Role of Soybean E-Genes in Regulating Onset and Duration of Leaf Senescence

Saratha Kumudini[1]

[1]Dept. of Agronomy, Univ. of Kentucky, Lexington, KY 40546-0312 e-mail: skumud@uky.edu
http://www.uky.edu/Ag/Agronomy/CropEcoPhys/kumudini/

Abstract

Genetic improvement in yield of a number of crop species including soybean has been associated with delayed leaf senescence ("stay-green"). Elucidation of the genetics regulating the onset and rate of leaf senescence will be of great agricultural importance for genetic improvement in yield. An interesting group of genes has been characterized in soybean that may prove beneficial in studying soybean leaf senescence. A series of loci with two alleles at each locus characterized by researchers working on photoperiod and light quality studies were given the prefix E. Studies on E-gene NILs have reported that plants with the dominant E alleles have higher dry matter accumulation in response to post-anthesis long-day photoperiod. It was hypothesized that this phenomenon was due to a delay in leaf senescence. In 2003, a randomized complete block, split plot experiment was conducted. The main plot was two different planting dates and the split-plot was nine E-gene NILs from two different genetic backgrounds ('Harosoy' and 'Clark'). Chlorophyll concentration (SPAD) and photosynthesis measurements were taken of NIL under field growing conditions. The E-gene dominant alleles did delay leaf senescence in terms of photosynthesis and chlorophyll concentration. The dominant E1 allele especially seemed to have a marked effect on maintaining a high photosynthetic rate. The dominant E1 allele has a role in delaying the onset of leaf senescence in soybean further in to the growing season, but accelerates the onset of leaf senescence after reaching first flower, and this pattern is apparent in both Clark and Harosoy backgrounds. The impact of the E1 locus on regulation of leaf senescence appears to be distinct from its impact on plant phenology.

Media summary

The E1 gene in soybean has been found to help the plant stay green for longer, and thus potentially increase soybean yield.

Keywords

Glycine max, Leaf carbon exchange rate

Introduction

Leaf senescence is a genetically regulated process that occurs at the later part of leaf development and culminates in programmed cell death. The process has been reported to occur in an age dependent manner but can be prematurely stimulated by abiotic stresses such as drought or heat stress, or delayed by application of growth regulators (Lim, et al., 2003). Leaf senescence, once induced, occurs as a highly ordered sequential process of disassembly and degradation of cellular components. The products of this degradation are translocated as nutrients to storage or reproductive organs. Thus, although senescence is a degenerative process, it helps remobilize/recycle valuable nutrients to important organs to maintain the reproductive "fitness" of the plant. Consequently it is important to maintain the integrity of the intact process when considering manipulating leaf senescence.

Soybean studies on delayed leaf senescence have previously met with difficulties. Much of this work has been conducted using mutant lines that were 'stay-green' phenotypes (Pierce et al. 1984; Phillips et al., 1984; Guiamet et al, 1991). Although these mutants worked to inhibit one or few genes that influenced breakdown of important components of photosynthesis, they did not improve either late season photosynthesis or yield under field conditions (Phillips et al., 1984; Guiamet et al., 1991, Guiamet and Gianibelli, 1996; Luquez and Guiamet, 2001). Failure of many 'stay-green' mutants to improve

photosynthesis or yield may be because delayed leaf senescence is not a trait controlled by one or a few genes. Leaf senescence is a complex regulated process and manipulation of this process needs to maintain the integrity of the whole process in order to effectively improve yield.

The group of e-genes that has been characterized in soybean over the last 30 years, may prove beneficial in studying soybean leaf senescence. The e-genes are a series of loci with two alleles at each locus characterized by researchers working on photoperiod and light quality studies. They are E1 and E2 (Bernard, 1971), E3 (Buzzell, 1971), E4 (Buzzell and Voldeng, 1980), E5 (McBlain and Bernard, 1987) and E7 (Cober and Voldeng, 2001). The dominant alleles at all loci were reported to confer late flowering and maturity (Buzzell and Voldeng, 1981; McBlain et al., 1987; McBlain and Bernard, 1987; Cober and Voldeng, 2001). Backcross-derived NIL's of the cultivars Clark and Harosoy have been developed by researchers in the United States and Canada in an attempt to characterize the function of these e-genes.

