Chapter 6
Limited-Transpiration Trait for Increased Yield for Water-Limited Soybean: From Model to Phenotype to Genotype to Cultivars

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Abstract Soybean (*Glycine max* (L.) Merr.) is the most widely grown grain legume in the world due to its many uses in feed, food, and industrial products. However, soybean yield is particularly sensitive to soil water deficits, and seemingly, opportunities exist to increase yield by improving specific plant traits. One trait that has proven to be especially useful is the limited-transpiration trait in which water loss by the plants is constrained by the plant under high atmospheric vapor pressure deficit conditions. This chapter reviews the integrated studies at several levels and disciplines to identify the trait, develop some physiological and genetic understanding of the trait, apply classical breeding approaches to develop germplasm expressing the trait, and a simulation analysis across the USA to identify where, how often, and how much the trait in soybean will benefit farmers. The research on the limited-transpiration trait has now led to higher yielding commercial germplasm for water-deficit environments based on expression of the limited-transpiration trait. As often suggested but rarely put into practice, a multi-level, multi-faceted approach was applied in the study of the limited-transpiration trait to generate scientific understanding that was applied in crop breeding to generate higher yielding genotypes.

6.1 Introduction

Soybean (*Glycine max* (L.) Merr.) is grown globally to the greatest extent by far among grain legumes, with a total annual production of about 250 million tons (Sinclair and Vadez 2012). The high protein and oil contents of its seeds cause it to have many uses. Its protein is used to feed both livestock and humans, and its oil is

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used in cooking. In addition, soybean oil has many industrial uses. Of course, a critical advantage of soybean is that it can express high rates of symbiotic nitrogen fixation alleviating the need for nitrogen fertilizer. Expanding consumption of meat and high prices of fossil fuel will result in continued increases in global production of soybean.

The increase in demand for soybean will likely require production in areas with uncertain rainfall patterns. Even now soybean is often subjected to sufficient soil drying that yields are constrained (Purcell and Specht 2004). One reason that soybean yield is especially sensitive to soil drying is the sensitivity of its symbiotic nitrogen fixation to even modest decreases in soil water content (Sinclair and Vadez 2012). Recently, we have given considerable attention to traits that result in early-season soil water conservation so that more water is available to complete seed fill under drought conditions, which occur more commonly late in the growing season. A specific trait that is especially promising in soybean is one in which transpiration rate is limited under high midday vapor pressure deficit. Partial restriction of transpiration rate under high vapor pressure deficit limits the rate of soil water use, allowing the crop to conserve water for sustaining physiological activity if late-season drought develops.

The objective of this chapter is to review the advances in developing the limited-transpiration trait in soybean. The approach to this trait generally followed the top-down approach leading to development of cultivars expressing the desired trait as originally proposed by Sinclair et al. (2004). In this approach, the sequence of steps undertaken include (1) initial exploration of the trait using a simulation model, (2) discovery of genotypic variation for the trait, (3) physiological studies on the nature of the trait, (4) genetic screen for the trait, and (5) development and assessment of cultivars with desired traits. Each of these steps is explored as essential components in progress in understanding the cropping system leading to yield increase.

6.2 Initial Model Exploration of Limited Transpiration Trait

The study of the limited-transpiration trait can be traced to a brainstorming session among Tom Sinclair, Graeme Hammer and Eric van Oosterom on one spring afternoon in 2004 under a tree at the University of Queensland in Brisbane, Australia. Dr. Sinclair was at the University of Queensland for two months with the rather ambiguous plan to "think" about plant traits that might improve crop yields under drought conditions. We ended up focusing on the idea that restricting transpiration under high atmospheric vapor pressure deficit (VPD) offered the possibility for conservation of soil water for use late in the growing season to support seed fill. We hypothesized that decreased transpiration rate under high VPD would have the double benefit of increasing transpiration water use efficiency, and conserving soil water for use later in the growing season.

We then undertook an initial assessment of the putative benefit of the limited-transpiration trait by simulating the development and growth of sorghum (*Sorghum* *bicolor* L.) at four locations in Australia for which 115 years of weather had been assembled (Sinclair et al. 2005). The analysis was done using a comparatively simple, mechanistic sorghum/maize model (Sinclair et al. 1997; Sinclair and Muchow 2001). In this model, the increase in leaf area development is simulated daily as a function of temperature and constrained when soil water reaches low levels. The leaf area is used to calculate the daily growth of the crop by multiplying intercepted solar radiation by the radiation use efficiency. Radiation use efficiency was held constant except when it was decreased as soil water content reached low levels. Transpiration was calculated as a function of the crop growth, which was shown by Tanner and Sinclair (1983) to be an obligatory relationship with an essentially constant coefficient for each crop species. Seed growth was simulated as a linear increase in harvest index during seed fill.

The limited-transpiration trait was simulated by adapting the model from daily time step calculations to hourly time steps. Models to extrapolate daily minimum and maximum temperature, and solar radiation were used to obtain hourly estimates from daily weather input. Hourly values of vapor pressure deficit were calculated from the estimates of hourly temperature and the minimum daily temperature. The limited-transpiration trait was imposed by simply setting a maximum hourly transpiration rate. Whenever the initsial calculation of transpiration exceeded this limit, the transpiration rate was set equal to the limit. Also, in these cases hourly carbon accumulation was decreased to correspond to the decrease in transpiration rate. As a consequence, during the midday period the transpiration rate could be constant as illustrated in Fig. 6.1.

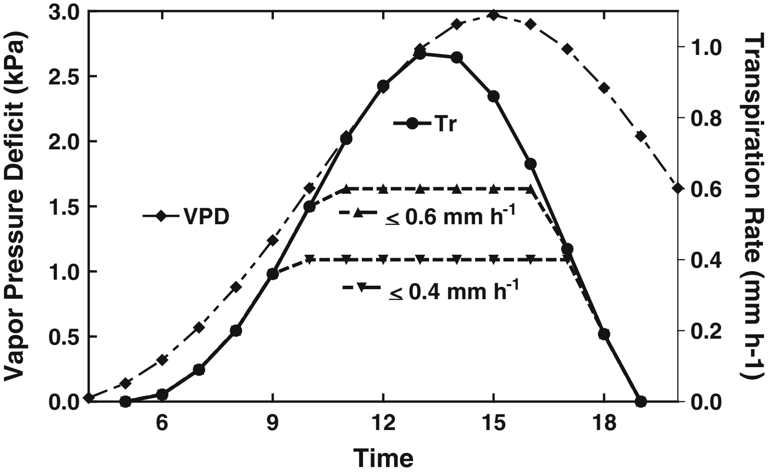

Fig. 6.1 Plot through the daily cycle of the transpiration rate of two limited-transpiration phenotypes (maximum transpiration rates of 0.4 or 0.6 mm h^{-1}) in which there is a constant transpiration rate once a maximum rate is reached (Sinclair et al. 2005). The limited-transpiration water loss pattern contrasts with the standard phenotype (*solid line*) in which there is no limitation on transpiration rate at high vapor pressure deficit. The vapor pressure deficit through the daily cycle is included as a reference

