# Influence of E-Genes on Onset and Rate of Leaf Senescence in near Isogenic Lines of Soybean

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### Introduction

Genetic improvement in yield of a number of crops species including soybean has been associated with delayed senescence, i.e., the 'stay-green' characteristic. Research on 'stay green' genes has focused primarily on genes involved with photosynthesis and chlorophyll degradation, but not genes involved in development. The current study explores the impact of a group of developmental genes, known as the E-gene series, on soybean leaf senescence.

# Objective

To determine the role of E-genes in the control of leaf senescence in soybean.

#### **Materials and Methods**



Table 1. Near isogenic lines (NILs) of Clark and Harosoy and their gene composition		
Name	Genetic Background	Gene composition
L71-920	Clark	e1,e2,e3, <mark>E4</mark> ,e5,E2
L62-667	Harosoy	e1,e2,e3,E4,e5,E2

E1.e2.e3.E4.e5.E7

E1,e2,e3,E4,e5,E7

180-5914 Clark

L71-802 Harosoy

Figure 1: Photographs taken on August 31, 2004 of the NILs planted at different times to obtain synchronous flowering and subjected to either A) ambient or B) ambient plus 3hr. incandescent day length extension post-flowering.

The experiment was conducted in a split-plot design with three replications. The main plots were two photoperiods imposed following R1; i) natural day length (Amb) and ii) incandescent day length extension of 3 hours (Amb+3). The split plots were the E-gene near-isogenic lines (NILs), planted on different dates to obtain synchronous flowering. Phenology, photosynthesis, fluorescence and leaf chlorophyll concentration (SPAD) measurements were taken. When photosynthetic measurements among the NILs under the Amb+3 treatment showed differential photosynthetic rates, leaf tissues were collected and frozen at -80C. Total RNA was isolated and c-DNA was subsequently hybridized to an Affymetrix soybean genome chip. Gene expression patterns were analyzed by 'Stratagene Array assist software' Ver. 3.2.

## **Results & Discussion**

Cultivars with the dominant E1 allele maintained functional photosynthesis for longer after flowering, such that full senescence (0 photosynthetic rate) was delayed by approximately 160 GDDs (Fig.2A, 2B). This phenomenon was observed under both photoperiod treatments and irrespective of the genetic background ('Clark' and 'Harosoy').



Figure 2: Net carbon exchange rate measurements of NILs with either e1,e2,e3,E4,e5,E7 or E1,e2,e3,E4,e5,E7 alleles grown under either the A) amb or B) amb+3 treatments. Data are averages of two genetic backgrounds (Clark and Harosoy).

Maintenance of functional photosynthesis by plants with the E1 dominant allele can be attributed to maintenance of high Electron Transport Rate (ETR) (fig 3a and 3b), as well as delayed decline in leaf chlorophyll concentrations (data not shown).



Figure 3: Electron Transport Rate of NILs with either e1,e2,e3,E4,e5,E7 or E1,e2,e3,E4,e5,E7 E-gene alleles grown under either the A) amb or B) amb+3 treatments. Data are averages of two genetic backgrounds (Clark and Harosoy).

Consistent with the effect on leaf senescence, the dominant alleles also reduced the rate of phenological development, such that R5 occurred later in genotypes with dominant alleles and under the Amb+3 treatment (Fig 4). Complete senescence occurred approximately 600 or 700 GDDs after R5 under Amb or Amb+3 treatments respectively.

Figure 4: Accumulated growing degree days from flowering to R5 and full senescence for NILs with either dominant or recessive E1 alleles under A) Amb, and B) Amb+3 treatments. The data are averaged over two genetic backgrounds (Harosoy' and 'Clark').

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The dominant E1 allele may delay leaf senescence directly or indirectly, through its delay of reproductive development (Fig.4).



Figure 6: Volcano plot showing nearly 360 transcripts (in red) with 4fold differential expression (P=0.01) between recessive and dominant E1 NILs (Harosoy') grown under Amb+3 treatment. Leaf tissues were collected 680GDDs after treatment induction.

The presence of the dominant E1 allele significantly delayed the onset of functional senescence post-flowering, under both photoperiod treatments tested. This delay in senescence may be directly or indirectly related to the impact of this gene on reproductive development. The microarray data revealed a large number of differentially expressed genes that will likely hold further information on this phenomenon.