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Insight

In search for drought-tolerant soybean: is the slow-wilting phenotype more than just a curiosity?

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Slow canopy wilting in response to decreased soil moisture is a complex conservation trait in soybean that is likely to involve multiple mechanisms. This trait is potentially extremely important in the development of more drought-tolerant crops, but it remains poorly characterized. Using QTL mapping, Ye *et al.* (2019) have shown that slow-wilting in soybean is associated with drought tolerance and involves at least two distinct water-conservation mechanisms.

Slow-wilting phenotype

Phenotyping plays a pivotal role in the selection of drought-resilient plant genotypes. It also provides a meaningful dissection of the quantitative genetic landscape that contributes to the adaptive response to drought in crops (Tuberosa, 2012). In agriculture, the term "drought" is defined as a situation in which the amount of water available through rainfall and/or irrigation is insufficient to meet the transpiration needs of the crop. This can result in significant yield losses. The development of crops with improved drought tolerance traits is therefore an important objective for plant breeders, but one that poses major challenges. Canopy wilting is the first visible stress symptom of soil water deficits caused by drought. The slow-wilting phenotype in soybean is defined as a delayed wilting response to decreased soil moisture content, when compared to an average soybean cultivar.

The slow-wilting phenotype in soybean was first reported in a Japanese landrace (PI 416937). Looking visually more vigorous in the field, when all other plants had wilted, PI 416937 not only wilted much more slowly under water deficit conditions but also maintained lower levels of solute potential, with a higher pressure potential and relative water content compared to fast-wilting soybean cultivars such as Forrest or Benning (Sloane *et al.*, 1990; Shin *et al.*, 2015) (Box 1). Simulation modeling predicted that the slow wilting phenotype could improve yield under drought conditions in most US regions by more than 80% (Sinclair *et al.*, 2010). Cultivars with low transpiration rates and a slow decline in whole plant water use in response to soil water deficits support the conservation of soil water (Box 2). The slow-wilting trait is therefore particularly suitable to low humidity environments, where water deficits commonly develop in the latter part of the season. The restricted transpiration rates of the slow-wilting soybeans in response to the high vapor pressure deficit occurring in the middle of the day in such environments will result in better water use efficiency and increased yields. These characteristics, coupled to the superior maintenance of cell turgor and a competitive yielding ability under drought conditions, made PI 416937 a useful genetic resource for the development of new drought-tolerant soybean varieties (Carter *et al.*, 2006; Fletcher *et al.*, 2007; King *et al.*, 2009; Sadok and Sinclair, 2009; Charlson *et al.*, 2009; Devi *et al.*, 2014).

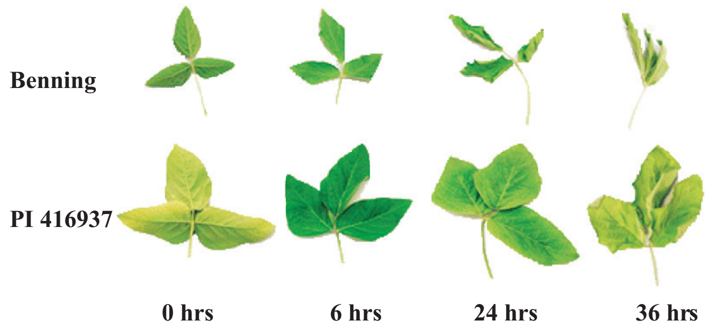
The observed ability of PI 416937 to maintain competitive yields under drought laid the foundations for further studies concerning the identification and characterization of new slow-wilting soybean accessions. Drought tolerance in soybean must be associated with traits that enhance yield stability rather than simply involving better survival (Sinclair, 2011; Ye *et al.*, 2018). Ye *et al.* (2019) have made a significant contribution to the identification of new drought-tolerant lines that maintain yield. Through the characterization of two new slow-wilting accessions (PI 567690 and 567731) of an early maturity soybean group (Pathan *et al.*, 2014), and the evaluation of a soybean accession with a fresh weight phenotype, these authors present convincing evidence in support of the hypothesis that the slow-wilting phenotype in soybean is linked to drought tolerance without affecting yield (Sloane *et al.* 1990).

Slow-wilting QTL

To speed up the development and release of superior cultivars, plant breeders rely on the identification and development of powerful genomic tools such as quantitative trait loci (QTLs). Identifying a particular QTL associated with the canopy-wilting trait has been a major task (Hwang *et al.*, 2015). Initial research indicated that the genetic mechanism controlling canopy wilting is most probably polygenic and is likely to be controlled by several QTLs (Charlson *et al.*, 2009). Seven QTLs were identified on different chromosomes in a

Box 1. Phenotyping response in slow-and fast-wilting wilting phenotypes under drought

Phenotypic response of Benning (sensitive) and PI 416937 (tolerant) soybeans after 0, 6, 24, and 36 hours of drying treatment (from Shin *et al.*, 2015).

