Cross-Species Identification of Mendel's I Locus

Ian Armstead, *et al.*

Science **315**, 73 (2007);
DOI: 10.1126/science.1132912

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.

Cross-Species Identification of Mendel's *I* Locus

Ian Armstead,[1]* Iain Donnison,[1] Sylvain Aubry,[2] John Harper,[1] Stefan Hörtensteiner,[2] Caron James,[1] Jan Mani,[2] Matt Moffet,[3] Helen Ougham,[1] Luned Roberts,[1] Ann Thomas,[1] Norman Weeden,[3] Howard Thomas,[1] Ian King[1]

We identified gene homologs in *Pisum sativum* (pea), *Arabidopsis thaliana*, and *Festuca pratensis* (meadow fescue), mutations of which partially disable plant senescence. The biochemical properties and map location of this gene in pea indicate that it is the same locus that determines yellow (*I*) and green (*i*) cotyledon color, as originally described by Mendel in his seminal paper (*1*). Staygreen (the gene *sgr*), the indefinite retention of greenness in senescing leaves and cotyledons, is a genetic variant that interferes with the normal expression of senescence. Biochemical (*2*, *3*) (Fig. 1A) and genetic analyses of *sgr* from pea and *F. pratensis* (*4*) show that it segregates as a discrete phenotype with equivalents in maize, sorghum, and rice (*5*, *6*). By using the *Lolium-Festuca* gene introgression system and extrapolating from genetic synteny, we show that *F. pratensis sgr* on chromosome (C)5 maps to a position corresponding to *sgr* on rice C9 (*4*, *6*, *7*). Fine mapping in *Festuca* delimits this to a region equivalent to circa 200 kb of the rice genome containing 30 annotated gene models, including a predicted senescence-inducible chloroplast-specific stay-green protein (*4*, *7*, *8*).

We investigated the temporal and tissue-specific expression patterns of the most similar *Arabidopsis* gene model, At4g22920, through the Genevestigator Meta-Analyzer database (*9*). These patterns indicate that At4g22920 is up-regulated in days 45 to 50 during maximal senescence in the *Arabidopsis* life cycle (fig. S1A) and that the strongest expression of this gene occurs in senescent leaves, although it is also detected in seeds, petals, and sepals. RNA interference (RNAi) was also used to silence the expression of At4g22920 in *Arabidopsis*, resulting in plants with leaves exhibiting prolonged chlorophyll retention upon dark incubation (Fig. 1B), phenotypically equivalent to *sgr F. pratensis*. Thus, alleles of *F. pratensis* and rice homologs of *Arabidopsis* At4g22920 likely underlie the monocot stay-green phenotypes.

In order to explore the parallels between Mendel's pea cotyledons and the stay-green leaf phenotypes in *Arabidopsis*, *Festuca*, and rice, we genetically mapped the pea homolog of *sgr* in two different pea populations segregating for cotyledon color polymorphism (*I* and *i*). No recombination between *I* and *sgr* was observed in 104 progeny, reflecting a theoretical maximum distance between *sgr* and *I* of 0.48 cM (fig. S1B). Northern analysis in pea indicates that *sgr* has reduced expression in mutant senescing leaves when compared with that of wild type (Fig. 1C).

This report characterizes a rare example of an unbroken chain from gene sequence to trait through biochemical, cell biological, and physiological definitions of phenotype, in which genomics and functional analysis in model species have played a critical role. This study results in the identification of a gene that plays a fundamental role in chlorophyll catabolism during plant senescence. In addition, it suggests that the cotyledon color trait described by Mendel reflects allelic variation in a pea gene, homologs of which are responsible for the stay-green phenotype in both dicots and monocots.