The results of the simulations for sorghum at the four locations in Australia were consistent. The mean yield increase over all seasons was in the range of 5 %. However more importantly, in growing seasons when yield was less than 450–500 g m^{-2} there was a yield increase in almost all growing seasons as a result of the transpiration-limited trait. The yield increase in these low-yielding seasons was generally around 10 %. In growing seasons above this threshold yield, yields were decreased due to the trait, but the decreases were generally quite small. Yield increases were simulated in about 75 % or more over all growing seasons. On balance, it was concluded that sorghum farmers in Australia would welcome the limited-transpiration trait since it resulted in yield increases in the economically threatening seasons of low yield, even if a small yield decrease was the price of this trait in the seasons of highest yield.

6.3 Studies of Limited-Transpiration Phenotype

Having shown in the sorghum model assessment that the limited-transpiration trait resulted in yield benefits in dry seasons, the next question was whether the idea also applies to soybean and if so, whether there is the possible expression of the trait in existing soybean germplasm. An experiment was done by one of the authors (TEC) that greatly narrowed the number of candidate genotypes that might express the limited-transpiration trait. A nursery of soybean genotypes was subjected to soil drying once full canopy had developed. During the drying cycle the onset of wilting for each genotype was observed. Two lines in particular were found to have delayed wilting with respect to all other lines: PI 416937 and PI 471938. Genotype PI 416937 was initially explored for several physiological traits for drought conditions but no specific trait was clearly identified to account for the delayed wilting (Sloane et al. 1990; Hudak and Patterson 1995).

Subsequently, the two ‘slow-wilting’ lines were investigated for the limited-transpiration trait (Fletcher et al. 2007). Genotype PI 416937 was found to express the desired limited-transpiration trait while PI 471938 did not (Fig. 6.2). In PI 416937, there was essentially a constant transpiration rate (Fig. 6.2c) at VPD greater than 2.1 kPa, which was an expression of the limited-transpiration trait explored in the sorghum model. Therefore, subsequent studies focused on PI 416937 in understanding the limited-transpiration trait. This genotype is a plant introduction from Japan with unknown parentage (Pantalone et al. 1999; Carter et al. 2003).

The hypothesis that was explored was that hydraulic conductance in the plants of PI 416937 was insufficient to allow water loss from leaves to be replenished under high VPD conditions. Three approaches were studied to determine if the hydraulic conductance in the leaves of PI 416937 was less than expressed in other genotypes (Sinclair et al. 2008). While all approaches indicated a low leaf hydraulic conductance in the leaves of PI 416937, the results for the temporal kinetics of rehydration of leaves were particularly interesting. The results showed that there were two distinct water compartments in soybean leaves, and both were much more

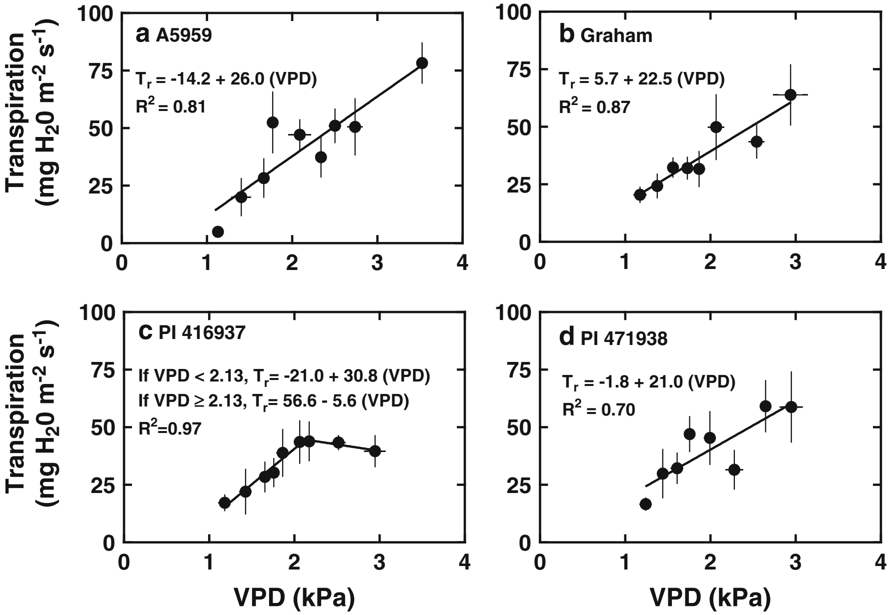

Fig. 6.2 Plot of transpiration rate for various vapor pressure deficits (VPD) of four soybean genotypes (Fletcher et al. 2007). Panel (**c**) presents the results for PI 416937 in which the transpiration rate was limited at a VPD threshold of 2.13 kPa. This result contrasts with other genotypes in which transpiration increased linearly over the whole range of tested VPD

slowly refilled in PI 416937 than in other genotypes. Of particular interest for the limited-transpiration trait, was the implication that hydraulic conductance between the xylem and the guard cells was low in PI 416937. A low hydraulic conductance in the leaves is likely to result in limited water flow to maintain turgor in guard cells for maximum pore opening.

Since no obvious unique features were observed in the leaf anatomy of PI 416937, the hypothesis was explored that the limited hydraulic conductance of PI 416937 may result from a unique population of aquaporins, i.e., water-transporting proteins in cell membranes. The population of aquaporins in PI 416937 was hypothesized to have lower capacity to transport water in the pathway from the xylem to guard cells. Sadok and Sinclair (2010a) subjected PI 416937 and a genotype without the limited-transpiration trait to two aquaporin inhibitors: mercury and silver. Transpiration of leaves of the two genotypes decreased equally when treated with mercury. However, when treated with silver ions the transpiration rate decreased substantially in the non-limited transpiration genotype while the transpiration rate of PI 416937 decreased only a small amount. Additionally, the comparative insensitivity of PI 416937 for decreasing transpiration rate to treatment with silver in comparison with many other soybean genotypes was documented by Sadok and Sinclair (2010b) (Fig. 6.3).

