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Temperature Control of Node Appearance and Initiation in Soybean

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TEMPERATURE CONTROL OF NODE APPEARANCE AND

INITIATION IN SOYBEAN

by

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TEMPERATURE CONTROL OF NODE APPEARANCE AND
INITIATION IN SOYBEAN

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Soybean demand remains strong and continues to grow as a source of protein and oil for food and feed. Soybean production is expanding into cooler and warmer environments, thus, it becomes critical to expand the current knowledge about the influence of temperature on soybean. Temperature is the main environmental factor effecting node appearance rate (NAR) and node initiation rate (NIR), which are key parameters controlling soybean growth and development. This study aims to assess the response of NAR and NIR to temperature and investigate the coordination between these two processes under controlled and field conditions. Two greenhouse experiments with four contrasting mean temperatures (15-26ºC) and two field experiments with different sowing dates (April 23 to June 19) were conducted using maturity groups 2.1 and 3.0. The number of appeared nodes was measured every 2 to 7 days from sowing to *ca.* R5 to estimate NAR (nodes d^{-1}). Stem apex was dissected every 4 to 7 days from sowing to *ca.* R3 to estimate NIR (nodes d^{-1}). A co-ordination model was fitted between the number of initiated nodes and number of appeared nodes. Significant relationship was found in response to temperature of NIR and NAR. A constant plastochron of 36ºCd and dual value for phyllochron (83 and 58ºCd) depending on ontogeny was observed, with base temperature of *ca.* 10ºC for both processes. There is a strong two-phase co-ordination between node initiation and node appearance. This work established the response of NAR and NIR to temperature which could improve prediction of phenology, leaf area, and yield by the current soybean simulation models.

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Overview of work

The projected annual growth rate of total world consumption of all agricultural products is 1.1% from 2005/2007-2050, which means that production in 2050 should be 60% higher than that of 2005/2007 (Alexandratos *et al.* 2012). Soybeans are expected to continue as one of the most dynamic crops, although its production is increasing at a more moderate rate than in the past. Soybean production, then, would need to increase by nearly 80% in 2050. Soybean production expansion was observed in cooler and warmer environments (Specht *et al.* 2014; Sinclair *et al.* 2013), thus, a more detailed observation on the influence of temperature on soybean growth and development is needed.

Leaf appearance is a key determinant of soybean growth and yield due to its influence in leaf area index (LAI) that controls light interception during the growing season (Barillot *et al.* 2014). Each leaf grows from a node in the stem where pods set, thus, the number of nodes a plant can potentially produce is important for yield determination (Bastidas *et al*. 2008). It is worthwhile, then, to study how environmental factors affect the dynamics of leaf appearance to help increase current yields and improve accuracy of existing soybean crop simulation models for decision making.

Temperature is the main environmental factor determining leaf or node appearance rate (NAR) and leaf or node initiation rate (NIR) in annual crop species (Granier & Tardieu 1998). Temperature sensitivity in soybean was first documented by Hesketh *et al.* (1973), who reported a phyllochron (thermal time between two successively appeared nodes) of 56ºCd, which was also reported by Thomas & Raper (1976). However, a lower base

temperature (T_b, below which rates equal zero) was reported by Hesketh *et al.* (1973) compared to Thomas & Raper (1976) (6ºC *versus* 9ºC). On the other hand, a temperature-insensitivity of field grown soybean was reported by Bastidas *et al.* (2008), and concluded a constant node appearance of 0.27 node d^{-1}. These contradicting results on soybean NAR response to temperature were puzzling, thus, this study was initiated. While there were previous reports about temperature effect on NAR, no study had focused on response of NIR to temperature and reported plastochron (thermal time between two successively initiated nodes) in soybean.

With the contrasting accounts on temperature effect on NAR and no clear effect on NIR, this study aimed to: 1). establish the response of both NAR and NIR under a wide range of temperature which would be useful for the observed soybean expansion in areas with contrasting temperature, and 2). investigate the co-ordination between the two processes in soybean which will make possible of NIR observation without any destructive sampling.

