IMPROVING SOYBEAN USING EXOTIC GERMPLASM

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ABSTRACT

Elite soybean [*Glycine max* (L.) Merr.] germplasm in North America has narrow genetic diversity while exotic germplasm has proven to be an important source of genes especially for disease and pest resistance. An example of the successful use of exotic germplasm in breeding is our research on resistance to soybean aphid [*Aphis glycines* Matsumura (Hemiptera: Aphididae)]. Soybean aphid was first reported in North America in 2000 and since that time, infestations have reached economic threshold annually. Modern cultivars and accessions from the soybean germplasm collection were screened for resistance. No resistance was found in modern cultivars but seven accessions with resistance were identified from the germplasm collection. Resistance in two accessions was characterised as antibiosis and a major resistance gene was mapped in one resistance source and this gene was named *Rag1*. With the use of marker-assisted selection (MAS), this resistance was rapidly introgressed into high yielding backgrounds. This selection was initially done using microsatellite markers but single nucleotide polymorphism (SNP) markers are now being used. *Rag1* is being fine mapped to identify closely linked markers that could be used in MAS and in the eventual cloning of this gene. These efforts are being aided by the recent sequencing of the soybean genome, an advancement which has lead to the rapid development of genomic tools that can be applied to breeding.

KEYWORDS

soybean, marker-assisted selection, soybean aphid, germplasm

INTRODUCTION

This paper will provide a general overview of the soybean crop and then give details on our research on soybean aphid resistance. We will first start with a discussion of the soybean industry, followed by information on soybean breeding and genetics research and on genetic diversity in this crop. The paper will then conclude with an overview of how we are utilizing new research resources in studying and breeding for soybean aphid resistance.

Soybean [*Glycine max* (L.) Merr.] is the leading oilseed crop produced worldwide (Wilcox, 2004). The crop was domesticated in China from the wild annual species *Glycine soja* Sieb. and Zucc. (Hymowitz, 2004). While significant production continues in China, countries in the Western Hemisphere have become the largest producers with the USA, Brazil and Argentina leading the world in soybean production (Wilcox, 2004). In the USA, soybean production is largely confined to the eastern half of the country where the climate is favourable for growth and development of the crop.

Soybean production and usage are continuing to increase. The total world production of soybean in the 2005-2006 cropping year was 220.2 million metric tons produced on 92 million hectares (Golbitz, 2007). This represents a production increase of almost 40% between the 1999-2000 growing season and the 2005-2006 season. A large portion of this change was due to increased production in Brazil where production increased 62% during this period (Golbitz, 2007). These production increases have been offset by consumption increases, especially in China. Most soybean seed produced worldwide is processed and used as a source of vegetable oil and a high protein feed source for animals (Sonka *et al.* 2004).

SOYBEAN BREEDING RESEARCH

Over the past thirty years, the development of soybean cultivars has shifted from being a public sector activity to an activity that is now dominated by the private sector. This shift has occurred through major investments in soybean breeding by private companies, which has resulted in a large increase in the number of experimental lines tested in the field by breeders annually. Although it is impossible to estimate the impact of these investments on the annual yield gains of cultivars enjoyed by the industry, industry breeding is likely having a major impact in fuelling these yield gains. The most recent estimate of yield gains in soybean was 23 kg ha^{-1} yr^{-1}(Specht *et al.* 1999).

Soybean has the most transgenic (or genetically modified organism, GMO) hectares grown of any crop. It is estimated that 91% of the crop grown in the U.S.A. in 2007 (http://www.ers.usda.gov/Data/BiotechCrops/Extentof AdoptionTable3.htm) and 53% in Brazil during the 2007-2008 growing season was transgenic. The vast majority of the GMO area was grown to Roundup Ready soybeans, which have a transgenic event that provides tolerance to the herbicide glyphosate (trade name is Roundup) (Padgette *et al.* 1995). During the next few years, companies will be releasing cultivars

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with new stacked transgenic events that combine tolerance to glyphosate and other herbicides as weeds with Glyphosate tolerance are becoming more common.

SOYBEAN MOLECULAR BIOLOGY AND GENOMICS

Advances in molecular biology and genetics are impacting soybean breeding dramatically through increased use of marker-assisted selection (MAS). MAS has been under development for many years and is now widely embraced by most breeders. The reasons for the wide use of MAS include reduced genotyping costs, the identification of more associations between markers and economically important traits, and the development of better methods for incorporating markers into breeding programs. An example of this wide use is that in 2003, Pioneer Hi-Bred reported gathering two million marker data points in support of their soybean breeding effort (Cahill and Schmidt, 2004). Since that time, the use of MAS at Pioneer and other seed companies has increased further.