One group of researchers working on photoperiod sensitivity of some of these NIL's (Clark background) reported that e-genes had a significant impact on leaf area duration (Ellis et al., 2000). Dominant alleles of the lines tested exhibited higher leaf area duration. Furthermore, these NIL's had a differential response to a post-anthesis long-day photoperiod stimulus. Leaf area duration was increased in the presence of the dominant allele but unaffected in the presence of the recessive allele. The dominant E1, E2, and E3 lines had greater dry matter accumulation post-anthesis, and increased seed yield per plant when grown under greenhouse conditions (Ellis et al., 2000).

It was speculated that the E-genes may play a role in the regulation (either directly or indirectly) of soybean leaf senescence. The objective of our study was to determine the impact of the E-genes on the onset and rate of soybean leaf senescence.

Methods

Nine E-gene NILs of soybean were tested in a randomized complete block, split plot design. The main plots were two planting dates, June 1, 2003 and June 24, 2003. The split plots were the five NILs. Plots were 4 rows (36cm row width) wide by 1.8 meters long. Due to poor emergence, an area of 0.36 m long and 0.72m wide of bordered plants was selected and all measurements were confined to this area.

Table 1. Near isogenic lines of Clark and Harosoy and their gene composition

Name	Genetic Background	Gene composition
OT94-47	Harosoy	e1,e2,e3,e4,e5,e7
OT89-5	Harosoy	e1,e2,e3,e4,e5,E7
L92-21	Clark	e1,e2,e3,e4,e5,E7
L62-667	Harosoy	e1,e2,e3,E4,e5,E7
L71-920	Clark	e1,e2,e3,E4,e5,E7
L71-802	Harosoy	E1,e2,e3,E4,e5,E7

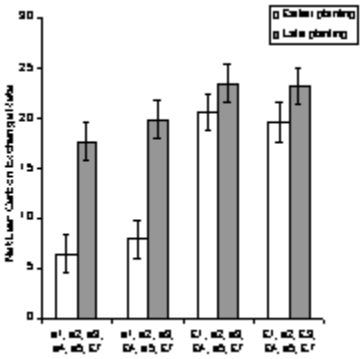
L80-5914	Clark	E1,e2,e3,E4,e5,E7
L67-2324	Harosoy	E1,e2,E3,E4,e5,E7
L66-432	Clark	E1,e2,E3,E4,e5,E7

Phenology data was taken every 3-4 days on 10 plants per plot based on the classification system defined by Fehr and Caviness (1977). Leaf SPAD values were taken on the third fully expanded leaf from the top. Five measurements were taken per leaf on three plants per plot. The measurements were taken every week after the plants reached the R1 stage. Photosynthetic rates were measured using a Li-6400 (Li-Cor 6400 Photosynthesis system, Lincoln, NE). The light levels were adjusted to 2000 µmol $m^{-2}s^{-2}$, using the LED light source and the CO_2 level was maintained at 400 µmol $m^{-2}s^{-2}$ on the reference side and the flow rate was set to 500 mmol m^{-2} s^{-2}.

Results

The dominant E1 alleles had a significant impact on leaf carbon exchange rate and leaf chlorophyll content as measured by net leaf photosynthetic rates (Fig. 1). The dominant E1 allele especially seemed to have a marked effect on maintaining a high photosynthetic rate, whereas the E4 and E7 dominant alleles did not appear to contribute to photosynthesis under field conditions. This pattern was evident when measured in both planting dates, but was more apparent under the earlier planting date. The plants in the earlier planting date were obviously further-on in development. Leaf greenness measurements confirmed that the differences in leaf senescence between genotypes with recessive versus dominant alleles at the E1 locus was apparent only later in the growing season (Fig. 2). The time course study of leaf greeness as measured by SPAD, as well as leaf carbon exchange rate, suggests that the dominant E1 allele has a role in delaying the onset of leaf senescence in soybean and this is apparent in both Clark and Harosoy backgrounds as measured in chronological time. However, it must be recognized that the plants of the various NILs were at different phenological stages when individual SPAD and photosynthesis measurements were taken. If leaf senescence is dependent on phenological stage and not on chronological age, then it would be necessary to evaluate leaf greeness relative to a phenological stage, e.g. first flower (R1).

