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Evaluation of Marker-Assisted Introgression of Yield QTL Alleles into Adapted Soybean

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ABSTRACT

Genetic diversity is limited in southern elite soybean [*Glycine max* (L.) Merrill]. Introgression of diverse alleles for yield may increase the rate of yield improvement. Beneficial yield alleles at three quantitative trait loci (QTL) from the northern cultivar Archer have been tagged with molecular markers. The objective of this research was to assess the value of the three Archer alleles for increased yield in southern environments and genetic backgrounds. Four sets of near isogenic lines (NIL) for each quantitative trait locus (QTL) were derived from heterozygous F_6 plants identified from the crosses of Archer × Asgrow A5403 and Archer × Pioneer 9641. The NIL sets were tested at four environments across 2 yr. Data was collected on yield, height, and maturity. None of the marker effects were significant for any of the three QTL for any trait, when averaged over all sets or for individual sets. The results suggest that the Archer alleles are not superior to the southern alleles when tested in southern environments. Archer has low relative yield in the South, while in the original mapping study Archer was the high-yield adapted parent. The superior genetic value assigned to the Archer yield QTL may not be readily transported to populations or environments where Archer is inferior. Recombination and epistasis may also have affected the ability of the Archer markers and QTL to improve yield. Our results indicate that it may be difficult to capture the value assigned to QTL alleles derived from diverse parents with variable relative genetic value when the alleles are introgressed into populations with different genetic backgrounds, or when tested in different environments.

Traditionally, soybean breeders have used high-yielding parental lines with good agronomic phenotypes to create new high-yielding cultivars. This approach has narrowed the genetic diversity of the Southern U.S. elite soybean population (Sneller, 1994; Gizlice et al., 1996). Increasing the diversity of elite soybean-breeding populations could increase the rate of yield improvement (Kisha et al., 1997). To increase genetic diversity, new beneficial alleles need to be identified and moved into elite soybean genomes. Plant introductions in the USDA germplasm collection are potential sources of beneficial diversity. In addition, northern U.S. cultivars (maturity groups 00–III) are a potential source of new alleles for southern soybean breeders (Sneller, 1994; Kisha et al., 1998).

Most plant introductions and northern elite varieties (even when adjusted for maturity) have lower yield than southern elite soybean cultivars (Sneller et al., 1997). Thus, a breeder trying to increase diversity for yield will be attempting to find a superior yield allele from a diverse parent with lower yield (inferior phenotype) than their adapted population. This scenario is quite different from using diversity for improving other traits, such as disease resistance, where the diverse parent has a better phenotype (say resistant or tolerant) than the elite population (susceptible).

Using phenotypic selection to introgress superior yield genes from diverse parents will require very large populations and extensive testing of lines derived from diverse × elite populations to identify the high-yielding transgressive segregant that is definitive proof that a desirable yield allele has been acquired from the diverse parent. Molecular markers have been used to identify beneficial alleles in genotypes with inferior phenotypes (de Vincente and Tanksley, 1993; Tanksley et al., 1996) and marker-assisted selection may allow for efficient introgression of such alleles from exotic germplasm. For such exotic alleles to be useful in improving an elite population, it must be shown that they will retain their superiority when compared with other alleles in the elite population and when tested in additional environments.

There are numerous reports on mapping QTL for yield and other traits, and on using marker assisted selection to introgress QTL. But there are few published reports that critically evaluate using markers to manipulate yield. Stuber et al. (1992) identified QTL alleles that were predicted to increase hybrid yield if introgressed into select maize (*Zea mays* L.) inbred lines. Markers were used to introgress the alleles into the inbred lines, and indeed the hybrids from the enhanced inbred lines yielded better than hybrids from inbred lines that lacked the marker-introgressed QTL (Stuber, 1994). Quantitative trait loci for yield have been identified in barley (*Hordeum vulgare* L.), and Zhu et al. (1999) indicated that some yield improvement resulted from using marker assisted selection for these QTL. It is important to note that in these maize and barley examples that QTL identification and subsequent assessment of MAS occurred in the same genetic background and similar environments. The value of these yield QTL in a broad array of genetic backgrounds and environments is not known.