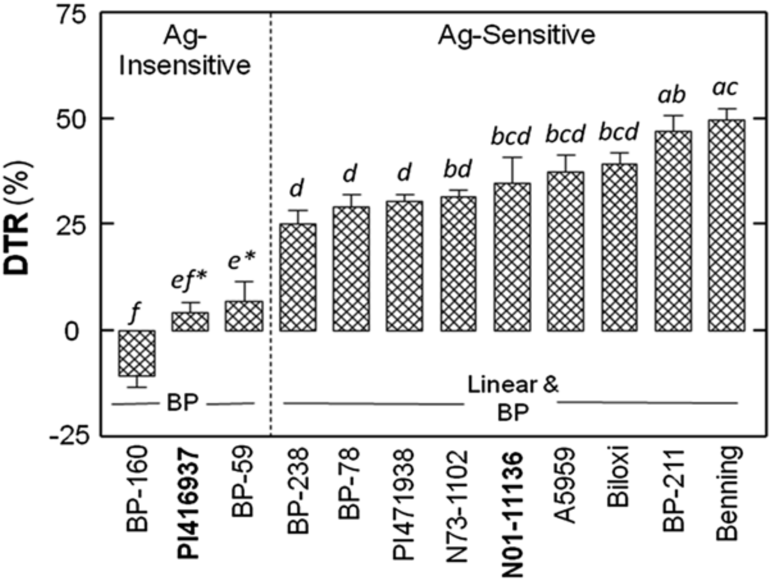

Fig. 6.3 Decrease in transpiration rate (DTR, expressed as percentage) of leaves of 12 soybean genotypes in response to the feeding with silver ion (Sadok and Sinclair 2010b). Line PI 416937 and two of its progeny lines (BP-160 and BP-59) were virtually insensitive to the silver treatment. These three lines were also found to express a breakpoint (BP) indicative of the limited-transpiration trait. The remaining lines had decreased transpiration rate with silver and these lines either expressed a BP or did not express a BP (i.e., a linear response to increasing vapor pressure deficit)

Sadok and Sinclair (2010b) hypothesized one explanation for the insensitivity of transpiration rate in PI 416937 to treatment with silver may be that it has fewer, or maybe none, of the aquaporins present in other genotypes that caused them to have decreased transpiration when treated with silver. The absence of the hypothesized silver-sensitive population of aquaporins in PI 416937 is consistent with the observation that the leaf hydraulic conductance of this genotype is less than that of other genotypes. That is, without the silver-sensitive aquaporins PI 416937 has restricted capacity for water movement to the guard cells resulting in limited transpiration rate under high VPD.

6.4 Studies of Limited-Transpiration Genotype

The possibility of sorting out the genetic expression of the limited-transpiration trait was explored in a recombinant inbred lines *(RILs)* population derived from the mating of PI 416937 and the cultivar Benning. While Benning also expressed a transpiration breakpoint in its response to increasing VPD (result not shown), the limitation on transpiration rate at high VPD was much less than in PI 416937. Sadok and Sinclair (2009) found in a comparison of 22 RIL lines from the mating of PI 416937×Benning that nine expressed the limited-transpiration trait at high VPD while thirteen did not. The breakpoint in the transpiration rate occurred at lower VPD (1.1–1.9 kPa) than for either parent.

A challenge in examining genotypic expression of the limited-transpiration trait is the limited capacity to measure directly the response of plants to a range of VPD. The possibility of using a screen based on transpiration response to silver treatment was explored since many genotypes (40+) could be tested in one day. Sadok and Sinclair (2010b) measured the response of five RILs to treatment with silver. The two RILs, which expressed the breakpoint in transpiration rate with increasing VPD, were also found to be insensitive to treatment with silver. The two RILs not expressing the breakpoint in transpiration with increasing VPD were quite sensitive to the silver treatment. However, one RIL that expressed a breakpoint was also sensitive to silver, indicating some ambiguity in interpreting the silver results.

Regardless of the specific mode of action of silver ions, the tests of Sadok and Sinclair (2010b) indicated that silver might be used as a rough screen of genotypes as a surrogate for direct measurements of the limited-transpiration trait. Therefore, a survey of the RIL population derived from PI 416937×Benning was done for transpiration response to silver treatment of leaves (Carpentieri-Pipolo et al. 2012). A wide range in response was observed in the initial screen of 147 RILs. Forty-eight lines were selected from the extremes of the initial screen for retest for silver response and quantitative trait loci (QTL) analysis. Ultimately, four major QTLs were identified as being associated with the segregation of RILs for their silver response. The phenotypic variation explained by each of these QTLs was greater than 16 % and the total variation explained by the four QTLs was 87.5 %. Two of the loci appeared to be associated with PI 416937 and two with Benning.

A complementary approach to the identification of genes involved in the limited-transpiration trait is transcriptomics. Analysis of the expression patterns under high and low VPD reveals not just the genes induced by the high VPD but also the genes whose transcription is inhibited. Identification of several stress specific genes could help understand the physiological networks involved in stress responses.

Illumina Hiseq sequencing was performed on leaf tissues of three soybean genotypes: PI 416937, PI 471938, and Hutcheson, which is fast wilting with a linear increase in transpiration rate with increasing VPD. Differential expression was tested between exposure of plants to low VPD and high VPD under which the limited-transpiration trait would be expressed. Of the 49,408 annotated genes, only one gene differentially expressed in Hutcheson between exposure of low and high VPD and 22 in PI 471938. In contrast, PI 416937 differentially expressed 944 genes between exposure to low and high VPD conditions (Devi et al. 2015a).