A



B

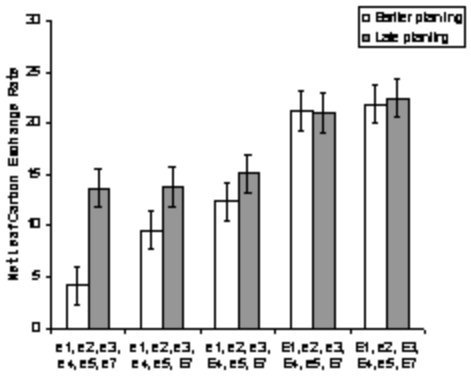
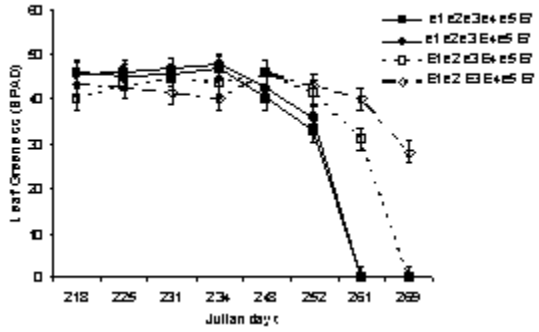


Fig. 1. Net leaf photosynthetic rate (µmol CO_2 $m^{-2}s^{-2}$) of near isogenic lines of soybean planted on June 2nd and June 24th 2003 from a) Harosoy and b) Clark background. Measurements taken Aug. 18, 2003. Bars represent standard error bars.

A



B

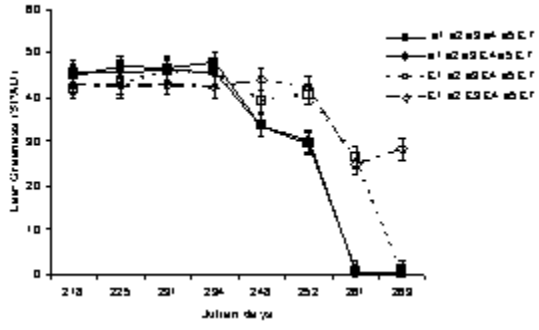


Fig. 2. Time course measurement of changes in leaf greeness (SPAD values) from a) Harosoy and b) Clark background taken on the third youngest fully expanded leaf from the top of NIL of soybeans (planted Julian day 153, 2003). The bars represent standard error bars.

When the data were adjusted for phenological stage, a completely different picture emerged. Although the dominant E1 allele maintained leaf greenness for a longer absolute duration, when leaf greenness was adjusted to reflect days from first flower, the E1 dominant alleles accelerated the rate of senescence

(Fig. 3), relative to those with the recessive e1 allele. Although leaf senescence began quicker after first flower under the influence of the dominant E1 allele, both recessive and dominant E1 allelic genotypes had similar duration of reproductive development (R1-R8, data not shown) at each planting date.