In this study, frequent node appearance and node initiation observation was done in a combination of greenhouse and field experiments with wide range of weather, soil, and management practices. The maturity groups used portrayed well those of the most dominant varieties grown in the US Corn Belt. In particular, results from this study will improve prediction of phenology, leaf area, and yield by the current soybean simulation models.

INTRODUCTION

Soybean is the most important legume crop globally, with respective harvested area and total production of 118 Mha and 280 million Mg (FAOSTAT 2014, http://faostat3.fao.org). Soybean is a key component of global food security as a source of protein and oil for food and feed, accounting for 56% of total oilseed production globally (Wilson *et al.* 2008). Meeting food demand on existing cropland area for a global population of 9.7 billion people by year 2050 will require current crop yields to increase 70-110% within the next 35 years (FAO 2009; Tilman *et al.* 2011; United Nations 2015, http://esa.un.org/unpd/wpp/Publications). Understanding leaf appearance dynamics can help elucidate ways to increase current soybean yield. On one hand, leaf appearance influences leaf area index which, in turn, controls light absorption during the growing season (Lafeur & Fransworth 2008; Barillot *et al.* 2014). On the other hand, each leaf is associated with a 'node' where pods and seeds can potentially be set; hence, the final number of nodes is a key component for yield determination (Board & Tan 1995; Board *et al*. 1999; Bastidas *et al*. 2008; Nico *et al.* 2015). Robust prediction of leaf appearance can help improve accuracy of existing soybean crop simulation models to inform producer decision-making and regional in-season yield forecasting (Sinclair 1987; Boote *et al*. 2003; Setiyono *et al*. 2010).

Leaf number at a given point of time depends upon the rate of (microscopic) leaf initiation at the stem apex (SA) and the rate of (macroscopic) leaf appearance. Temperature is the main environmental factor determining leaf appearance and initiation rates in annual crop species (Kiniry *et al*. 1991; Sadras & Villalobos 1993; Granier &

Tardieu 1998), while photoperiod was reported to have negligible effect on leaf appearance (Thomas & Raper 1983; Nico *et al.* 2015). Typically, initiation and appearance rate linearly increase over a range of temperature delimited by a base temperature (T_b), below which rate equals zero, and an optimal temperature (T_{opt}), above which the rate decreases (Kiniry *et al.* 1991; Slafer & Rawson 1994). The inverse of the slope of the linear relationship between leaf appearance and initiation rates *versus* temperature represents the phyllochron (defined as the thermal time [ºCd] between two successively appearing leaves) and the plastochron (thermal time between two successively initiated leaves), respectively. Plastochron and phyllochron are relatively constant across management practices, environmental conditions, and cultivars of the same crop species (Kiniry *et al.* 1991; Ritchie & NeSmith 1991; Slafer 1995), although genotypic variation has been reported for maize and wheat (Padilla & Otegui 2005; Slafer & Rawson 1997). With soybean production expanding into cooler (Specht *et al.* 2014) and warmer (Sinclair *et al.* 2013) environments, it becomes critical to bolster the current knowledge about the influence of temperature on soybean leaf appearance and initiation.

In soybean, cotyledons and unifoliolate leaves at the first two stem nodes are opposite (*i.e.*, in pairs, borne on two nodes directly 180º opposite from each other) while subsequent trifoliolate leaves are alternate (*i.e.*, singly, borne on nodes alternating 180º from each other) (Fehr *et al.* (1971) (Table 1). To distinguish between leaf initiation and appearance rates, the two hereafter are termed as the node initiation rate (NIR, initiated node d^{-1}) and node appearance rate (NAR, appeared node d^{-1}), respectively. Node appearance starts at emergence (VE) and ceases around flowering (R1 stage) and