With an objective to display differentially expressed genes onto pathways and to obtain an overview of genes affected in response to high VPD in PI 416937, the MapMan tool was used on the 944 genes in PI 416937 for which differential expression values were available. A total of 425 transcripts had up-regulated expression variation with log 2 fold changes from 2 to 9 and 519 with significant down-regulated expression with log 2 fold of −2 to −10. The overview map of Mapman

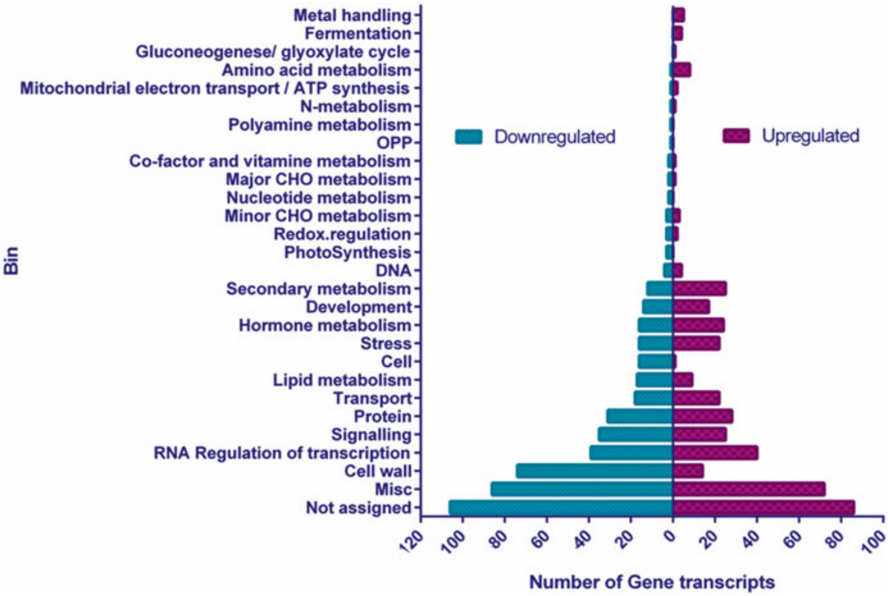

Fig. 6.4 Number of gene transcripts up or down regulated under high vapor pressure deficit conditions in soybean phenotype exhibiting limited transpiration trait. Annotated genes are categorized in to 28 functional classes (BINs) using MapMan

showed differentially expressed genes assigned into 28 classes of a total of 36 functional classes, referred as BINs (Thimm et al. 2004; Usadel et al. 2009). Of these classes, the majority of the genes were classified as unknown or not assigned, while the remaining were identified as belonging to known metabolic pathways (Fig. 6.4). This allowed exploration of gene categories that are activated during high VPD conditions and may have been involved in the processes associated with the limited-transpiration trait.

Based on the assigned genes to different BINs, an attempt was made to understand differentially expressed genes of key metabolic reactions that often modulate normal cellular functioning under the high VPD conditions. As a result, cell, cell wall and development, RNA, lipid metabolism, secondary metabolism, stress-related genes, protein, signaling and transport categories were analyzed in detail.

6.4.1 Cell, Cell Wall and Development

Changes in the magnitudes of cell and cell wall transcripts have been identified to play crucial roles in cellular metabolism. The genes related to cell metabolism (17) are mostly involved in the process of cell organization and are down regulated.

Genes coding cell wall (74) had decreased transcript abundance and the majority of those belong to cell wall degradation and modification (39) and some cell wall proteins are abundant in both up- and down-regulated categories. Some of the genes related to development mostly late embryogenesis abundant (LEA) proteins and storage proteins were found in both the up- and down-regulated categories.

6.4.2 Secondary, Hormone and Lipid Metabolism

Flavonoids and isoflavonoids are known to play a significant role in plant defense responses to pathogens (Dixon and Steele 1999; Uppalapati et al. 2009). Several genes related to secondary metabolism such as phenylpropanoids, flavanoids and simple phenols which are mostly over expressed were observed in response to high VPD. Several genes involved in phenylpropanoid metabolism, such as phenyl ammonia lyase, coumarate:CoA ligase, lignin biosynthesis, were observed in the study. All the aforementioned genes are in both over expressed and suppressed lists but commonly appeared in induction. Hormonal genes which are differentially over expressed are ethylene and jasmonate metabolism related genes. All abscisic acid, gibberellin, auxin and brassino-steroid related genes are repressed, that included some jasmonate metabolism related genes too. It is already well known that these hormones, especially ABA, are involved in stomatal regulation (Wang and Irving 2011). Lipid metabolism related genes include lipid degradation, sphingolipids, fatty acids (FA) synthesis and FA elongation and phospholipid synthesis which can be found both in expressed and inhibited category. FA may be an important determinant of responses of photosynthesis and stomatal conductance to environmental stresses such as high VPD (Poulson and Edwards 2002).

6.4.3 RNA Regulation of Transcription

Expression of limited transpiration responsive genes under high VPD environment was shown to be regulated by many transcription factors. Many genes (79) assigned to RNA regulation were identified. Genes coding for the zinc-finger family protein, MYB domain containing family, WRKY, ethylene response factor, bZIP were identified (Tran et al. 2004; Mochida et al. 2010). A high number of genes belonging to WRKY transcription factor and MYB domain category were found and they up-regulated under high VPD. Transcripts related to the basic helix-loop family were mostly decreased and the remaining transcripts like bZIP, zinc finger, and auxin/IAA were up and down regulated.

6.4.4 Protein, Signaling and Transport

Protein and signaling related genes were both induced and suppressed in high VPD samples. Protein genes are mostly involved in either post translational changes or degradation. Signaling genes include receptor kinases, leucine rich repeats and those involved in nutrient physiology. There were especially large numbers of the receptor kinases which are involved in improving plant performance under drought and also defense mechanism (Marshall et al. 2012). Several transport related transcripts are regulated under high VPD conditions and it was found that in PI 416937 most of the transcripts are induced (22).

Upregulated transcripts include nitrate, amino acids, ABC transporters, anion, cation, oligopeptides and phosphates. However, the majority of the differentially down-regulated transporters are major intrinsic proteins i.e., water channel proteins which probably could be the reason for the limited transpiration in PI 416937 under high VPD environments. In an aquaporin study in PI 416937 by Devi et al. (2015b), plasma membrane intrinsic proteins (PIP), especially PIP 2 were down regulated. PIPs and TIPs (Tonoplast Intrinsic Proteins) are said to play major roles in water transport (Maurel et al. 2008) and are responsive to different environmental conditions including VPD.

6.4.5 Stress Genes

Molecular responses to stress factors such as heat shock, anaerobiosis, plant pathogens, oxygen free radicals, heavy metals, water stress and chilling in plants have been assessed in various plant species (Matters and Scandalios 1986). Sixteen down-regulated and 22 up-regulated genes with biotic and abiotic stress-related annotations were grouped in to stress genes. Most of the biotic stress genes that are pathogen resistance responsive proteins were found to be more induced than suppressed, while abiotic stress showed an inverse pattern. The induced genes in the abiotic stress group include heat, drought and salt majorly involving heat shock proteins and dehydration responsive elements. The genes involved in the stress group are conserved and evidenced in earlier stress response studies of soybean (Le et al. 2012; Cal et al. 2013).

