

## Synchronism between adventitious root and leaf development in hydroponic sorghum

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### Introduction

Plant studies rarely investigate how vegetative development and root development affect each other. Better characterization of this relation is a key point in understanding how plants adapt to diverse environmental conditions. The difficulty is that roots are not directly accessible in the natural environment and the traditional methods used to study roots are difficult, destructive and time consuming. As a result, the acquired information is generally limited to root biomass or length distribution in the soil.

Hydroponic culture methods could be a way of more easily gaining information on root characteristics and growth. They allow the rooting potential of plants to be expressed and the genetics of root traits to be investigated as done by Tuberosa et al. (2002). Blum et al. (1977a, 1977b) used them to study the effect of maturity genes and heterosis on the sorghum (*Sorghum bicolor*) root system. They carried out interesting observations on the appearance of adventitious roots, which are produced in sequence in concentric whorls with a lag between whorl initiation events in juvenile sorghum plants. They thus defined the concept of the root plastochron and noted that it appeared constant over the experimental period (limited to 38 days after germination).

In our study, we grew a sorghum genotype under hydroponic conditions to more accurately determine its root plastochron, which we called the rhizochron, and we investigated the relationship between root and vegetative development.

### Materials and methods

The sorghum genotype used for this study was an Australian line (R999218) derived from an initial cross between an elite tester (31945-2-2) and a wild sorghum (*Sorghum arundinaceum*). It is short and has poor tillering ability. The same seed sample was used for two experiments.

For each experiment, seeds were germinated in a box on crinkled paper and kept in a germination room (day/night temperature respectively 28°C/20°C and day/night 13 h/11 h). Seedlings of uniform size were transferred to hydroponic vessels four days after the beginning of germination. The vessels consisted of plastic boxes measuring 40 cm long by 35 cm wide by 35 cm tall. With 8 seedlings per box on a carpet of foam and four boxes, we observed 32 plants per experiment for 28 days. The boxes were rearranged daily to avoid any border effect or light heterogeneity effect in the growth chamber.

Each box contained 30 liters of nutrient solution (Hoagland type, pH adjusted to 6.5), with continuous water circulation.

In each experiment, the same six random plants were regularly measured (each day if possible, except at the week-end). The adventitious roots were tagged with a record of the time of emergence and length. At the end of the experiment, the diameters of each adventitious root per plant were measured at their emergence point. For the six plants, the appearance of the tip and ligule of each leaf was also recorded for each plant.

The first experiment was conducted in a growth chamber in the summer of 2006, with a day/night air temperature of 28°C/20°C and relative air humidity at around 70%. Illumination in the growth chamber provided an average PAR (photosynthetically active radiation) of 450  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at the top of the plant. Photoperiod duration was adjusted to 13 h/11 h (day/night).

Temperature measurement was done by using thermocouples placed at the bottom of the canopy of the seedlings. Light was supplied with metal halide lamps around 0.8 m from the plant tops. Radiation and temperature conditions were recorded with hourly integration, using sensors and a CR10X data logger (Campbell Scientific, North Logan, UT, USA).

In the summer of 2007, a second experiment was carried out under the same conditions except that illumination was increased to provide an average PAR of 600  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at the top of the plants.

Similarly to the plastochron, we defined the rhizochron as the thermal time expressed in Celsius degree-days (°Cd) separating the appearance of the elemental adventitious roots of two consecutive phytomers, on the main stem. A phytomer is the elemental structural unit repeated on a given stem. Each phytomer potentially has a node, internode, subtending leaf and axillary bud. Consequently, in order to determine the rhizochron, it was necessary to have the right determination of the phytomer of origin for each adventitious root on juvenile sorghum plants such as ours. As adventitious roots from a given phytomer have traits that group together closely, but differently from adventitious roots of other phytomers (Blum et al. 1977b), we used a multivariate method (PCA: Principal Component Analysis) to illustrate how adventitious roots cluster according to their phytomer, and consequently determine the rank of their original phytomer. The traits considered were especially discriminating, ie, time of emergence, maximum length and diameter at the end of the experiment. The PCA method was performed on these traits using XLSTAT (Addinsoft Inc.). This allowed to assign each adventitious root to its original phytomer. To calculate the appearance date of the roots for each phytomer, we averaged the individual times of the visual appearance of the adventitious root from each phytomer.