MAS in soybean has thus far been largely used to select traits with fairly simple inheritance, such as disease resistance. One trait that MAS has been especially successful and now used widely is resistance to soybean cyst nematode (SCN, *Heterodera glycines* Inchinohe) (Cregan *et al.* 1999; Glover *et al.* 2004). Although the genetics of SCN resistance is not very complex, phenotyping plants for SCN resistance is tedious, time consuming, and expensive, making MAS a quick and effective method to increase resistance (Concibido *et al.* 2004). MAS is now being used to select genotypes that carry SCN resistance, and resistance to other pathogens, early in the breeding process resulting in yield testing of only resistant genotypes.

Fuelled by new high throughput DNA sequencing and analysis technologies, advancements in soybean genetics and genomics are producing valuable resources that can be applied to soybean improvement. A major recent advancement that will greatly impact the soybean research community is the sequencing of the soybean genome by the US Department of Energy Joint Genome Institute. Using Sanger sequencing technology, they have released a draft sequence of the soybean genome (http://www.phytozome.net/soybean). This sequencing will impact soybean improvement in a number of ways. Firstly, this will lead to a much greater availability of genetic markers. Although over one thousand simple sequence repeat (SSR) markers are available for soybean research (Song *et al.* 2004), marker availability has often been limited in genetic regions of interest in specific backgrounds. The sequencing of the soybean genome coupled with resequencing of additional genotypes using ultra high throughput sequencing technology should result in the identification of hundreds of thousands of SNPs. This will aid in soybean improvement and the use of MAS by making it possible for breeders to identify markers that are polymorphic between their parents and are very tightly linked to genes of interest. These SNP markers will be especially useful in fine mapping projects, which requires many markers on a sub-centiMorgan interval level. In addition, the genome sequencing also will speed the cloning of genes of agronomic interest. Cloning will give breeders perfect markers that can be used in MAS and will make it possible to screen germplasm for the presence of cloned genes and to identify new allelic diversity.

The increased availability of SNP markers and improved SNP marker detection technology has resulted in the soybean research community beginning to embrace SNP markers instead of SSR markers in research and breeding. SNP markers can be valuable tools for situations where mapping populations need to be tested with hundreds of markers to map genes or QTL. New technologies such as the Illumina Beadstation SNP detection system can be used to rapidly test populations and a soybean GoldenGate assay with 1,536 SNP markers has been developed for this system in the lab of Perry Cregan (USDA-ARS). With the GoldenGate assay, a researcher can genotype 96 DNA samples in approximately two days. For MAS, SNP markers can be used efficiently to genotype many individuals with few markers using TaqMan (Meksem *et al.* 2001) or melting curve assays.

SOYBEAN GENETIC DIVERSITY

The genetic base of modern soybean cultivars in North America is narrow due to its limited initial domestication base and several decades of intensive breeding and selection. Gizlice *et al.* (1994) showed that over 86% of the genes present in modern North American soybean cultivars could be traced to a collection of 17 ancestors and first progeny. Since genetic variability is necessary for genetic progress, this limitation of genetic diversity may impede future genetic gains in soybean breeding unless new sources of genetic variability are introduced into breeding programs.

The United States Department of Agriculture-Agricultural Research Service (USDA-ARS) maintains a large soybean germplasm collection at the University of Illinois. This collection includes over 21,000 accessions collected throughout the world (http://www.ars-grin.gov/cgi-bin/npgs/html/site.pl?SOY). Included in this collection are related perennial species, annual wild *G. soja*, and almost 19,000 soybean accessions that range from primitive Asian landraces to elite cultivars, making the collection a resource that can be used to help overcome the lack of diversity in elite North American germplasm. Breeders and geneticists in North America have been successful in utilizing exotic soybean germplasm as sources of new alleles, especially for disease resistance and other relatively simply inherited traits (Carter *et al.* 2004). However, it still remains a challenge to use the collection to improve complex traits such as seed yield.