A clear trend in expression of all transcription factors together was not observed. However, overall, the differential regulation of many transcription factors under high VPD is similar to that seen with dehydration in soybean (Le et al. 2012).

6.5 Application of Limited-Transpiration Trait

While the initial simulations of the limited-transpiration trait with sorghum provided encouragement to study the trait, the value of the trait in developing cultivars for drought-tolerant lines and the possible benefit in soybean production needed to be assessed. Two lines of evidence were developed in evaluating the practical benefit of the limited-transpiration trait. The first evidence involved the development of breeding lines that have superior performance under water-limited conditions. The breeding effort based on PI 416937 was initiated even before the results of the physiological studies were available. The second evidence was obtained for a detailed modeling of soybean production across the various environments of the USA to determine the amount and probability of yield increase that might be expected from the limited-transpiration trait.

6.5.1 Breeding Progress

Deliberate efforts to mitigate the impact of drought on soybean via breeding are relatively recent even though the problem has long been recognized by farmers and scientists (Carter 1989; Carter et al. 1999; Orf et al. 2004; Chen 2013). Until recently, the prevailing view among breeders was that yield data collected from drought stricken environments had little or no value because genetic repeatability or heritability of seed yield in these environments was thought to be (and often was) lower than in high-yielding irrigated counterparts. This view is exemplified by the practice of discarding yield trials from USDA regional testing (starting in the 1940s) whenever the average yield of the experiment was 170 g m^{-2} (25 bu/ac) or less. Minimum-stress environments were viewed as allowing expression of yield and greater separation of genotypic means. Sneller and Dombeck (1997) and Specht et al. (1986) offered arguments that generally supported this view. While they found some evidence for drought tolerance in the applied breeding pools in Arkansas and Nebraska, heritability and genetic variance for seed yield were generally greater in high-yielding irrigated environments.

A paradigm shift began with the discovery of the delayed wilting phenotype of PI 416937 (USDA 2012a) and PI 471938 (USDA 2012b) in the 1980s and 1990s (Sloane et al. 1990; Carter et al. 1999). Although genetic variation for seed yield was still regarded as greatest in high-yielding environments, the prospect of making agronomic yield improvements in drought-stressed environments gained substantial currency. Funding from the United Soybean Board (a non-profit farmer organization), starting in the mid 1990s, plus the identification of drought-prone field sites which had sufficient uniformity to support field breeding, set the stage for public breeders in Nebraska, Minnesota, North Carolina, Arkansas, and Georgia to begin the process of developing drought-tolerant cultivars.

One important field site identified for this work was North Carolina State University's Sandhills Research Station with deep uniform sandy soils and low water holding capacity. The station has moderate drought during August pod filling in 2 of 4 years, and extreme August drought in 1 of 4 years, on average. The USDA soybean breeding program in North Carolina began its drought breeding project in 1989 by hybridizing PI 416937 from Japan (identified in 1983 at a drought prone field site at Clinton, NC) with adapted USDA breeding line N77-144, known to be an elite performer in high-yielding environments. F4-derived lines were evaluated under drought at the Sandhills station in replicated trials over 2 years, and eventually cultivar N7001 was released in 2003 from this effort (Carter et al. 2003). N7001 has good yielding ability and excellent overall yield stability and was the first USA cultivar that had in its pedigree as much as 50 % exotic pedigree since the 1950s. The USDA program has evaluated approximately 5000 yield plots annually at Sandhills research station since 1992. Several additional breeding lines have been developed, tested regionally, and shown to have high yield under drought (Devi et al. 2014). Some of these lines are being made available through Materials Transfer Agreements to major commercial seed companies and others for use as parental stock in their breeding programs. As such, this program is a major source of new genetic materials for the soybean industry and as genetic resources for physiological investigations into mechanisms of drought tolerance.

Using the cultivar N7001 as a parent, new cultivars N7002, N8001, and Woodruff were developed which are now among the top-yielding public cultivars in maturity groups VII and VIII (Carter et al. 2007, 2008). Both N7002 and N8001 have been high-yielding ‘check’ or control cultivars in USDA regional trials, in their respective maturity groups, for the past several years. A new breeding line, N05-7432, was developed more recently from the mating of N7002 (a derivative of PI 416937) with N98-7265 (a derivative of slow wilting PI 471938). This new breeding line is a top yielder in maturity group VIII (Gillen and Shelton 2012), surpassing check cultivar N8001 by 7 % ($p < 0.05$) averaged over more than 45 year-location combinations, which is quite large by breeding standards. The yield advantage of N05-7432 is quite stable, numerically out yielding N8001 in eight of the ten locations used in the multi-year testing trials. Further, expression of the limited-transpiration trait by N05-7432 has been documented (Devi et al. 2014). It also happens that N05-7432 is very tolerant to soil manganese deficiency (Masson 2014).

Line PI 416937 is perhaps the only exotic plant introduction being used as parental stock in USA at present which has the limited-transpiration trait. The full impact of this specific trait on improved agronomic drought response in its many progeny has not been ascertained as yet. Certainly, the ability of PI 416937 to conserve soil water to a greater extent than other soybean genotypes has been demonstrated in field experiments (King et al. 2009; Ries et al. 2012).

Parallel research in conjunction with field breeding has identified QTLs from PI 416937 for slow-wilting aquaporin response, prolific rooting, and aluminum tolerance in recent years, and all traits appear multi-genic in nature (Abdel-Haleem et al. 2011, 2012, 2013; Carpentieri-Pipolo et al. 2012). Current QTL research involves fine mapping of genetic markers for developing of factorial combinations of these QTL through breeding, so that their relation to the limited transpiration trait can be ascertained. In conjunction, the QTLs are being adapted to marker assisted selection in order to facilitate and enhance current field breeding programs aimed at improved drought tolerance.

6.5.2 Model Assessment of Benefit in the USA

Having investigated the physiology of the limited-transpiration trait and shown that the trait can be genetically transferred in a breeding program, key questions remain about where and by how much can soybean yield be expected to be impacted as a result of the trait. Considering the breadth of geographical area and environments in which soybean is grown in the USA, such an assessment can only be done by using simulations done over the range of locations and weather conditions likely to be experienced by soybean. Such an assessment using a mechanistic model has been done for soybean production in the USA (Sinclair et al. 2010).