beginning of seed filling (R5 stage) in determinate and indeterminate soybean cultivars, respectively (Sinclair 1984a; Bastidas *et al*. 2008). Temperature influence on soybean NAR was first documented in the early 1970s by Hesketh *et al*. (1973). These researchers grew soybean indeterminate cv Wayne (maturity group [MG] 3.0) in a series of greenhouse and growth chamber experiments where plants were exposed to a specific temperature that range from 13ºC to 30ºC. The results obtained in this study indicated a linear relationship between NAR and temperature, with an estimated phyllochron of 56ºCd successive node, and T_b of 6ºC. A few years later, Thomas & Raper (1976) reported an identical phyllochron for determinate cv Ransom (MG 7.0), but with a slightly higher T_b (9ºC), based on experiments conducted in growth chambers, set to specific temperatures that ranged from 16 to 28ºC. All of these values should, however, be treated with caution as it is not clear whether the temperature reported in these two studies were actually measured at or near the SA height position during the growing season or, instead, were just targeted (but not measured) temperatures. Moreover, the phyllochron estimates derived from these studies have not been validated for field-grown soybean crops. Despite these uncertainties, the phyllochron of 56ºCd derived by Hesketh *et al.* (1973) was subsequently used to model main stem node appearance in soybean (Sinclair 1987; Wilkerson *et al*. 1989; Jones *et al*. 1991; Piper *et al*. 1996; Setiyono *et al*. 2007, 2008). Recently, Bastidas *et al*. (2008) measured NAR in field experiments conducted in 2003 and 2004 in Lincoln NE (USA), which included four sowing dates of late April, mid-May, late May, and mid-June and indeterminate cultivar MGs (from 3.0 to 3.9) during two crop growing seasons. These researchers concluded that NAR was insensitive to temperature, reporting a near-constant NAR of 0.27 node d^{-1} (*i.e.*, 3.7 day

node^{-1}) across cultivar x sowing date x year combinations. However, the range of mean

temperature during the period of node appearance (from V1 to R5) was relatively narrow

(from 22 to 26 ℃) across sowing date x year combinations, and this small temperature

range was likely insufficient for robustly assessing the influence of temperature on NAR.

Still, the NAR temperature-insensitivity observed in this study and the NAR temperature-

sensitivity are conflicting, and further research is warranted that would involve

coordinated experiments conducted in both controlled conditions and field conditions.

Few researchers have looked at node initiation in soybean, and then focusing only on

soybean cultivars grown in controlled environments, and none explicitly evaluated the

influence of temperature on NIR. Miksche (1961) reported a NIR of 0.29 node d^{-1} (*i.e.*,

3.5 days between successive nodes) but only from the first to the second initiated

trifoliolate nodes, and thereafter observed a constant NIR of 0.50 node d^{-1} (*i.e.*, 2 days).

Johnson *et al*. (1960) observed that just 35 days after planting (about V5), the SA had

already produced all the 19 node primordia that were eventually to appear. Based on this

early observation, Lersten & Carlson (2004) inferred a NIR of 0.50 node d^{-1} (*i.e.*, 2 days).

Unfortunately, air temperature was not reported in those studies. Thomas &

Kanchanapoom (1991) and Chiera *et al*. (2002) also reported a NIR of 0.50 node d^{-1}

under 26/22 ℃ day/night temperature and 0.52 node d^{-1} under 24/22 ℃ day/night

temperature, respectively.

A strong synchronization between node initiation and appearance has been reported in the

literature. Kirby (1990) and Hay & Kirby (1991) found that NIR was faster relative to

NAR (*i.e.,* 1.7 primordia per appeared node) across different wheat cultivars grown in a wide range of environments. This observation suggested that node appearance and initiation were coordinated, with SA development controlled by emerging leaves or *vice versa* (later known as the "coordination model"). Subsequent studies have confirmed the strong coordination between leaf initiation and appearance in maize (Padilla & Otegui 2005), sunflower (Sadras & Villalobos 1993), rice (Nemoto *et al*. 1995), and pea (Turc & Lecoeur 1997). Remarkably, no study had looked at the coordination between node appearance and initiation in indeterminate soybean where the SA continues to differentiate new node primordia, while floral differentiation progresses from the lower to the upper nodes (Saitoh *et al*. 1991).