Recently, Orf et al. (1999b) mapped QTL for soybean yield and identified beneficial alleles from the northern elite cultivar Archer and in 'Minsoy' and 'Noir I'. These three genotypes are all diverse from southern U.S. cultivars on the basis of pedigree analysis. In particular, they reported that Archer had QTL alleles for increased yield associated with the simple sequence repeat markers Satt002 (on linkage group D2, Cregan et al., 1999) and Satt144 (on linkage group F, Cregan et al., 1999), and a region flanked by Sct_33 and SOYHsp176 (on linkage

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Abbreviations: cM, centimorgan; QTL, quantitative trait locus/loci; NIL, near isogenic line; PCR, polymerase chain reaction.

group F, Cregan et al., 1999, but unlinked to the Satt144 QTL, G. Lark, 1996, personal communication). The QTL linked to Satt002 and Satt144 accounted for 8 and 13% of the phenotypic yield variation, respectively (Orf et al. 1999b), while the Sct_33/SOYHsp176 region accounted for up to 10% of the phenotypic yield variation in some environments (G. Lark, 1996, personal communication). These QTL were not associated with other agronomic traits such as height, maturity, or stem termination that influence soybean yield (Orf et al., 1999b).

These QTL alleles may be useful in increasing the diversity of southern elite soybean populations since they may be diverse from southern soybean and have been associated with increased yield. The objective of this research was to assess the value of the three Archer QTL alleles for increased yield in southern environments and genetic backgrounds.

MATERIALS AND METHODS

Near Isogenic Lines

In 1996, 300 F_6 plants developed by single pod descent from the crosses Archer (Cianzio et al., 1991) × Asgrow A5403 and Archer × Pioneer 9641, were field-grown in Fayetteville, AR. Archer is a group I indeterminate cultivar, Asgrow A5403 is a group V determinate cultivar, and Pioneer 9641 is a group VI determinate cultivar. DNA was isolated from each plant and each was genotyped with the simple sequence repeat markers Satt144, Satt002, Sct_33, and SOYHsp176 to identify individuals that were heterozygous at these loci. All plants were harvested as $F_{6:7}$ families. In 1997, the $F_{6:7}$ families were field grown at the research farm (fine-silty, mixed, thermic, Typic Glossaqualf) in Fayetteville, AR. DNA was extracted from 20 to 60 F_7 plants from each family that was segregating for one of the selected markers. F_7 plants that were homozygous for either the Archer or the southern parent allele at the target locus were harvested as $F_{7:8}$ families. Thus, each NIL set consisted of $F_{7:8}$ families, derived from the same F_6 plant, that contrasted at the assayed marker loci (Table 1). Multiple sets of NILs, each derived from a different F_6 plant, were developed for each marker and the Archer and southern genotypes were represented by multiple $F_{7:8}$ families within each set (Table 1). The selected $F_{7:8}$ families were grown in Costa Rica in the winter of 1997-1998 and harvested as $F_{7:9}$ bulks that were used in the 1998 field trials. $F_{7:10}$ families were harvested from the 1998 trials and used in the 1999 field trials.

Table 1. Parentage, stem termination, and number of soybean lines with either the Archer or southern allele at Satt144, Satt002, or SOYHsp176/Sct_33 for each near isogenic line set.

Marker	Set	Southern parent	Stem termination	Number of lines	
				Archer	Southern
Satt144	1	9641†	indeterminate	1	2
	2	9641	indeterminate	3	2
	3	9641	determinate	1	1
	4	9641	determinate	3	2
Satt002	1	A5403	determinate	1	2
	2	9641	indeterminate	4	3
	3	9641	determinate	2	3
	4	9641	determinate	1	1
Sct_33/SoyHsp176	1	A5403	determinate	2	2
	2	9641	determinate	2	2
	3	9641	indeterminate	1	1
	4	9641	indeterminate	4	6

† 9641 = Pioneer 9641, A5403 = Asgrow A5403.

The NILs were developed from $F_{6:7}$ families selected for low lodging, low shattering potential, all were in maturity group V, and all were adapted to Arkansas.