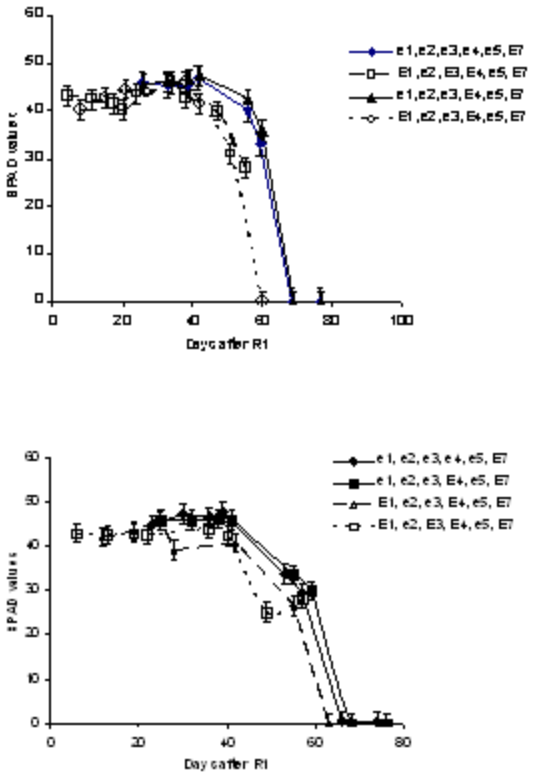


Fig. 3. Changes in leaf greeness (SPAD values) after first flower (R1) from a) Harosoy and b) Clark background. Measured on the third youngest fully expanded leaf of E-gene NILs of soybean(planted June 2nd 2003). The bars represent standard error bars.

Summary

The dominant E1 allele impacts functional leaf senescence. The NILs with the E1 allele had a longer vegetative period, and similar duration of reproductive development. The genotypes with the E1 allele maintained leaf greenness for a longer absolute duration during the growing season but exhibited an accelerated onset of leaf senescence after first flower as measured by leaf greenness (SPAD). There is evidence that the flowering and maturity E1-gene is involved in the regulation of the onset of leaf senescence. The data from the current study suggest that regulation of leaf senescence by E-genes appears to be distinct from its regulation of flowering.

References

Bernard RL (1971) Two major genes for time of flowering and maturity in soybeans. Crop Sci. **11**, 242-244.

Buzzell RI (1971) Inheritance of a soybean flowering response to fluorescent-daylength conditions. Can. J. Genet. Cytol. **13,** 703-707.

Buzzell RI, Voldeng HD (1980) Inheritance of insensitivity to long daylength. Soybean Genet. Newsl. **7,** 26-29.

Cober ER, Voldeng HD (2001) A new soybean maturity and photoperiod-sensitivity locus linked to E1 and T. Crop Sci. **41**, 698-701.

Ellis RH, Asumadu H, Qi A, Summerfield RJ (2000) Effects of photoperiod and maturity genes on plant growth, partitioning, radiation use efficiency and yield in soyabean [Glycine max. (L.) Merrill] 'Clark'. Annals of Bot. **85**, 335-343.

Fehr, W.R., and C.E. Caviness. 1977. Stages of soybean devlopment. Spec. Rep. No. 80, Coop. Ext. Serv., Agric. & Home Econ. Expn. Stn., Iowa State Univ., Ames IA.

Guiamet JJ, Giannibelli MC (1996) Nuclear and cytoplasmic 'stay green' mutations of soybean alter the loss of leaf soluble proteins during senescence. Physi. Plant. **96,** 655-661.

Guiamet JJ, Schwartz E, Pichersky E, Nooden LD (1991) Characterization of cytoplasmic and nuclear mutations affecting chlorophyll and chlorophyll-binding proteins during senescence in soybean. Plant Phys. **96**, 227-231.

Lim OP, Woo HR, Nam HG (2003) Molecular genetics of leaf senescence in Arabidopsis. Trends in Plant Sci. **8**, 245-299.

Luquez VM, Guiamet JJ (2001) Effects of the 'stay green' genotype GGd1d1d2d2 on leaf gas exchange, dry matter accumulation and seed yield in soybean (Glycine max L. Merr.). Annal Bot. **87**, 313-318.

McBlain BA, Bernard RL (1987) A new gene affecting the time of flowering and maturity in soybeans. J.Hered. **78**, 160-162.

Phillips DA, Pierce RO, Edie SA, Foster KW, Knowles PF (1984) Delayed leaf senescence in soybean. Crop Sci. **24**, 518-522.

Pierce RO, Knowles PF, Phillips DA (1984) Inheritance of delayed leaf senescence in soybean. Crop Sci. **24**, 515-517.