SOYBEAN APHID RESEARCH

As an example of how we have been able to combine the use of exotic germplasm, genetic markers, and other genomics tools in plant improvement, we will describe our research on resistance to soybean aphid (*Aphis glycines* Matsumura) in detail. Soybean aphid is a relatively new soybean insect pest that was first discovered in North America in 2000 (Hartman *et al.* 2001) and has rapidly spread throughout the Midwestern USA and southern Canada since its first report (Venette and Ragsdale, 2004). Feeding by soybean aphid may reduce seed yield directly by causing plant stunting, leaf distortion, and reduced pod set (Hartman *et al.* 2001; Hill *et al.* 2004). An additional threat posed by the soybean aphid is its ability to transmit plant viruses such as *Alfalfa mosaic virus,* and *Soybean mosaic virus* to soybean (Sama *et al.* 1974; Hartman *et al.* 2001). Insecticide applications are the only method currently available to growers to control soybean aphids. During the 2003 soybean aphid outbreak, nearly 3 million ha of soybeans in the U.S.A. were sprayed to control the pest (Landis *et al.* 2003).

Plant resistance can provide an effective, economical, and environmentally sound method of insect control. Shortly after finding soybean aphids in North America, accessions in the USDA-ARS soybean germplasm collection were screened for resistance to soybean aphid. From a screening of over 1,500 genotypes, Hill *et al.* (2004) reported the identification of seven aphid resistant soybean germplasm accessions. Among the resistant genotypes, the ancestral cultivars Dowling and Jackson were characterised as having resistance that is primarily antibiosis in action based on choice and non-choice greenhouse experiments (Li *et al.* 2004). Using a bulked segregant analysis with SSR markers, a single major resistance gene conferring resistance was mapped to the same genetic position in both Dowling and Jackson and the gene from Dowling was named Rag1 (Li *et al.* 2007).

The mapping of Rag1 from Dowling resulted in the identification of genetic markers that are useful in MAS for aphid resistance breeding. These markers allowed us to rapidly backcross Rag1 into elite cultivars adapted to the Midwestern U.S.A. through the selection of F1 plants that carried Rag1 during each backcross generation. By combining MAS with the rapid cycling of generations in a greenhouse, we were able start with F2 plants from a two-way cross and produce F1 plants developed through five backcrosses (BC5F1) in 15 months. We now routinely use MAS and SNP markers to select plants carrying Rag1 in segregating populations. The SNP markers are detected with TaqMan (Meksem *et al.* 2001) or melting curve assays eliminating the need for gel electrophoresis. The Rag1 gene is currently being fine mapped in our laboratory with the goals of identifying new genetic markers more tightly linked to the gene and eventually cloning it. Our strategy for fine mapping Rag1 first involves screening thousands of F2 plants developed through backcrossing with markers known to flank the resistance gene. Plants identified with a recombination event in the region containing the gene are then selected and grown to maturity, and the progeny from these plants are tested for resistance. Because the Joint Genome Institute sequencing efforts have provided the DNA sequence across the interval where Rag1 maps, we have been able to quickly identify additional SNP markers close to the gene by designing primers based on this sequence information. These efforts have allowed us to map the gene to a 152 kb region.

We have recently shown that there is biotype diversity for soybean aphid in North America (Kim *et al.* 2008) and there is currently at least one biotype that can overcome Rag1 resistance. This finding has brought focus to the need for identifying additional aphid resistance genes. Further screening of the soybean germplasm collection has resulted in the identification of over 40 new sources of resistance to the new aphid biotype. The current challenge is to develop methods that will allow us to efficiently determine the location of resistance alleles among these new sources of resistance allowing us to identify which germplasm lines carry new resistance alleles that can be used to broaden our diversity for resistance alleles in varieties.

REFERENCES

Cahill, D.J. and D.H. Schmidt. 2004. Use of marker assisted selection in a product development breeding program. Proceedings of the 4th International Crop Science Congress, 26 Sept.-1 Oct. 2004, Brisbane, Australia. http://www.cropscience.org.au/icsc2004/symposia/3/4/133_schmidtdh.htm (verified 1 July 2008).

Carter, T.E., R.L. Nelson, C.H. Sneller and Z. Cui. 2004. Genetic diversity in soybean. pp. 303-416. *IN* H.R. Boerma and J.E. Specht (eds.) Soybeans: improvement, production, and uses. 3rd ed. Agron. Monogr. 16. ASA, Madison, WI.

Cregan, P.B., J. Mudge, E.W. Fickus, D. Danesh, R. Denny and N.D. Young. 1999. Two simple sequence repeat markers to select for soybean cyst nematode resistance conditioned by the rhg1 locus. Theoretical and Applied Genetics 99: 811-818.

Concibido, V.C., B.W. Diers and P.R. Arelli. 2004. A decade of QTL mapping for cyst nematode resistance in soybean. Crop Sci. 44: 1121-1131.