DNA Isolation and Marker Genotyping

Soybean genomic DNA was extracted by the methods described by Keim et al. (1989). The extraction procedure was modified as described by Sneller et al. (1997). Trifoliate leaves were collected from each selected plant. Leaves were freeze dried and powdered. DNA was isolated with a standard CTAB (hexadecylatrimethylammonium bromide) extraction buffer [1% (w/v) CTAB, 0.7 *M* NaCl, 50 m*M* Tris pH 8.0, 1 μL mL^{-1} 2-mecaptoethanol, 1 m*M* phenanthroline]. DNA quantification was done with a TKO 100 fluorometer (Hoefer Scientific Instruments, San Francisco, CA). Wavelength of the fluorometer was set between 365 and 460 nm. All samples were diluted with water to a concentration of 500 ng of DNA/μL or 100 ng of DNA/μL depending on the original concentration.

Reaction mixes for polymerase chain reaction (PCR) included: 50 ng of soybean genomic DNA; 2.5 m*M* Mg^{+2}; 0.5 μ*M* of 3′ and 5′ primer; 100 μ*M* of each nucleotide; 1× PCR buffer (Promega Corporation, Madison, WI); 1× dye (Promega Corporation); and 0.07 μL of *Taq* DNA polymerase in a total volume of 11 μL. A Hybaid OMN-E thermalcycler (Hybaid Limited, Teddington Middlesex, UK) was used to perform all PCR reactions. The thermalcycler program had one 2-min denaturation period at 94°C, and 32 cycles of 25-s denaturation at 94°C, 25-s annealing at 47°C, and 25-s elongation at 72°C with a 2-min final extension at 72°C.

Separation of the PCR products (11 μL/lane) was on a 6% (w/v) polyacrylamide gel (19:1 crosslinking ratio) with 0.5 × TAE (Tris-acetate EDTA) running buffer. Ten microliters of the PCR product was loaded per lane and separated by gel electrophoresis for 100 min at 300 v. After 100 min the gel was stained with SYBER Green I nucleic acid gel stain (FMC Bio-Products, Rockland, ME) for 10 min under dark conditions. The stained gel was visualized under a UV light and photographed with Polaroid 667 film. Size of PCR products was estimated by comparing them to a ϕX174/*Hae*III ladder (Promega Corporation) containing 11 fragments ranging from 72 to 1353 base pairs. Genotypes were determined by comparing the banding pattern of each plant or family to the pattern of the parents.

Field Trials

Field trials of the NILs were preformed at the experiment stations in Rowher (very-fine, smectitic, nonacid, thermic Veric Haplaquept) and Keiser (very-fine, montmorillonitic, nonacid, thermic Vertic Haplaquept), Arkansas, in 1998 and 1999. The NILs for each marker were planted together, and data for each marker were analyzed separately. For each marker, the NILs were tested in a split plot design, with different sets being whole plots. The lines within a set were then randomly assigned to subplots. The check cultivar Hutcheson and the parents Asgrow A5403 and Pioneer 9641 were tested with each marker in a separate whole plot. Two replications were used in 1998, and three replications were used in 1999. At the Keiser location, plots consisted of four rows, each 6.1 m long with 96.5 cm between rows. The middle two rows were harvested for yield after end trimming to a final length of 4.8 m. At the Rowher location, plots consisted of five rows, each 6.1 m long with 48.3 cm between rows. The middle three rows were harvested for yield after end trimming to a final length of 4.8 m. Data were collected on height and maturity. Height was measured as the distance from the soil to the tip of the mainstem

Table 2. Results from the analysis of variance of yield, height, and maturity of sets of near isogenic soybean lines for the Satt002, Satt114, and Sct_33/SOYHsp176 markers.

	Satt144			Satt002			Sct_33/Hsp176		
Source	**Yield**	**Height**	**Maturity**	**Yield**	**Height**	**Maturity**	**Yield**	**Height**	**Maturity**
Environment (E)	**	ns†	**	**	ns	**	**	ns	**
Marker (M)	ns	ns	ns	ns	ns	ns	ns	ns	ns
E × M	ns	ns	ns	ns	ns	ns	ns	ns	ns
Set (S)	ns	**	ns	ns	**	ns	ns	ns	ns
S × M	ns	ns	ns	ns	ns	ns	ns	ns	ns
S × E	ns	**	**	**	**	ns	*	**	**
S × M × E	ns	ns	ns	ns	ns	ns	ns	ns	ns
Family (S M)	ns	ns	ns	**	**	ns	ns	**	ns

*** Significant at the $P < 0.05$ level.**
**** Significant at the $P < 0.01$ level.**
† ns, not significant.

at maturity. Maturity was measured as the number of days after 31 August when 95% of the pods had attained their mature color.