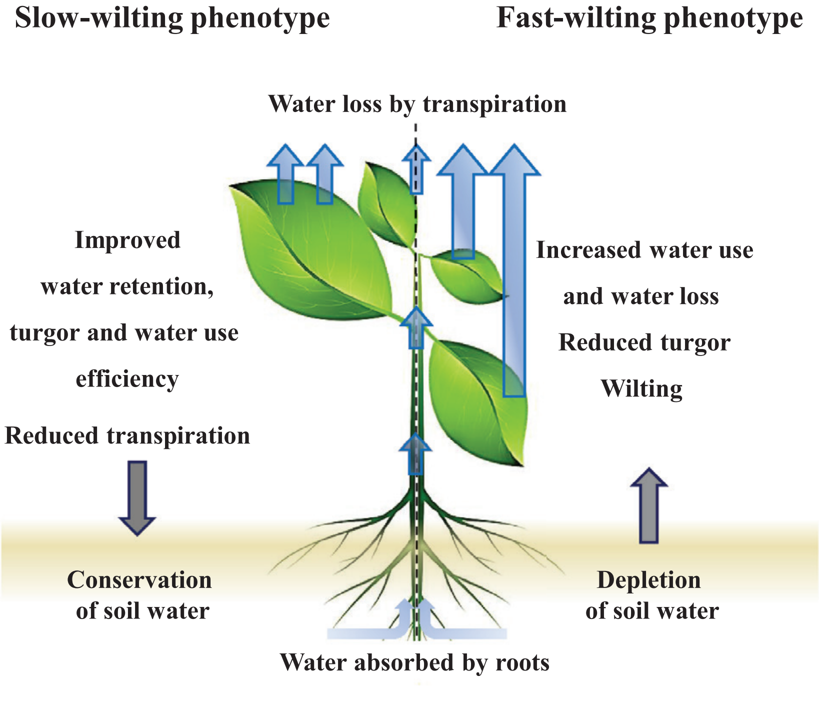


recombinant inbred line from a cross between PI 416937 and Benning (Abdel-Haleem *et al.*, 2012). These QTLs explained 75% of the variation in the canopy-wilting trait. This result provided strong evidence that a QTL from PI 416937 could be a powerful tool for the development of drought-tolerant soybean cultivars. In addition to showing the complexity of the slow-wilting trait, these early studies also provided an indication that the canopy-wilting trait might also be associated with additional morpho-physiological traits. Moreover, exotic soybean landraces may house such useful traits.

Ye *et al.* (2019) identified two new slow-wilting QTLs (qSW_Gm06 and qSW_Gm10) mapped on chromosomes 6 and 10 from a Magellan × PI 567731 cross. This result demonstrates the feasibility of using marker assisted selection (MAS) in soybean breeding to incorporate the complex slow-wilting trait. Findings further demonstrate that stacking these two

Box 2. Slow-wilting phenotype in soybean

Comparison of a slow- and fast-wilting phenotype with respect to transpiration, turgor and water use under drought conditions. Plants showing the slow-wilting phenotype has improved water retention, turgor and water use efficiency.



QTLs could enhance plant performance under selected experimental conditions in comparison with incorporating either QTL alone. The new study by Ye *et al.* (2019) therefore provides strong support for the hypothesis that stacking slow-wilting QTLs can significantly improve drought tolerance (Valliyodan *et al.*, 2017).

Unfortunately, QTLs seldom provide clues about underlying molecular mechanisms. To obtain information concerning which genes are located in or are controlled by a specific QTL, it is necessary to undertake a much more intensive investigation. This would need the QTL to be mapped to a much higher resolution on its chromosomal location. However, the first important insights with regard to the specific genes residing in such QTLs have been provided by the recent excellent work of Kaler *et al.* (2017). Applying genome-wide association studies, these authors identified significant SNPs associated with canopy-wilting across different environments and tagged 23 putative loci related to the trait. Six of these loci were located within previously reported chromosomal regions associated with canopy wilting through bi-parental mapping. Several significant SNPs were also located within or very close to genes that had reported biological connections to transpiration or water transport. In this new study, Ye *et al.* (2019) also presents a promising strategy to clone the QTL by developing near-isogenic lines (NILs) for the two major QTLs (*qSW_Gm06* and *qSW_Gm10*), which can facilitate the fine-mapping process at more synchronized genetic backgrounds.

Slow-wilting mechanisms

The hypothesis that slow-wilting is a basis for drought tolerance is interesting and exciting even though key questions still remain concerning the exact mechanisms responsible for the observed phenotype. While previous studies have failed to identify a specific physiological mechanism that fully explains the slow wilting phenotype (Ries *et al.*, 2012; Sadok *et al.*, 2012), several traits appear to be involved, such as better water resource exploration by a larger root system (Pantalone *et al.*, 1996), lower stomatal conductance (Tanaka *et al.*, 2010), constant transpiration rates under vapor pressure deficit (VPD) conditions above 2.0 kPa (Fletcher *et al.*, 2007), as well as lower radiation use efficiency (Ries *et al.*, 2012). In the absence of a mechanistic explanation of the slow-wilting phenotype, a hypothesis involving water transport and anatomical features related to specific water transport properties may be required to explain slow-wilting in PI 471938.