Accurate prediction of NAR and NIR across a wide range of weather, management, and varieties is crucial for accurate prediction of soybean phenology, leaf area, and seed yield. However, as noted, little is known about the influence of temperature on node initiation and appearance in indeterminate soybean and the coordination between these two processes. To bridge this knowledge gap, the aim of this study was to investigate the response of NAR and NIR to temperature in indeterminate soybean with a combination of experiments conducted in controlled environments and in field conditions. Our working hypothesis was that node appearance and initiation rates are temperature-dependent, and that these two processes are coordinated.

2. MATERIALS AND METHODS

2.1. Overview

A series of greenhouse (GH) and field experiments (including producer fields) were carried out in Nebraska (USA) during 2015 to assess the influence of temperature on soybean node initiation and appearance (Table 2). Three experiments (Expt. 1-3) were conducted at the greenhouse (GH) and field facilities located at the University of Nebraska-Lincoln (Lincoln NE). The same two indeterminate soybean varieties (IA2102 and IA3024), with contrasting MGs (2.1 and 3.0, respectively), were grown across these three experiments. GH experiments (Expt. 1 and 3) involved four treatments with contrasting temperature regimes (range from 15 to 26ºC) under constant photoperiod (15 h). The field experiment (Expt. 2) involved six planting dates spaced out at *ca.* 10-day intervals, where plants were exposed to the natural change in temperature and photoperiod during the growing season. The last experiment (Expt. 4) was conducted in four producer irrigated soybean fields in NE (Table 2). Different sowing dates and locations for field experiments (Expt. 2 and 4) allowed to have a relatively wide temperature range during the period of node appearance across experiments. Altogether, the four experiments explored a wide range of weather, soil, and management practices. Range of cultivar MGs across experiments portrayed well that of most dominant commercial varieties grown in the US Corn Belt, with the latter region accounting for 35% of global soybean production. In this study, the range in mean photoperiod explored across treatments and experiments was very narrow (14.4 to 15 h). Previous studies have documented that photoperiod does not influence node appearance in soybean (Thomas &

Raper, 1983; Nico *et al.* 2015), which agreed with the results from this study (see section 3.3).

Soil and air temperature was measured every 30 minutes in all experiments, starting at sowing and ending at physiological maturity (R7). Soil temperature sensors were placed at seed depth while air temperature sensors were adjusted at SA height. Average daily temperature was calculated by averaging the (48) temperature records within a day. For all calculations, daily soil temperature was only used for the time period when SA was below soil surface (*i.e.*, from sowing to VE) while daily air temperature was used afterwards. In all experiments, crops received optimal pest, water, and nutrient management in order to keep plants free of abiotic and biotic stresses.

2.2 Experiments

Experiment 1. Plants were grown in 21-L plastic pots (0.3 m height and diameter) filled with sandy loam soil. Pots were placed in four GH rooms simulating a crop stand of 36 plants m^{-2}, with rows spaced 0.15 m apart. A total of 64 pots were allocated to each GH room and the experiment was completely surrounded by a border row of plants. The experiment followed a randomized complete block design to account for the E-W temperature gradient inside GH rooms. Each of the four block was treated as a replicate for the temperature treatment selected for each GH room. Three pre-germinated seeds (radicle = 5 mm) were initially sown on February 21 and was thinned to a single plant at VE, resulting in 32 plants of the same MG per block. Plants were watered twice daily by a drip irrigation system using amounts of water that were periodically adjusted to meet seasonal variations in evaporative demand. Plants were fertilized with a total dressing of

1.5 g N, P and K per pot, applied at V1 stage. Plants received prophylactic applications of fungicides and insecticides periodically.

Each room was exposed to a different day/night temperature regime throughout the growing season: high (HT; 28/20ºC), moderate (MT; 23/15ºC), and low temperature (LT; 18/10ºC). The daily oscillation in each GH was set following the normal change in daytime/nighttime temperature in soybean fields in the US Corn Belt region. The temperature range falls within T_b (5ºC) and T_{opt} (32ºC) for soybean development (Setiyono *et al.* 2007). A fourth treatment included an increasing temperature (IT) regime, in which temperature was raised by 2ºC every week, from 18/10ºC (VE- V3 stage) up to 28/20ºC (*ca.* R5 stage). This treatment attempted to mimic the typical temperature increase during the soybean growing season in the US Corn Belt region. Photoperiod was artificially extended after sunset in all treatments using incandescent lamps to achieve a constant value of 15 h from sowing to maturity. The 15-h photoperiod was equivalent to the daylength to which soybean crops are exposed around the summer solstice across most of the US Corn Belt region. Light level measured at plant height increased as the season progressed due to the natural increase in incident radiation during the spring.