Data Analysis

Analyses of variance were run using SAS (SAS institute Inc. Cary, NC). Data from each marker were analyzed separately. Within each marker, different NIL sets were considered random affects, as were different lines with the same marker genotype within each set. Each location and year combination was considered an environment, and environments were considered random. Within each marker analysis, marker genotype (Archer or southern) was considered fixed. The different marker classes were represented by a different number of lines in some sets. When comparing marker classes across sets, we averaged by marker class within sets prior to averaging over sets so that unequal sampling of set effects would not influence the marker comparison.

RESULTS AND DISCUSSION

The main effect of marker genotype and the interactions of marker genotype with other factors were not significant ($P > 0.05$) for yield, height, or maturity for any of the three markers (Tables 2 and 3). The absence of significant marker genotype × set interactions indicates that the effect of the marker genotype did not vary by NIL set. This occurred despite the fact that the NIL sets differed from one another for stem termination and genetic background, including different southern elite parents for the NILs for the Satt002 and Sct_33/SOYHsp176 markers. Marker genotype effect was tested separately for each set and each trait and all marker genotype main effects were found to be not significant.

Set effects were significant for height for the Satt144 and Satt002 markers, primarily because the NIL sets for these markers varied by stem termination (Table 1). The set × environment interaction was significant in seven of nine tests (Table 2). This is not important to the evaluation of the putative yield QTL as it simply indicates that different NIL sets have different phenotypic response to the environments. This is expected as the NIL sets are derived from different F_6 plants and should behave as recombinant inbred lines from an inbred population.

The yield difference between the NIL with the Archer marker alleles and the NILs with the southern marker allele was small, ranging from 9 to −81 kg ha^{-1} (Table 3) and appear agronomically and statistically unimportant. In the original mapping populations, the average yield advantage with five environments of the Archer allele was 168 kg ha^{-1} for the Satt144 QTL, 140 kg ha^{-1} for the Satt002 QTL, and 136 kg ha^{-1} for the Sct_33/SOYHsp176 QTL (G. Lark, 2000, personal communication). The LSD values from our tests (Table 3) were small enough to have found such differences significant for the Satt144 and Satt002 markers, had they occurred. The LSD for the Sct_33/SOYHsp176 marker was higher in our tests than for the other two markers, still NILs with the Archer Sct_33/SOYHsp176 marker allele yielded 12 kg ha^{-1} less than the NILs with the southern marker.

There are several possible reasons why the yield superiority of Archer alleles at these QTL reported by Orf et al. (1999b) was not repeated in this study. Archer was the high-yield elite parent in the original mapping populations that uncovered these QTL. In our study, the southern parents Asgrow A5403 and Pioneer 9641 are the elite parents. Sneller et al. (1997) estimated that Archer would yield only 58% of the yield of Asgrow A5403, and 65% of the yield of Pioneer 9641 if Archer had a southern maturity and was grown in southern environments. Thus, while Archer alleles at these loci may be superior to Minsoy or Noir alleles when tested in environments where Archer is adapted, that superiority may not exist when these alleles are evaluated in southern environments and contrasted to alleles of adapted southern cultivars.

Southern and northern environments differ dramati-

Table 3. Yield, height, and maturity averaged over sets and environments of near isogenic soybean lines with either the Archer or southern parent allele at Satt002, Satt144, and Sct_33/SOYHsp176 marker loci.