For each experiment, the thermal time up to a developmental event was established by accumulating daily mean temperatures from sowing up to that event according to the following calculation:

$$TT = \sum_{i=1}^n (T_{i,min} + T_{i,max})/2 - T_b$$

with a Tbase of 10°C;  
 $T_{i,min}$  = Daily minimum temperature; and  
 $T_{i,max}$  = Daily maximum temperature.

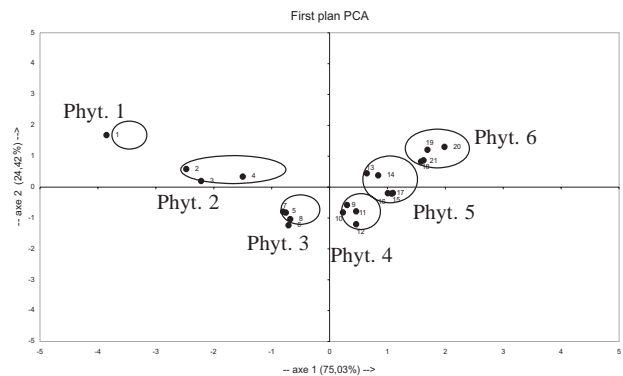
In this study, the phyllochron was expressed as the slope of thermal time up to the appearance of the leaf tip

versus the leaf number, based on the linear and early-established relationship between the two variables (Clerget et al. 2008). We also calculated the ligulochron as the slope of thermal time up to the emergence of the leaf ligule versus leaf number. Like the plastochron, the rhizochron was expressed as the slope of thermal time up to the mean appearance of the adventitious roots per phytomer versus phytomer rank on the linear relationship.

## Results

Figure 1, obtained from the PCA method used per plant, illustrates how the adventitious roots of a given plant (ie, fourth plant – 2006 experiment), ranked according to their thermal time of appearance, were clustered according to the phytomer number of origin.

For some plants, clustering of the first adventitious roots (first to fourth or fifth roots) was less obvious than that of the following roots because there was some disturbance in the root emission of the first two phytomers.



**Figure 1.** F1 and F2 factorial plane of the PCA for traits related to adventitious roots of the fourth plant in the 2006 experiment.

**Table 1. Phyllochron, ligulochron and rhizochron values per plant (in °Cd) studied in the 2006 and 2007 experiments.**

| Plant              | 2006 experiment |             |            | 2007 experiment |             |            |
|--------------------|-----------------|-------------|------------|-----------------|-------------|------------|
|                    | Phyllochron     | Ligulochron | Rhizochron | Phyllochron     | Ligulochron | Rhizochron |
| 1                  | 33.37           | 47.20       | 44.12      | 37.30           | 45.00       | 46.50      |
| 2                  | 34.81           | 47.60       | 44.95      | 37.84           | 43.71       | 47.50      |
| 3                  | 33.20           | 47.60       | 45.94      | 41.43           | 43.71       | 43.50      |
| 4                  | 35.32           | 47.60       | 49.13      | 43.66           | 45.00       | 45.00      |
| 5                  | 33.37           | 46.00       | 46.64      | 37.58           | 43.71       | 48.50      |
| 6                  | 33.70           | 47.20       | 43.20      | 38.00           | 43.71       | 45.00      |
| Mean               | 33.91           | 47.20       | 45.66      | 39.30           | 44.14       | 46.00      |
| Standard deviation | 0.91            | 0.62        | 2.10       | 2.62            | 0.67        | 1.84       |

As a result, the assignment of adventitious roots to their phytomers of origin is presented per plant in Figure 2. There was an increase in the number of adventitious roots from the first phytomer (one to three roots) to the fifth (four to six roots).

After calculating the mean thermal time of appearance of the adventitious roots per phytomer and per plant, we established the rhizochron values and compared them to the phyllochron and ligulochron values. Figure 3 gives a graphic representation of the results for the third plant in the 2006 experiment. The linear relationship between phytomer rank and thermal time of their leaf or root production is confirmed. Moreover, it appeared that the rhizochron (45.9°Cd) was very close to the ligulochron (47.6°Cd). This applied for each plant from the two experiments (2006 and 2007), as shown in Table 1.

Another presentation of the results illustrates the linear relationships between phytomer and thermal time

leaves of phytomer n were somewhat synchronized with the emission of the adventitious roots of phytomer n-1 in the 2006 experiment and with the emission of the adventitious roots of phytomer n-2 in the 2007 experiment.

