu> (verified 7 March 2005) and their sequences are deposited in Genbank at <http://www.ncbi.nlm.nih.gov/> (verified 7 March 2005).

Two soybean large-insert libraries have been constructed from Forrest in the pCLD04541 (V41) binary vector after partial digestion of genomic high-molecular-weight DNA with *Bam*HI or *Hind*III (Meksem et al., 2000). The libraries contain 76 800 clones with an average insert size of 125 kbp, and therefore represent 9.5-fold haploid genome equivalents. Another soybean BAC library was constructed from Forrest in pBELOBACII vector after digestion with *Eco*RI. The library contains 38 400 clones with inserts of 153 kbp representing

Table 3. The number of QTL for six traits that were evaluated in the Essex × Forrest mapping population.

Trait	Number of QTL	Reference
Sudden death syndrome (SDS)	3	Chang et al., 1996, 1997
	2	Hnetkovsky et al., 1996
	2	Njiti et al., 1998
	6	Iqbal et al., 2001
	9	Kassem et al. (unpublished)
Soybean cyst nematode (SCN)	2	Chang et al., 1997
	2	Meksem et al., 1999, 2001b
	2	Prabhu et al., 1999
Seed yield	3	Kassem et al. (unpublished)
	3	Yuan et al., 2002
Seed isoflavones content	6	Kassem et al. (unpublished)
	2	Njiti et al., 1999
Resistance to Mn toxicity	6	Meksem et al., 2001a
	8	Kassem et al., 2004b
	2	Kilo and Lightfoot, 1996
Foliar trigonelline content	5	Kassem et al., 2004a
	2	Cho et al., 2002

5.8 haploid genome equivalents (Wu et al., 2004a, 2004b). These BAC libraries were used to generate a physical map containing anchored microsatellite markers. Updates to the physical map, the BAC clones, gene fingerprints, and the libraries are publicly available through request at <http://bioinformatics.siu.edu>; verified 25 March 2005.

QTL Mapped in the E×F RIL Population

Twenty-five published QTL among six traits identified in this population include six QTL for resistance to SDS (Hnetkovsky et al., 1996; Chang et al., 1996, 1997; Iqbal et al., 2001), three QTL for high yield in Southern Illinois (Yuan et al., 2002), eight QTL for seed isoflavone content (Kassem et al., 2004a), two QTL for trigonelline content and water deficit tolerance (Cho et al., 2002), four QTL for resistance to manganese toxicity (Kassem et al., 2004b), and two for bigenic resistance to SCN (Meksem et al., 2001c). Among these QTL, we have verified in a second population the positions of four of six SDS resistance QTL (Njiti et al., 1998), one of two yield QTL (Yuan et al., 2002), and both SCN resistance genes

Table 2. Description of 20 linkage groups mapped in the Essex × Forrest mapping population. The map distances and markers distribution for the linkage groups were generated from analysis of the 100 F₅-derived progeny from E × F.

Linkage group	Published NIL populations	Map distance	Total	Number of markers†				
				SSR	RFLP	RAPD	BES	EST
		cM						
A1		73.8	14	4	3	7	458	13
A2	2	259.0	22	10	8	4	757	0
B1		164.0	16	11	2	3	234	7
B2		53.4	12	7	1	4	156	3
C1	1	150.1	13	10	0	3	136	0
C2		213.2	30	19	4	7	565	14
D1a+Q		140.0	17	14	0	3	625	30
D1b+W		87.4	14	8	1	5	124	1
D2		245.4	19	15	0	4	122	0
E		97.4	9	6	0	3	362	11
F		219.9	29	16	5	8	369	0
G	4	242.5	37	19	12	6	1 126	33
H		98.3	9	6	111	2	427	9
I	1	116.9	16	11	0		192	6
J		40.7	7	3	1	5	577	3
K		150.9	18	13	0	3	590	1
L		103.8	12	9	0	5	91	3
M		105.2	10	6	1	3	87	9
N		145.1	21	9	2	3	156	0
O		116.4	13	10	0	10	566	9
Total		2823.4	337	206	41	3	7 720	152
Unlinked	2004	0	0	0	0	0	10 529	485

† ESTs and BES may appear at 2 or more locations on the linkage map if they appear in homeologous regions of different linkage groups

(Webb et al., 1995; Meksem et al., 1999). The remaining QTL have not been tested to verify to date.

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