Gizlice, Z., T.E. Carter, Jr. and J.W. Burton. 1994. Genetic base for North American public soybean cultivars released between 1947 and 1988. Crop Sci. 34: 1143-1151.

Glover, K.D., D. Wang, P.R. Arelli, S.R. Carlson, S.R Cianzio and B.W. Diers. 2004. Near isogenic lines confirm a soybean cyst nematode resistance gene from PI 88788 on linkage group. J. Crop Sci. 44: 936-941.

Golbitz, P. 2007. Soya and Oilseed Bluebook. Soyatech, Inc., Bar Harbor, ME.

Hartman, G.L., L.L. Domier, L.M. Wax, C.G. Helm, D.W. Onstad, J.T. Shaw, L.F. Solter, D.J. Voegtlin, C.J. D'Arcy, M.E. Gray, K.L. Steffy and P.L. Orwick. 2001. Occurrence and distribution of Aphis glycines on soybean in Illinois in 2000 and its potential control [Online]. Available at http://www.plantmanagementnetwork.org/pub/php/brief/aphisglycines/ (verified 1 July 2008).

Hill, C.B., Y. Li and G.L. Hartman. 2004. Resistance to the soybean aphid in soybean germplasm. Crop Sci. 44: 98-106.

Hymowitz, T. 2004. Speciation and cytogenetics. p. 97-136. In H.R. Boerma and J.E. Specht (ed.) Soybeans: improvement, production, and uses. 3rd ed. Agron. Monogr. 16. ASA, Madison, WI.

Kim, K.S., C.B. Hill, G.L. Hartman, M.A. Mian and B.W. Diers. 2008. Discovery of soybean aphid biotypes. Crop Sci. 48: 923-928.

Landis, D., M. Brewer and G. Heimpel. 2003. Soybean Aphid Parasitoid Questionnaire 2003 [Online] http://www.ncera125.ent.msu.edu/StateRpts2003MI.htm. (verified 1 July 2008).

Li, Y., C.B. Hill and G.L. Hartman. 2004. Effect of three resistant soybean genotypes on the fecundity, mortality, and maturation of soybean aphid (Homoptera: Aphididae). J. Econ. Entomol. 97: 1106–1111.

Li, Y., C.B. Hill, S.R. Carlson, B.W. Diers and G.L. Hartman. 2007. Soybean aphid resistance in the soybean cultivars Dowling and Jackson map to linkage group M. Mol. Breeding 19: 25-34.

Meksem, K., E. Ruben, D.L. Hyten, M.E. Schmidt and D.A. Lightfoot. 2001. High-throughput genotyping for a polymorphism linked to soybean cyst nematode resistance gene Rhg4 by using Taqman probes. Molecular Breeding 7: 63-71.

Padgette, S.R., K.H. Kolacz, X. Delannay, D.B. Re, B.J. LaVallee, C.N. Tinius, W.K. Rhodes, Y.I. Otero, G.F. Barry, D.A. Eichholtz, V.M. Peschke, D.L. Nida, N.B. Taylor and G.M. Kishore. 1995. Development, identification, and characterisation of a glyphosate-tolerant soybean line. Crop Sci. 35: 1451-1461.

Sama, S., K.M. Saleh and P. van Halteren. 1974. Research Reports 1969-1974. p. 171-172. *IN* Varietal screening for resistance to the aphid, *Aphis glycines*, in soybean. Agricultural Cooperation, Indonesia, the Netherlands.

Song, Q.J., L.F. Marek, R.C. Shoemaker, K.G. Lark, V.C. Concibido, X. Dalanay, J.E. Specht and P.B. Cregan. 2004. A new genetic linkage map for soybean. Theor. Appl. Genet. 109: 122-128.

Sonka, S.T., K.L. Bender and D.K. Fisher. 2004. Economics and marketing. p. 919-947. In H.R. Boerma and J.E. Specht (ed.) Soybeans: improvement, production, and uses. 3rd ed. Agron. Monogr. 16. ASA, Madison, WI.

Specht, J.E., D.J. Hume and S.V. Kumundini. 1999. Soybean yield potential – a genetic and physiological perspective. Crop Sci. 39: 1560-1570.

Venette, R.C. and D.W. Ragsdale. 2004. Assessing the invasion by soybean aphid (Homoptera: Aphididae): where will it end? Ann. Entomol. Soc. Am. 97: 219-226.

Wilcox, J.R. 2004. World distribution and trade of soybean. p. 1-14. In H.R. Boerma and J.E. Specht (ed.) Soybeans: improvement, production, and uses. 3rd ed. Agron. Monogr. 16. ASA, Madison, WI.