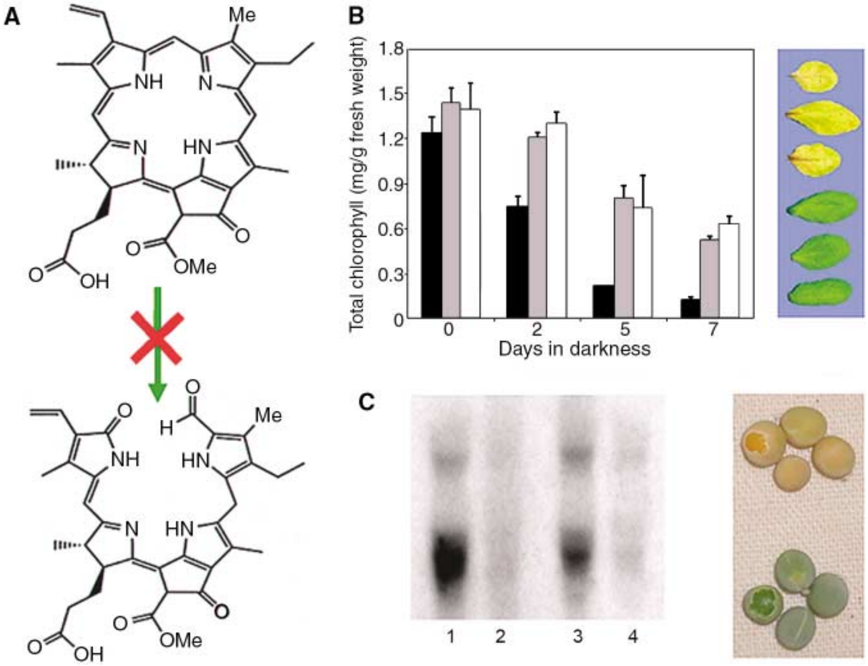

Fig. 1. (**A**) In mutant and silenced genotypes, inhibition (X) of the ring-opening step between (top) pheophorbide and (bottom) red chlorophyll catabolite in the chlorophyll breakdown pathway (*3*) leads to retention of greenness in senescing leaves. (**B**) RNAi silencing of *Arabidopsis* At4g22920 causes a stay-green phenotype. (Left) Degradation of chlorophyll in control (black) and two independently RNAi-silenced genotypes (gray and white) during dark-induced, detached leaf senescence. Error bars indicate standard deviation. (Right) Wild-type (top) and stay-green (bottom) leaf phenotypes of *Arabidopsis* after 5 days of dark-induced, detached-leaf senescence. (**C**) (Left) Northern analysis using RNA extracted from senescing leaves of wild-type (lanes 1 and 3) and stay-green (lanes 2 and 4) pea plants. (Right) Wild-type (top) and stay-green (bottom) pea cotyledons illustrating Mendel's *I* and *i* phenotypes, respectively.

References and Notes

1. G. Mendel, *Verh. Naturforsch. Ver. Brünn.* **4**, 3 (1866).
2. H. Thomas, M. Schellenberg, F. Vicentini, P. Matile, *Bot. Acta* **109**, 3 (1996).
3. S. Hörtensteiner, *Annu. Rev. Plant Biol.* **57**, 55 (2006).
4. I. P. Armstead *et al.*, *New Phytol.*, **172**, 592 (2006).
5. H. Thomas, C. M. Smart, *Ann. Appl. Biol.* **123**, 193 (1993).
6. K. W. Cha *et al.*, *Theor. Appl. Genet.* **104**, 526 (2002).
7. Materials and methods are available as supporting material on *Science* Online.
8. Institute for Genomic Research (TIGR), gene model LOC_Os09g36200, www.tigr.org.
9. Eidgenössische Technische Hochschule Zurich, www.genevestigator.ethz.ch/.
10. This work was supported by the Biotechnology and Biological Sciences Research Council (UK), the Swiss National Science Foundation (3100A0-105389), and Cooperative State Research, Education, and Extension Service, U.S. Department of Agriculture, for Hatch Project MONB00235.

Supporting Online Material

www.sciencemag.org/cgi/content/full/315/5808/73/DC1
Materials and Methods
Fig. S1
Table S1
References

24 July 2006; accepted 16 October 2006
10.1126/science.1132912

---

[1] Institute of Grassland and Environmental Research, Aberystwyth SY23 3EB, UK. [2] Institute of Plant Sciences, University of Bern, Altenbergrain 21, CH-3013 Bern, Switzerland. [3] Department of Plant Sciences and Plant Pathology, Montana State University, Bozeman, MT 59717, USA.

*To whom correspondence should be addressed. E-mail: ian.armstead@bbsrc.ac.uk