

Mutant screening / phenology key

Delphine Moreau and Nathalie Munier-Jolain

INRA, URLEG, Unité de Recherche en Génétique et Ecophysiologie des Légumineuses, F-21065 Dijon, France.

moreau@dijon.inra.fr

munierjo@dijon.inra.fr

http://www.dijon.inra.fr/english/genecophy_uk.htm

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1. Mutant screening for morphological traits

A standard terminology and a standard system for the notation of the plant developmental stages have been presented in Chapter “Morphology, development and plant architecture of *M. truncatula*”. This phenology key can be of practical use for identifying variations in plant developmental stages and for describing individual organs with specific characteristics of development and growth.

2. Mutant screening for the timing of establishment of the vegetative organs

As such, the phenology key does not allow understanding the origins of the differences in plant developmental stages between genotypes. In order to be able to explain phenotypic differences between lines at a given date, it is necessary to outline the temporal pattern of the plant developmental stages.

How taking in account time when analysing plant development?

The number of calendar days since sowing is a commonly used means of expressing time. Using calendar days can be relevant for comparing genotypes in a given experiment, if environmental conditions remain constant throughout the plant culture. However plant development is highly dependent upon environmental conditions. As a consequence, using calendar days does not allow comparing genotypes that have been grown under fluctuating environmental conditions (glasshouse and even growth chamber) or in different experiments.

Temperature is the main factor affecting plant vegetative development; but other environmental factors, such as photoperiod and light intensity, can in a lesser extent affect plant vegetative development (for review, see Kiniry et al. 1991). Therefore, an appropriate way for expressing time is the use of thermal time (for review, see Bonhomme 2000). Thermal time consists in expressing time by taking in account temperature. It is expressed in degree-days and is calculated as the sum of the mean daily effective temperatures, namely mean daily temperatures (T_m) minus the base temperature (T_b) as follows:

$$\text{Thermal time} = \int_0^t \max[0; (T_m - T_b)] dt$$

The base temperature is defined as the temperature below which the development is supposed to be nil. It has been estimated at 5°C for the line A17 (Moreau et al. 2006).

Modelling the timing of establishment of the vegetative organs using thermal time

The timing of establishment of the vegetative organs was analyzed for the line A17. Towards this goal, the number of leaves on the first branch B0 was observed in 5 experiments conducted under varied conditions of temperature and light intensity, mean temperature ranging from 12 to 22 °C according to the experiment (Moreau et al. 2006). The number of leaves was then related to time expressed in calendar days (Figure 1a): a constant rate of leaf appearance was obtained for each experiment but the rate of leaf appearance varied between experiments when time was expressed in days. Instead of analyzing plant development as a function of the number of days, it was related to thermal time expressed in degree-days (Figure 1b). The use of thermal time unified the rate of development: the rate of leaf appearance became constant in spite of environmental differences among experiments. Because the rate of leaf appearance was constant, it can be considered as a genotypic characteristic of the line A17 (Tardieu 2003).

How screening mutants using an ecophysiological model?

Here, an example of practical application of the model is presented. The phenotypic differences exhibited by two nodulation mutants (*sun-2* and *sun-4*) were analysed in comparison with A17. When analysis relied on the global observation of the plants at a given date (Figure 2), the establishment of the leaf area was delayed for the mutants as compared with A17. However, the physiological basis of the phenotypic differences is not apparent from this observation alone.

In order to better characterize the phenotypic differences, the phenotypes were analysed using ecophysiological tools: plant development stage was described using the system of notation (see section 1.3.1) and the number of leaves on the two first primary branches (B0 and B1) was then related to thermal time. Each line was characterized by four parameters: the date of appearance of B0 and B1 (in degree-days) and the phyllochron of B0 and B1 (thermal time interval in degree-days between the appearance of two leaves):

The parameters of the mutants were then compared to those of A17 (Table 1):

- ✓ *sun-2* / A17: a delay in the date of appearance of B0 and B1 was shown for *sun-2*. Conversely, the rate of leaf appearance did not significantly vary between the two lines: the phyllochron of *sun-2* was very close to that of A17. So, the delay in the establishment of the leaf area for *sun-2* was to linked to a later appearance of the branches.
- ✓ *sun-4* / A17: a great delay in the date of appearance of B0 and B1 was shown for *sun-4*. Additionally, the rate of leaf appearance was slower for *sun-4*: its phyllochron was higher than that of A17. So, the delay in the establishment of the leaf area for *sun-4* was linked both to a later appearance of the branches and to a slower rate of leaf appearance.

Conclusion on the different practical uses of the model

The framework methodology can be helpful for:

- ✓ Analysing mutants with alterations in the timing of establishment of the vegetative organs: for "obvious" mutants, the model can be used for identifying the origins of phenotypic differences; but it can also be helpful for making easier the discovery of "subtle" mutants

that are inconspicuous without a sensitive method for identifying slight alterations in the development timing;

- ✓ Comparing genotypes according to ecophysiological parameters that are independent upon environmental conditions (such as the rate of leaf appearance);
- ✓ Predicting plant development, using solely temperature data, and therefore scheduling both plant observation and data collection better;
- ✓ If temperature has been measured throughout the plant development, *a posteriori* evaluating the plant developmental stage and the physiological age of the organs that have been used for metabolic or gene expression analyses; thus, this model can be of practical importance when checking if data produced with plants cultivated under different environments have been obtained using organs with similar physiological ages, making the comparison of data arising from different experiments more relevant.