Experiment 2. The field experiment followed a split-plot randomized block design, with four replicates (*i.e.,* blocks). Main plot were six sowing dates spaced at *ca.* 10-day interval, starting on April 23 and ending on June 19, a period during which Corn Belt producers typically sow soybean (Tables 2 & 3). The subplots were the two MGs (2.1 and 3.0). The six-row subplot row length was 4.6 m, with an interrow spacing of 0.76 m,

resulting on a subplot area of *ca*. 21 m^2. Seeds were sown at 2.5 cm spacing (39 plants m$^-$ ^{2}) and 6 cm sowing depth and thinned to 30 plants m^{-2} at VE. The last row for both sides of each subplot served as border rows. The measured yields ranged from 3.5 to 4.6 Mg ha^{-1}. A pre-emergent herbicide (Dual II Magnum) was applied before sowing and weeds were manually controlled during the rest of the season. Irrigation was provided using a drip irrigation system and was scheduled as needed to replenish soil moisture with adjustments made for local rainfall and crop evapotranspiration.

Experiment 3. This GH experiment was identical to Expt. 1 except for (i) sowing date was September 16 which caused plants to be exposed to progressively lower light levels as the season progressed; (ii) non pre-germinated seeds were sown due to the lack of difference in dates of emergence between pre-germinated and non-pre-germinated seeds observed in Expt. 1; and (iii) two treatments (IT and LT) were terminated around 85 days after sowing (before R1 occurred) due to severe powdery mildew infestation caused by fungus *Microspaera diffusa*.

Experiment 4. Experiments were conducted in four producer pivot-irrigated fields in Nebraska. These four fields were selected based on their consistent high soybean yields in previous years (>5 Mg ha^{-1}). Selected fields portrayed well the range of weather, soil, and management practices across soybean fields (Table 2). There was one sampling site at each of the four fields. Each sampling site had four blocks. Each of the 22-row block, with 0.76 m inter-row spacing, was 16.8 m wide and 19.1 m in length. The viable seeding rate was 27 to 34 plants m^{-2}. Fields were sown and managed by producers. Sowing date

and varieties varied across the four fields as a result of differences in early-season weather and recommended varieties for each region. Crops were watered frequently to maintain available soil water content above 65% of soil plant available water holding capacity in the 0-90 cm soil depth throughout the season. Application of 19 to 30 kg ha^{-1} P was done along with 4 to 67 kg ha^{-1} N (*i.e.,* either as a fertilizer or a credit from irrigation). Producers applied pre- and post-emergence herbicide to control weeds and prophylactic in-season foliar fungicide and insecticide at R3. Measured end-of-season soybean yields ranged from 5.1 to 5.9 Mg ha^{-1} across fields, with the latter reported at standard grain moisture content of 130 g H$_2$O kg^{-1} grain.

2.3. Determination of appeared and initiated nodes

Node appearance and phenology was tracked following Fehr *et al.* (1971) vegetative (V) staging system based on node accrual in the main stem. In this system, V-number (Vn) is given to a node when the leaflets at the next node above it have unfolded or unrolled. Sinclair (1984b) noted that unrolled leaflets have a length of 21 mm, which was corroborated in the present study (data not shown). In order to account for the first (cotyledonary) node and the last node with unrolled leaflets, which were missed by Fehr *et al.* (1971) V-staging system, the number of appeared nodes was computed as follows:

Number of appeared nodes: Vn + 2   (1)

where Vn is the vegetative stage calculated following Fehr *et al.* (1971) V-staging system. Plants were staged every 2 d (Expt. 1, 2 & 3) and 7 d (Expt. 4) from sowing until cessation of node appearance. In Expt. 1 and 3, eight plants located in the middle of each block were staged for each MG. In Expt. 2 and Expt. 4, ten contiguous plants located in

one of the center rows in each block were used for staging. In Expt. 3, no data on node

appearance were collected for two treatments (IT and LT) starting at R1 because of

severe powdery mildew infestation.