The soybean model used in this study was originally presented by Sinclair (1986) and has been shown to be robust in several studies (Muchow and Sinclair 1986; Sinclair et al. 1987, 1992, 2007; Salado-Navarro and Sinclair 2009). The structure of the model is virtually the same as the sorghum model discussed earlier. The major modification was the simulation of nitrogen accumulation by the crop that reflected the high sensitivity of nitrogen fixation to soil drying. Loss of nitrogen fixation activity to soil drying caused an inhibition on leaf area development and ultimately less nitrogen in the plants to support seed growth. Again, the model was modified to run on an hourly basis to allow the limited-transpiration trait to be expressed in simulation during daytime hours under high VPD. In these simulations, the VPD breakpoint was assumed to occur at 2.0 kPa.

A key feature of the soybean simulations was the use of the GIS data base assembled by Pioneer DuPont (Loffler et al. 2005). In this data base, the areas in the US in which soybean is grown was segmented into 2655 blocks of 30 km × 30 km. In each block, 50 years of weather were developed to give daily minimum and maximum temperature, and precipitation. Solar radiation on each day was synthesized from temperature using the function developed by Bristow and Campbell (1984). In addition to weather information, the data base for each block included sowing date, maturity group, and available soil water storage.

Each set of simulations required the model to be run for each of the 50 years in each of the blocks for a total of more than 130,000 runs. The initial simulations were for a ‘standard’ soybean with a linearly increasing transpiration rate with increasing VPD. The limited-transpiration rate was simulated by imposing a VPD breakpoint at 2 kPa, above which there was no further increase in transpiration rate. For each year in each geographical block, the difference between the yield with the limited-transpiration trait and the standard soybean was calculated. A probability of yield increase was calculated for each block based on the fraction of years in which the yield increased as a result of the limited-transpiration trait. The results of the soybean simulations assessing the probability of yield increase as a result of the limited-transpiration trait are shown in Fig. 6.5a. The probability of yield increase was 80 % or greater in virtually all areas of the USA. The only places where there was not a high probability of yield increase were on the coasts in the southeast, the very

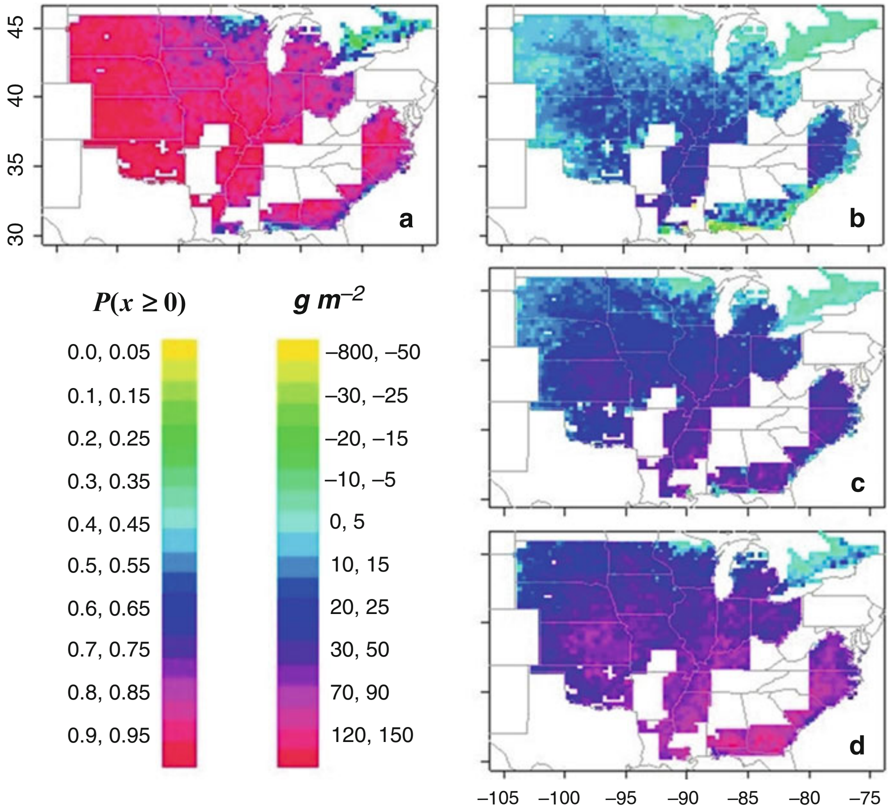

Fig. 6.5 Simulation results of soybean grain yield with limited-transpiration trait for 50 years in 30 × 30 km blocks across the USA (Sinclair et al. 2010). Panel (**a**) presents the probability of yield increase over the standard soybean in each block. Panels (**b**), (**c**), and (**d**) present the 75 % (wet), medium, and 25 % (dry) percentile yield increase (in g m^{-2}) over the standard soybean within each block

northern blocks, and a few scattered blocks in Iowa and Minnesota. Overall, the probability results show the limited-transpiration trait to be beneficial in nearly all the soybean production areas in the USA.

In addition to calculating the probability of yield increase, within each block the yield difference of each growing season was ranked from lowest to highest. This ranking gave the distribution yield from which specific percentile rankings could be compared. In Fig. 6.5b–d are shown the yield difference for each block for the 75 % (wet years), median, and 25 % (dry years) percentile, respectively. In the 25 % percentile, or drier years, the model predicted increases of about 90 g m^{-2} (dry grain weight) in many locations especially in the southern areas. The southeast, mid-south, and Kansas showed the greatest benefit of the limited-transpiration trait. The remainder of the country had yield increases in the 25 % percentile years in the range of 30–90 g m^{-2}.

The benefit of the limited-transpiration trait was also positive in most areas in the USA in the median year. In these years the yield benefit was simulated to be in the range of roughly 20–70 g m^{-2}. Even in the 75 % percentile year, the limited-transpiration trait was simulated to be beneficial although the yield increase tended to be in the range of 0–25 g m^{-2}. The largest benefit in the 75 % percentile year was in the North Carolina & Virginia, the Mississippi Delta region, and a belt from southern Indiana to Kansas. While not shown in these maps, the yield decrease simulated in the wettest years was small.

6.6 Conclusions

This study of the limited-transpiration trait in soybean illustrates the benefit of a systematic approach involving approaches and disciplines of study. Simulation studies were done initially to assess the potential value of the trait. Potential germplasm sources for the trait were evaluated in field screens and candidate lines were subjected to detailed studies of transpiration response across various levels of VPD. Physiological investigations of the trait led to hypotheses for investigation of the limited-transpiration trait and its potential physiological explanation. Finally, breeding has progressed to the release of soybean lines that express the limited-transpiration and have increased grain yields under dry conditions. Another series of simulations were done specifically for the production areas for soybean in the USA. These simulations indicated where, how often, and how much the trait in soybean will benefit farmers. The results of the studies on the limited-transpiration trait were advanced due to a comprehensive research program that involved investigations in several disciplines, under field and controlled conditions, and at several levels of sophistication. As often suggested but rarely put into practice, a multi-level approach in the study of the limited-transpiration trait involving a multi-faceted research effort resulted in progress in scientific understanding leading to benefit for farmers.