### Discussion

The multivariate analysis method employed in this study was useful for determining the phytomer number of origin of adventitious roots and then facilitated calculation of the rhizochron. However, some inaccuracies existed for the assignment of the first roots. This may have been due to the change in the nutrition mode of the seedlings when they became autotrophic, which probably disrupted the emission and characteristics of the first adventitious roots. These roots emerged between 100° and 200° days after germination

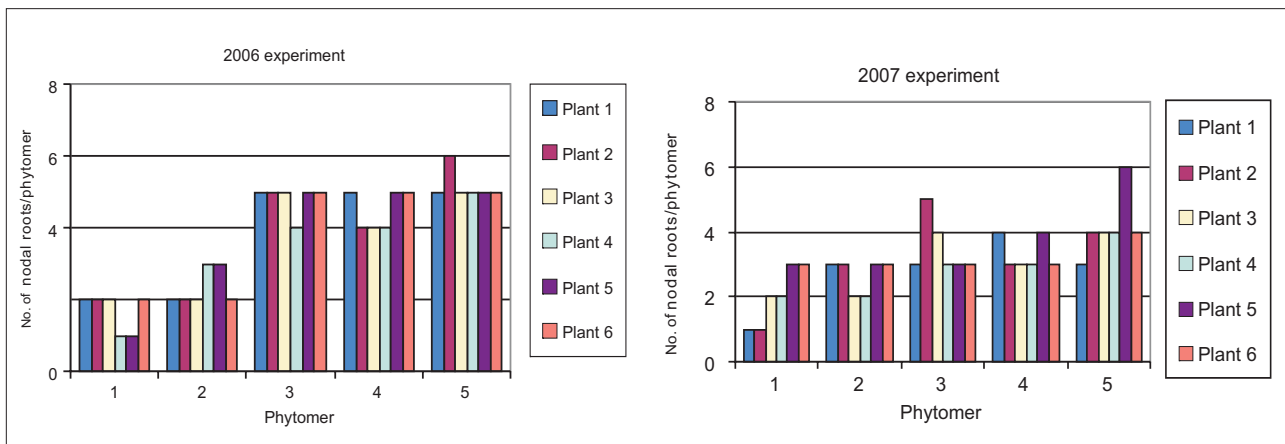


Figure 2. Number of adventitious roots per phytomer for each juvenile plant in the 2006 and 2007 experiments.

for organ production (leaves and adventitious roots) averaged per phytomer and plants (six) for each experiment (Fig. 4). When averaged on an experiment level, the results were the same as those established per plant. The emission of adventitious roots per phytomer was linear in thermal time with a rhythm similar to the rhythm of totally deployed leaves: rhizochron ~ ligulochron. For a given phytomer, a time difference existed between the full deployment of its leaf and the emission of its adventitious roots. The difference was almost constant for each experiment, but differed from one to the other (around 66°Cd and 98°Cd respectively in the 2006 and 2007 experiments). This corresponded to 1.4- to 2-fold the ligulochron, which means that the

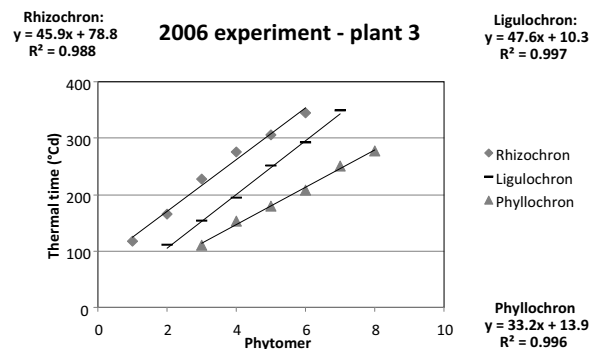


Figure 3. Thermal time of leaf appearance, total leaf expansion and appearance of adventitious roots per phytomer (phyllochron, ligulochron and rhizochron respectively) for the third plant in the 2006 experiment.

at the three-leaf stage, which marks the end of the seed dependent phase in sorghum (Vanderlip and Reeves 1972). As a result, the rhizochron could be more accurately determined by discarding the adventitious roots of the first two phytomers in the calculation.