Number of initiated nodes was determined through destructive main SA sampling. SA

samples were collected and placed in 8 to 12 mL glass vials, and preserved in a standard

solution of formaldehyde (100 mL L^{-1}), ethyl alcohol (500 mL L^{-1}), glacial acetic acid (50

mL L^{-1}), and distilled water (350 mL L^{-1}). Each SA sample was dissected under a

binocular microscope (Nikon SMZ-10) at 10-60X magnification. Initiated nodes were

counted starting from the cotyledonary node up to the last initiated node on the flank of

the SA. Following Sun (1957), a new node was initiated when the leaf primordia has

reached a height of *ca.* 80 µm. Hence, final number of initiated node was calculated as

follows:

Number of initiated nodes: a + 1   (2)

where a is the number of nodes that have a height $\geq$ 80 µm. SA samples were collected

every 4 d (Expt. 1, 2 & 3) and 7 d (Expt. 4), from sowing until cessation of node

initiation in the main terminal SA. Samples were collected from plants located in the

center rows of the block.

2.4. Data analysis

2.4.1. Dynamics of node appearance and initiation. Temporal dynamics of node

appearance followed a generic tri-phasic pattern, with a different set of parameters (*i.e.*,

slopes and breakpoints) for each treatment. The following tri-segment linear regression

model provided the best fit to portray the seasonally observed pattern of node main stem accrual:

$$Y = Y0 + B1*X \qquad\qquad \text{IF } X<X1$$
$$Y = Y0 + B1*X1 + B2*(X-X1) \qquad \text{IF } X1{\leq}X{\leq}X2 \qquad\qquad (3)$$
$$Y = A_F \qquad\qquad\qquad\qquad \text{IF } X>X2$$

where Y is the number of appeared nodes, Y0 is the y-intercept, X is the time after sowing (d), B1 and B2 are the slopes corresponding to the two successive linear phases (node d^{-1}), X1 and X2 are the breakpoints separating the initial and subsequent linear phases (d), and A_F is the season-end final number of appeared nodes. The B1 and B2 coefficients represent the node appearance during the first (NAR1) and second phase (NAR2), respectively. The model was fitted to the observed data from VE until the cessation of node appearance. Model-fitting was implemented with GraphPad Prism (GraphPad Software® v. 6.07). Fitting the tri-segment linear model was not possible in Expt. 3, due to the unstable GH temperature setting during earlier soybean stages and early termination of the experiment. The difference in the number of appeared nodes between VE and V2 was divided by the number of days between the same crop stages to compute NAR1, whereas NAR2 was computed as the difference in the number of appeared nodes between V2 and R5 (or earlier if R5 was not achieved) divided by the number of days between the two crop stages. For accrual of just the trifoliolate-bearing main stem nodes, the calendar dates of stages V2 and R5 constitutes the timeframe spanning the first and last of those leaves.

Node initiation followed a one-segment linear pattern during the growing season until cessation of node initiation around R3. Thus, only a simple linear function was fitted:

$$Y = Y0 + B1*X \tag{4}$$

where Y is the number of initiated nodes, Y0 represents the number of already initiated

nodes in dormant seed (before germination commences), which was fixed at a constant

value of 3, X is time after sowing (d), and B1 is the regression coefficient corresponding

to the linear phase (node d^{-1}), which is functionally treated as an estimate of NIR. A two-

segment linear regression model (*i.e.,* two phases with different slopes) provided a better

fit for the IT treatments in Expt. 1 and 3. NIR for increasing temperature (IT) treatment

was calculated as the average of the two slopes weighted by the time period

corresponding to each slope. The final number of initiated nodes (I_F) was calculated

based on the maximum number of initiated nodes until initiation ceased around R3.