References

Abdel-Haleem H, Lee GJ, Boerma HR (2011) Identification of QTL for increased fibrous roots in soybean. Theor Appl Genet 122:935–946

Abdel-Haleem H, Carter TE Jr, Purcell LC, King CA, Ries LL, Chen P, Schapaugh W Jr, Sinclair TR, Boerma HR (2012) Mapping quantitative loci for canopy wilting trait in soybean (*Glycine max* L. Merr.). Theor Appl Genet 125:837–846

Abdel-Haleem H, Carter TE Jr, Rufty TE, Boerma HR, Li Z (2013) Quantitative trait loci controlling aluminum tolerance in soybean: candidate gene and SNP marker discovery. Mol Breed 33:851–862

Bristow KL, Campbell GS (1984) On the relationship between incoming solar radiation and daily maximum and minimum temperature. Agric For Meteorol 31:159–166

Cal AJ, Liu D, Mauleon R, Hsing Y-IC, Serraj R (2013) Transcriptome profiling of leaf elongation zone under drought in contrasting rice cultivars. PLoS One 8, e54537

Carpentieri-Pipolo V, Pipolo AE, Abdel-Haleem H, Boerma HR, Sinclair TR (2012) Identification of QTLs associated with limited leaf hydraulic conductance in soybean. Euphytica 186:679–686

Carter TE Jr (1989) Breeding for drought tolerance in soybean: where do we stand? In: Pascale AJ (ed) Proceedings of the world soybean conference IV, Asociacion Argentina de la Soja, Buenos Aires, 1856, pp 1001–1008

Carter TE Jr, De Souza PI, Purcell LC (1999) Recent advances in breeding for drought and aluminum resistance in soybean. pp 106–125. In: Kauffman H (ed) Proceedings of the world soybean conference VI Chicago, IL. 4–7 Aug. 1999. Superior Print, Champaign, pp 542

Carter TE Jr, Burton JW, Bowman DT, Cui Z, Zhou X, Villagarcia MR, Fountain MO, Niewoehner AS (2003) Registration of ‘N7001’ soybean. Crop Sci 43:1126–1127

Carter TE Jr, Burton JW, Fountain MO, Rzewnicki PE, Villagarcia MR, Bowman DT (2007) Registration of ‘N7002’ soybean. J Plant Reg 1(2):93–94

Carter TE Jr, Burton JW, Fountain MO, Rzewnicki PE, Villagarcia MR, Bowman DT (2008) Registration of N8001 soybean. J Plant Regist 1(2):23–23

Chen P (2013) Breeding for drought tolerance in soybean. In: World soynbean conference Durbin, South Africa, Jan 2013

Devi JM, Sinclair TR, Chen P, Carter TE (2014) Evaluation of elite southern maturity soybean breeding lines for drought-tolerant traits. Agron J 106:1947–1954

Devi JM, Sinclair TR, Taliercio E (2015a) Comparisons of the effects of vapor pressure deficit on gene expression among fast wilting and slow wilting soybean leaves. PloS One 10(10), e0139134

Devi JM, Sinclair TR, Taliercio E (2015b) Silver and zinc inhibitors influence transpiration rate and aquaporin transcript levels in intact soybean plants. Env Exp Bot (in press)

Dixon RA, Steele CL (1999) Flavonoids and isoflavonoids – a gold mine for metabolic engineering. Trends Plant Sci 4:394–400

Fletcher AL, Sinclair TR, Allen LH Jr (2007) Transpiration responses to vapor pressure deficit in well watered ‘slow-wilting’ and commercial soybean. Environ Exp Bot 61:145–151

Gillen AM, Shelton GW (2012) Uniform soybean tests – Southern States, 2011. USDA-ARS, Stoneville, MS. Available at http://www.ars.usda.gov/Business/Business.htm?modecode=64-02-15-00&docid=4357&page=3. Verified 29 Nov 2013

Hudak CM, Patterson RP (1995) Vegetative growth analysis of a drought-resistant soybean plant introduction. Crop Sci 35:464–471

King CA, Purcell LC, Brye KR (2009) Differential wilting among soybean genotypes in response to water deficit. Crop Sci 49:290–298

Le DT, Nishiyama R, Watanabe Y, Tanaka M, Seki M, Yamaguchi-Shinozaki K, Shonozaki K, Tran LS (2012) Differential gene expression in soybean leaf tissues at late developmental stages under drought stress revealed by genome-wide transcriptome analysis. PLOS One 7, e49522

Loffler CM, Wei J, Fast T, Gogerty J, Langton S, Bergman M, Merrill B, Cooper M (2005) Classification of maize environments using crop simulations and geographical information systems. Crop Sci 45:1708–1716

Masson R (2014) Heritability and selective genotyping QTL analysis of resistance to manganese deficiency derived from soybean breeding line SC97-1821. North Carolina State University, Raleigh

Marshall A, Aalen RB, Audenaert D, Beeckman T, Broadley MR, Butenko MA, Caño-Delgado AI, de Vries S, Dresselhaus T, Felix G (2012) Tackling drought stress: receptor-like kinases present new approaches. Plant Cell Online 24:2262–2278

Matters GL, Scandalios JG (1986) Changes in plant gene expression during stress. Develop Genetics 7:167–175

Maurel C, Verdoucq L, Luu DT, Santoni V (2008) Plant aquaporins: membrane channels with multiple integrated functions. Ann Rev Plant Biol 59:595–624

Mochida K, Yoshida T, Sakurai T, Yamaguchi-Shinozaki K, Shinozaki K, Tran LSP (2010) LegumeTFDB: an integrative database of Glycine max, Lotus japonicus and Medicago truncatula transcription factors. Bioinformatics 26:290–291

Muchow RC, Sinclair TR (1986) Water and nitrogen limitations in soybean grain production. II. Field and model analysis. Field Crops Res 15:143–156