Marker	Allele	Yield	Height	Maturity
		kg ha^{-1}	cm	d after 8–31
Satt002	**Archer**	**2291**	**43.4**	**34.4**
	Southern	**2372**	**43.1**	**35.1**
	LSD (0.05)	**112**	**0.6**	**0.8**
Satt144	**Archer**	**2496**	**46.5**	**36.4**
	Southern	**2487**	**45.5**	**35.5**
	LSD (0.05)	**90**	**1.3**	**1.1**
Sct_33/SOYHSP176	**Archer**	**2150**	**48.5**	**35.4**
	Southern	**2162**	**49.8**	**35.7**
	LSD (0.05)	**166**	**1.8**	**1.0**

cally in soil type, temperature, and photoperiod, as well as other factors. It is possible that these QTL may be effective only in northern environments. The yield advantage of the Archer markers in the original mapping populations was quite consistent across five testing environments (G. Lark, 2000, personal communication). It is impossible in our study to determine whether the southern testing environments, or the contrast to the southern alleles nullified the superiority of the Archer alleles. Perhaps we have overextended our extrapolation of the results from the Orf et al. (1999b) study by applying them to southern environments and southern germplasm. Marker-assisted selection for these Archer yield QTL alleles may be more successful in northern environments and northern genetic backgrounds.

The Archer and southern alleles at these QTL appeared to have equal value. Yet it is unlikely that the Archer and southern alleles are identical by descent, as the coefficient of parentage between Archer and Pioneer 9641 was 0.038, and the coefficient of parentage between Archer and Asgrow A5403 was 0.160. Archer also appears diverse from the two southern parents, based on molecular markers (Kisha et al., 1998). It is possible that Archer and southern alleles at these loci have the same effect, yet derive from different ancestors.

Recombination between the markers and the QTL could also have affected our results. If recombination occurs between a marker and a QTL, then selection based on the marker will not be effective. Recombination seems an unlikely explanation though, because each QTL and its respective marker are closely linked. Satt144 and Satt002 were 14 and 5 centimorgans (cM), respectively, from their respective QTL (G. Lark, 2000, personal communication). Nearby markers were not polymorphic in these populations, so we could not use flanking markers to minimize the effect of recombination between the Satt002 and Satt144 markers and their QTL. The markers Sct_33 and SOYHsp176 are 7.4 cM apart and were deemed to flank the yield QTL. Recombination seems unlikely to have affected the value of these two markers.

Epistasis could have also affected our ability to use these markers to improve yield. The yield QTL were not affected by epistasis in the Archer, Minsoy, and Noir genetic backgrounds, but epistasis may affect their value in the Asgrow A5403 or Pioneer 9641 backgrounds. Our NIL sets differed from the original mapping populations not only by the southern allele at the three markers, but also by theoretically having southern alleles at one half of the other genes in the genome versus one half Minsoy or Noir alleles. This may have created opportunities for epistasis. Researchers have demonstrated that yield QTL in soybeans can be effected by interactions of alleles at different loci (Lark et al., 1995; Orf et al., 1999a).

The four different NIL sets for each marker were in different, randomly generated genetic backgrounds. The lack of a marker by set interaction for all three QTL (Table 2) suggests that epistasis or recombination are unlikely explanations for our results. The value of a QTL would be expected to vary across random genetic backgrounds with epistasis or if recombination was affecting the linkage disequilibrium between the marker and the QTL alleles. This did not occur in our study, as the effect of the marker on yield was not significant for any NIL set.

There is little published research that critically evaluates marker-assisted selection to improve complex traits such as yield. Most published reports (Stuber, 1994; Zhu et al., 1999) evaluate marker-assisted selection for complex traits in populations of similar genetic background and/or similar testing environments that were used to first identify the QTL. Our results indicate that it may be difficult to extrapolate the results of marker analyses of complex traits such as yield to populations with different genetic backgrounds or to different testing environments. We need to be cognizant of the reality of introgressing a diverse allele for improved yield into an elite population. For an exotic allele to have value in an elite population, it must have superior value to all other alleles in the elite population. Universal value of the diverse allele (e.g., superior value relative to all other elite alleles) may be safely inferred for some traits where the diverse line has a superior phenotype to elite lines. For example, we would expect an allele for disease resistance from a diverse line to have universal value in an elite population where all elite lines are susceptible to the disease. Universal value of a diverse allele that appears superior relative to one elite allele in a single mapping population may be less common for traits where the elite population is phenotypically superior to the diverse line, as is often the case for yield.