Recent speculation regarding the mechanisms that contribute to the slow-wilting phenotype have included the possibility that accumulation of minerals (such as K, Ca, B, Na) or organic compounds (such as sucrose, raffinose and stachyose and oleic acid) under drought stress in slow-wilting genotypes could maintain cell turgor, conserve water and achieve osmoregulation (Bellaloui *et al.*, 2013). The higher leaf water potentials observed in slow-wilting indicate that the genotypes with this trait are able to retain more water by better water conservation, as well as better nutrient homeostasis. The unique response of PI 416937 to drought has also been attributed to factors that affect transpiration, such as the distribution and/or expression of aquaporins, as well as the limited hydraulic conductance between the xylem and the leaf guard cells (Sinclair *et al.*, 2008; Fletcher *et al.*, 2007). The absence of a specific silver-sensitive population of aquaporins in PI 416937 may explain the limited-maximum transpiration rates of PI 416937 under increasing vapor pressure deficits (Sadok and Sinclair, 2010). The paper by Ye *et al.* (2019) adds exciting new information to this hypothesis. Although the two new slow-wilting soybean accessions (PI 567690 and 567731) share the same water conservation strategy of limited-maximum transpiration rates (similar to PI 416937), transpiration in these accessions was shown to be sensitive to an aquaporin inhibitor. This finding supports the concept that more than one distinct water-conservation mechanism is involved in the soybean slow-wilting trait, as previously suggested (Charlson *et al.*, 2009).

Filling the gaps

Despite the progress made to date regarding the mechanisms involved in the slow-wilting phenotype, many "unknowns" remain. Many questions—ranging from a fundamental understanding of the phenotype at a molecular level to the practical implementation of the trait in field grown commercial cultivars with enhanced drought tolerance—remain unaddressed. How can we proceed to fill these knowledge gaps?

The finding by Ye *et al.* (2019) that the stacked QTLs (*qSW_Gm06* and *qSW_Gm10*) provide improved resistance compared with each QTL alone warrants further investigation. As the authors suggest, the analysis of a larger sample size under better-controlled drought stress conditions is required to fully assess the potential of these QTLs in marker-assisted soybean breeding for the slow-wilting phenotype. The involvement of gene sets has been proposed. While data on the differences between the slow- and fast-wilting phenotypes exist in the literature, the identification of specific gene targets is still required. The identification of the exact mechanism(s) involved will require a significant amount of more work. Up to now, only a handful of studies have been published that have compared differences between genotypes at a transcriptome level. The first useful insights were provided by Shin *et al.* (2015) who identified five genes that clearly exhibited a GxE response when PI 416937 and the cultivar Benning were investigated. These genes provide good candidates for future studies designed to advance our understanding of the slow-wilting phenotype. Another important resource are the SNP data, which could provide significant alleles for gene pyramiding studies, as well as for the identification of parental genotypes in breeding programs (Kaler *et al.*, 2017). The review of genes that have already been reported to confer drought tolerance (Lawlor, 2013) could also be useful in the identification of gene targets involved in the slow-wilting phenotype.

The regulation of specific soybean genes and how they may influence the plant response to drought was discussed by Gallino *et al.* (2018). These authors compared two contrasting soybean genotypes (slow-wilting N7001 and drought sensitive TJS2049). Although both cultivars generated similar transcriptomic responses to long-term drought stress, a eukaryotic translation initiation factor, iso4G (GmeIFiso4G-1a), was shown to be specifically induced in the slow-wilting genotype, N7001. This finding is consistent with the report of Shin *et al*. (2015), who showed that the dehydration-induced transcriptional profiles of soybean accessions with different canopy wilting phenotypes vary at different time points after the initiation of stress. This result provides further support to the hypothesis that only certain genes respond during the early stages of water deficit in order to establish a defense response, whereas many of the later-induced genes respond only to the physiological consequences of drought stress. Increasing our knowledge on the early-induced genes that are responsible for the initiation of the drought defense response, and their possible associations with the slow wilting phenotype, is urgently required. This should be a major research focus going forward.

Slow-wilting is a highly valuable and easily measurable trait for the screening of drought tolerance in soybean breeding programs. This trait will be particularly helpful in breeding programs in less developed countries searching for affordable and visible marker systems for improved drought tolerance. Although considerable progress in our understanding of the slow-wilting soybean phenotype has been made in the recent years, fundamental questions have to be answered, particularly relating to the genes and mechanisms involved. There is no doubt that the new study reported by Ye *et al*. (2019) is an exciting and important stepping stone for researchers seeking to unravel the slow-wilting phenotype.

Keywords: Aquaporins, delayed wilting response, drought tolerance, drought-tolerant soybean, slow-wilting phenotype, slow-wilting soybean, slow wilting QTL, water-conservation mechanism

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