3. References

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- Kiniry J.R., Rosenthal W.D., Jackson B.S. & Hoogenboom G. (1991) Predicting leaf development of crop plants. In *Predicting Crop Phenology* (ed T. Hodges), pp. 29-42. CRC Press, Boca Raton.
- Moreau D., Salon C. and Munier-Jolain N. (2006). Using a standard framework for the phenotypic analysis of *Medicago truncatula*: an effective method for characterizing the plant material used for functional genomics approaches. *Plant, Cell and Environment* **29**: 1087-1098.
- Tardieu F. (2003) Virtual plants: modelling as a tool for the genomics of tolerance to water deficit. *Trends in Plant Science* **8**: 9-14.

4. Figures and Tables

Figure 1

Changes over time in the number of appeared leaves on the first primary branch (B0), in five experiments identified by different symbols. (a) Time is expressed in calendar days from the date of appearance of the first trifoliolate leaf on the main axis. (b) Time is thermal time expressed in degree-days (with a base temperature of 5 °C) from the date of appearance of the first trifoliolate leaf on the main axis (from Moreau et al. 2006).

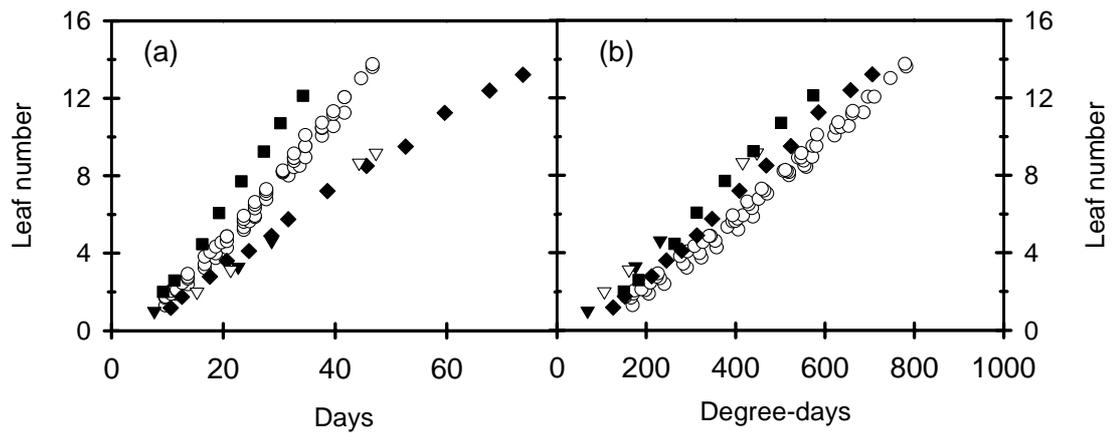


Figure 2

Global observation of the phenotypic differences between the reference line A17 and two mutants of *Medicago truncatula* 34 days after sowing (from Moreau et al. 2006).

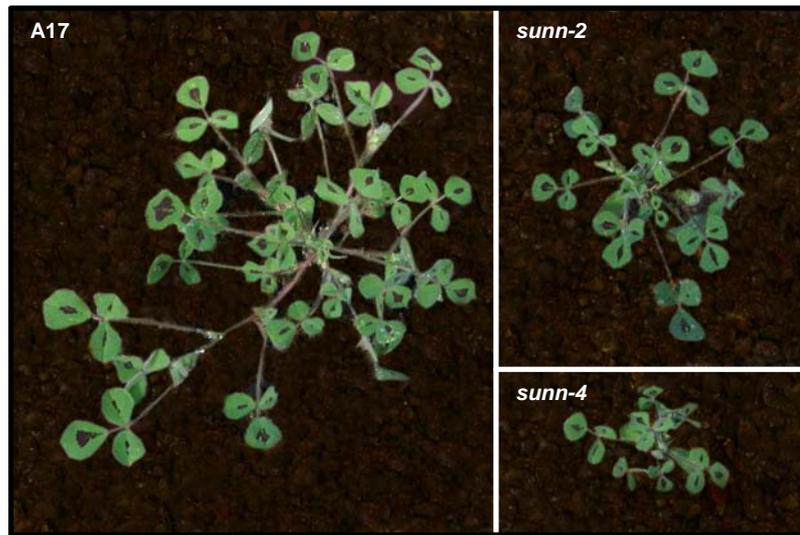


Table 1

Detection of phenotypic differences in the vegetative development between the reference line A17 and two mutants of *Medicago truncatula*
 From Moreau et al. (2006)

Axis	Genotype	Date of appearance (degree-days)			Phyllochron (degree-days)		
		Mean ^a	SD ^b	Mean comparison ^c	Mean	SD ^b	Mean comparison ^c
B0	A17	123	11	a	47	3	a
	<i>sunn-2</i>	158	15	b	49	2	a
	<i>sunn-4</i>	194	10	c	56	3	b
B1	A17	162	5	a	47	3	a
	<i>sunn-2</i>	205	17	b	48	3	a
	<i>sunn-4</i>	245	9	c	53	2	b

^a Mean time interval between the date of appearance of the first trifoliate leaf on the main axis and the date when the branches carried one appeared leaf.

^b SD, standard deviation.

^c Means were classified using a Student-Newman-Keuls test (P<0.05): for a given axis and a given developmental process (date of appearance or phyllochron), identical letters indicate no significant differences between genotypes