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Drought and Heat Responses in the Wild Wheat Relative *Aegilops geniculata* Roth: Potential Interest for Wheat Improvement

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ABSTRACT

Wild wheat (*Triticum aestivum* L.) relatives could represent a valuable source of genetic variation for improvement of abiotic stress tolerance in cultivated wheat. A better knowledge of the adaptive strategies developed by these species is needed. A collection of 157 *Aegilops geniculata* accessions originating from different ecogeographical regions was studied during two successive years for several traits related to water status, chlorophyll content, and plant thermal regulation under Mediterranean field conditions. Close association was found between the studied traits and the origin of accessions. Two adaptive strategies were distinguished. Accessions originating from harsh environments had low biomass, low grain production and high water-use efficiency (low C isotope discrimination). They were early, with small, thick leaves exhibiting low chlorophyll content, high surface temperature and low epidermal transpiration. We suggest that in these accessions, decreased leaf chlorophyll content could limit the energy load from strong sunlight. In accessions originating from regions with a mild Mediterranean climate, thermal regulation of the leaf may rather depend on transpiration, as suggested by high C isotope discrimination values. These accessions also were characterized by high chlorophyll content, leaf area, and biomass production. Associations between the physiological traits observed could help to better understand the relationship between abiotic stress tolerance and yield in cultivated wheats. Results obtained confirmed the potential value of *Aegilops geniculata* for improvement of high temperature and drought stress tolerance in wheat and could contribute to the choice of traits to be introgressed and the accessions to be used in wide hybridization programs.

The genus *Aegilops* is closely related to *Triticum* (Kerby and Kuspira, 1988). Interest has developed in recent years in exploiting *Aegilops* spp. as important genetic resources for wheat improvement (Comeau et al., 1993; Mujeeb-Kazi, 1993; Farooq et al., 1996). *Aegilops geniculata* Roth (= *Ae. ovata* L.) is an annual, selfing (Hammer, 1980) allo-tetraploid species (2n = 4x = 28) with MU genome (Van Slageren, 1994). Among the 22 species of the genus *Aegilops* it is particularly interesting as a source of disease and pest resistance (Valkoun et al., 1985; Dimov et al., 1993). Some information is also available concerning its response to drought (Rekika et al., 1998b) and salinity (Farooq et al., 1996), suggesting that this species could represent a valuable reservoir of genes for resistance to these stresses. A better understanding of the adaptive features of *Ae. geniculata* may promote its use for wheat genetic improvement. *Aegilops geniculata* grows in Mediterranean regions (Van Slageren, 1994) characterized by a dry summer season with high temperature and high irradiance. As with other wild species, it can acclimate to these constraints by escape, avoidance, and tolerance (Blum, 1988).

Escape and avoidance traits are likely to play an important role in adaptation to specific environments (Monneveux and Belhassen, 1996). Escape from water, heat, and high radiation stresses can be achieved under Mediterranean conditions by shortening of the growing cycle. Earliness, however, reduces light absorbed by the crop and consequently total biomass and potential yield. Drought avoidance comprises mechanisms involved in water potential maintenance, such as the reduction of stomatal conductance and leaf area. Stomatal closure, which is the most efficient way to reduce water loss, negatively affects CO_2 assimilation (Yin et al., 1995). Stomatal transpiration also plays a major role in plant temperature regulation (Chetti et al., 1997; Singh et al., 1997). In wheat, stomatal conductance correlates with canopy temperature depression in a wide range of climatic conditions (Amani et al., 1996; Lu et al., 1998). Epidermal transpiration comprises both a nonstomatal (cuticular) and a stomatal component. The first corresponds to water loss through the leaf epidermis, and the second to transpiration due to incomplete closure of stomates (Kirkham et al., 1980; Muchow and Sinclair, 1989). Epidermal transpiration has proved to be an im-

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Abbreviations: BIOM, plant biomass; CHL, total chlorophyll content; DH, days to heading; Δ, carbon isotope discrimination; EPT, epidermal transpiration rate; GW, grain weight per plant; LA, leaf area; LCO, leaf colour; LL, leaf length; PTD, plant temperature depression; RWC, relative water content; SLDW, specific leaf dry weight.