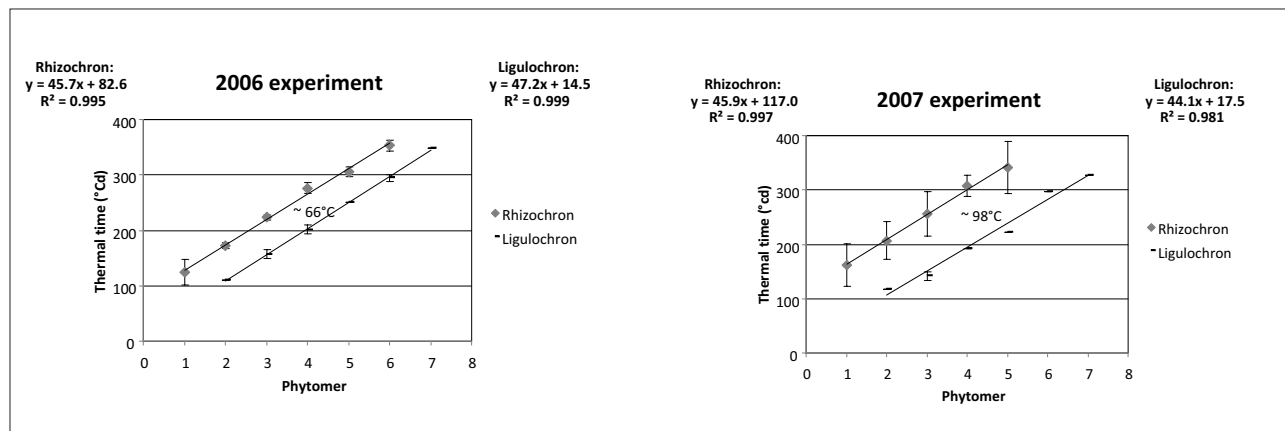
The increasing number of adventitious roots depending on phytomer rank was observed in the 2006 and 2007 experiments with generally one or two roots for the first phytomer and four or five for the fifth phytomer. Their spatial arrangement was not randomized. Two adventitious roots for a given phytomer tended to be opposite (180°), three roots at 120°, four at 90° and so on ... From one phytomer to the next, the trend was also for roots not to be superposed at their insertion points.

There were some differences in the phyllochron and ligulochron values depending on the two experiments: 33.9°Cd and 47.2°Cd in the 2006 experiment and 39.3°Cd and 44.1°Cd in the 2007 experiment. This can be partly explained by differences in the observation of leaf emergence and expansion, which were more regularly scored in the 2006 experiment. The phyllochron values in the two experiments were lower than those under field conditions as calculated by Clerget et al. (2008), who determined a mean value of 46.3°Cd with  $T_b=11^\circ\text{C}$  for three different sorghum varieties. The rapid rhythm of leaf emergence under hydroponic conditions in a growth chamber highlighted development and growth up to potential when no limiting factors were involved. In conformity with this result, Clerget et al. (2012) have recently observed that soil fertility affected phyllochron in sorghum.

The rhizochron displayed similar traits in the two experiments. Calculated over thermal time, it was constant and became established early, as already observed by Blum et al. (1977a, 1977b). Moreover, it

was synchronized with vegetative development, as shown by the rhizochron values, which were very similar to the ligulochron values. Synchronism between the development of adventitious roots and leaf development also exists in rice (*Oryza sativa*) and is constant across environments within each rice cultivar (Nemoto et al. 1995). For a given phytomer, the interval of total leaf expansion and adventitious root appearance was almost constant, but differed in the two experiments (around 66°Cd in 2006 and 98°Cd in 2007). As a result, the beginning of adventitious root emission was later in 2007 than in 2006, which might indicate slightly poorer growth conditions in the second experiment, maybe because the seeds were a year older. This difference also explains why the total leaf expansion of phytomer “n” was roughly synchronized with emission of the adventitious roots of phytomer “n-1” in the 2006 experiment and with emission of the adventitious roots of phytomer “n-2” in the 2007 experiment.

These results were obtained from just one sorghum variety under hydroponic conditions. We also determined the rhizochron of the same variety in a greenhouse pot culture. Under these conditions, the preliminary results showed that the rhizochron was constant and was established early, but was higher than the ligulochron, which was itself higher than that under hydroponic conditions. A synchronic scheme between leaf and root development is confirmed but with some differences compared to hydroponic culture. To conclude, synchronism appears to link adventitious root development to leaf development in sorghum. Any environmental conditions that accelerate leaf production should accelerate adventitious root emission. Moreover, if genetic diversity exists for the phyllochron in sorghum, the varieties characterized by a significant rapid



**Figure 4.** Relationship between phytomer and mean appearance (in thermal time) of their adventitious roots and total expansion of their leaves. (Note: Results are averaged for the six plants of the 2006 experiment and the six plants of the 2007 experiment. Vertical lines represent the 95% confidence interval of the means.)

phyllochron should also be characterized by rapid adventitious root emission.

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