Orf JH, Hutton SF, Carter TE Jr (2004) Breeding for soybean drought tolerance: update on U.S. experience. pp 260–266. In: Moscardi F, Hoffmann-Campo CB, Ferreira Saraiva O, Galerani PR, Krzyzanowski FC, Carrao-Panizzi MC (ed) Proceedings of the world soybean conference VII, Foz do Iguassu, PR, Brazil, February 29–March 5 2004. Embrapa Soybean, Londrina, pp 1344

Pantalone VR, Rebetzke GJ, Burton JW, Carter TE Jr, Israel DW (1999) Soybean PI 416937 root system contributes to biomass accumulation in reciprocal grafts. Agron J 91:840–844

Poulson ME, Edwards GE (2002) Photosynthesis is limited at high leaf to air vapor pressure deficit in a mutant of Arabidopsis thaliana that lacks trienoic fatty acids. Photosyn Res 72:55–63

Purcell L, Specht JE (2004) Physiological traits for ameliorating drought stress. In: Boerma HR, Specht JE (eds) Soybeans: improvement, production, and uses. American Society of Agronomy, Madison, pp 569–620

Ries LL, Purcell LC, Carter TE Jr, Edwards JT, King CA (2012) Physiological traits contributing to differential canopy wilting in soybean under drought. Crop Sci 52:272–281

Sadok V, Sinclair TR (2009) Genetic variability of transpiration response to vapor pressure deficit among soybean (Glycine mas [L.] Merr.) genotypes from a recombinant inbred line population. Field Crops Res 113:156–160

Sadok W, Sinclair TR (2010a) Transpiration response of ‘slow-wilting’ and commercial soybean (*Glycine max* (L.) Merr.) genotypes to three aquaporin inhibitors. J Exp Bot 61:821–829

Sadok W, Sinclair TR (2010b) Genetic variability of transpiration response of soybean [*Glycine max* (L.) Merr.] shoots to leaf hydraulic conductance inhibitor AgNO3. Crop Sci 50:1423–1430

Salado-Navarro LR, Sinclair TR (2009) Crop rotations in Argentina: analysis of water balance and yield using crop models. Agr Syst 102:11–16

Sinclair TR (1986) Water and nitrogen limitations in soybean grain production. I. Model development. Field Crops Res 15:125–141

Sinclair TR, Muchow RC (2001) System analysis of plant traits to increase grain yield on limited water supplies. Agron J 93:263–270

Sinclair TR, Vadez V (2012) The future of grain legumes in cropping systems. Crop Pasture Sci 63:501–512

Sinclair TR, Muchow RC, Ludlow MM, Leach GJ, Lawn RJ, Foale MA (1987) Field and model analysis of the effect of water deficits on carbon and nitrogen accumulation by soybean, cowpea, and black gram. Field Crops Res 17:121–140

Sinclair TR, Salado-Navarro LR, Morandi EN, Bodrero ML, Martignone RA (1992) Soybean yield in Argentina in response to weather variation among cropping seasons. Field Crops Res 30:1–11

Sinclair TR, Muchow RC, Monteith JL (1997) Model analysis of sorghum response to nitrogen in subtropical and tropical environments. Agron J 89:201–207

Sinclair TR, Purcell LC, Sneller CH (2004) Crop transformation and the challenge to increase yield potential. Trends Plant Sci 9:70–75

Sinclair TR, Hammer GL, van Oosterom EJ (2005) Potential yield and water-use efficiency benefits in sorghum from limited maximum-transpiration rate. Funct Plant Biol 32:945–952

Sinclair TR, Salado-Navarro LR, Salas G, Purcell LC (2007) Soybean yields and soil water status in Argentina: simulation analysis. Agr Syst 94:471–477

Sinclair TR, Zwieniecki MA, Holbrook NM (2008) Low leaf hydraulic conductance associated with drought tolerance in soybean. Physiol Plant 132:446–451

Sinclair TR, Messina CD, Beatty A, Samples M (2010) Assessment across the United States of the benefits of altered soybean drought traits. Agron J 102:475–482

Sloane RJ, Patterson RP, Carter TE Jr (1990) Field drought tolerance of a soybean plant introduction. Crop Sci 30:118–123

Sneller CH, Dombeck D (1997) Use of irrigation in selection for soybean yield potential under drought. Crop Sci 37:1141–1147

Specht JE, Williams JH, Weidenbenner CJ (1986) Differential responses of soybean genotypes subjected to a seasonal soil water gradient. Crop Sci 26:922–934

Tanner CB, Sinclair TR (1983) Efficient water use in crop production: research or re-search? In: Taylor HM, Jordan WR, Sinclair TR (eds) Limitations to efficient water use in crop production. American Society of Agronomy, Madison, pp 1–27

Thimm O, Blasing O, Gibon Y, Nagel A, Meyer S, Kruger P, Selbig J, Muller LA, Rhee SY, Stitt M (2004) MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. Plant J 37:914–939

Tran LS, Nakashima K, Sakuma Y, Simpson SD, Fujita Y, Maruyama K, Fujita M, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2004) Isolation and functional analysis of *Arabidopsis* stress-inducible NAC transcription factors that bind to a drought-responsive *cis-element* in the early responsive to dehydration stress promoter. Plant Cell 16:2481–2498

Uppalapati SR, Marek SM, Lee HK, Nakashima J, Tang YH, Sledge MK, Dixon RA, Mysore KS (2009) Global gene expression profiling during Medicago truncatula–Phymatotrichopsis omnivore interaction reveals a role for jasmonic acid, ethylene, and the flavonoid pathway in disease development. Molecular Plant Microbe Interact 22:7–17

USDA-ARS National Genetic Resources Program (USDA-ARS NGRP) (2012a) Glycine max (L.) Merr. PI 416937 Germplasm Resources Information Network (GRN). National Germplasm Resources Laboratory, Beltsville. Available at http://www.ars-grin.gov/cgi-bin/npgs/acc/display.pl?1314868. Verified 28 Sept 2013

USDA-ARS National Genetic Resources Program (USDA-ARS NGRP) (2012b) Glycine max (L.) Merr. PI 471938 Germplasm Resources Information Network (GRIN). National Germplasm Resources Laboratory, Beltsville. Available at http://www.ars-grin.gov/cgi-bin/npgs/acc/display.pl?1366874. Verified 28 Sept 2013

Usadel B, Poree F, Nagel A, Lohse M, Czedik-Eysenberg A, Stitt M (2009) A guide to using MapMan to visualize and compare omics data in plants: a case study in the crop species, maize. Plant Cell Environ 32:1211–1229

Wang YH, Irving HR (2011) Developing a model of plant hormone interactions. Plant Signal Behav 6:494–500