2.4.2 Phyllochron and plastochron estimation. NAR1 and NAR2 were derived from

the estimated B1 and B2 coefficients in equation 3. Likewise, NIR was derived from

estimated B1 coefficient in equation 4. For each treatment, mean temperature was

calculated for the time period between VE and X1(NAR1), X1 and X2 (NAR2), and

between sowing to end of node initiation around R3 (NIR). In Expt. 3, mean temperature

was computed from VE to V2 and V2 to R5 (or earlier if experiment was terminated

before R5) for NAR1 and NAR2, respectively. A separate analysis and combined

analysis of the performed field or GH data indicated no statistically significant difference

in the estimated slope and T_b for the relationships between NAR and NIR *versus*

temperature ($P \geq 0.30$). Likewise, there was no statistically significant difference between

the MG 2.1 and MG 3.0 cultivars for any of the previous parameters ($P \geq 0.44$).

Therefore, data across treatments and experiments were pooled and a generic linear

regression model was fitted to portray the response of NAR and NIR to mean temperature. NIR was much lower in HT treatments (Expt. 1 and 3) relative to field treatments that explored the same temperature range (Expt. 2, SD4-SD6) due to an interaction of very low radiation and high temperature (Table 4). Given the unlikely scenario of having very low radiation coupled with high temperature during the entire soybean crop season, NIR data from HT in Expt. 1 and 3 were excluded from the NIR regression analysis. Similarly, NAR data for MG 2.1 from HT and MT treatments in Expt. 3 were excluded from the NAR2 regression analysis because of the short time period between X1 and X2, which made unfeasible a reliable estimation of NAR2.

Phyllochron and plastochron (ºCd) were estimated as the inverse of the slope of the linear model fitted to the relationship between NAR and NIR, respectively, and mean temperature. T_b for node appearance and node initiation was estimated by extrapolating the fitted regression model and estimating the respective x-intercept values.

2.4.3 NAR and NIR coordination model development. A two-segment linear relationship between initiated and appeared nodes was evident after visual inspection of the data. Statistical analysis indicated that the two-segment model (F-test, $P <0.001$) was better than a simple linear model for modelling the observed trend. Hence, a two-segment linear model was fitted to the relationship between number of initiated nodes and appeared nodes:

$$Y = Y0 + B1*X \qquad\qquad IF\ X \leq X1 \qquad\qquad\qquad (5)$$
$$Y = Y0 + B1*X1 + B2*(X-X1) \qquad IF\ X>X1$$

where Y is the number of initiated nodes, Y0 represents the number of initiated nodes in the dormant seed, which was fixed to a constant value of 3, X is the number of appeared nodes, B1 and B2 are the two regression coefficients corresponding to the two successive linear phases (initiated node per appeared node), and X1 is the breakpoint (number of appeared nodes) separating the two linear phases. Data on node appearance after reaching the final number of initiated nodes were not used when the two-segment model was fitted. The two-segment model was fitted separately to the data from each experiment. Likewise, regression analyses was performed separately using data from GH and field experiments. Since parameters of the model did not differ among experiments ($P \geq 0.15$) or experimental conditions ($P \geq 0.45$), data were pooled across experiments and a single generic two-segment linear model was fitted to the pooled data. Finally, a small dataset on initiated and appeared node number reported as average of six varieties (*i.e.*, determinate and indeterminate) used by Johnson (1960) was added to the plot for comparison against the data collected in the present study.

Treatment effects on measured and calculated variables were tested by analysis of variance (ANOVA) in each experiment separately using InfoStat Professional v1.1 (Table S1-S3). Tukey's tests were used to determine significant differences ($\alpha = 0.05$) between treatment means.

Table 1. Description of vegetative (V) and reproductive (R) soybean stages following Fehr *et al.* (1971) system.

Stage	Description
VE	Emergence. Cotyledons 'pulling free' above the soil surface. Total number of nodes: 1 [†]
VC (V0)	Cotyledon. Unifoliolate leaves unrolled sufficiently so the leaf edges are not touching. Total number of nodes: 2 [†]
V1	First node. Fully developed leaves at unifoliolate node. Total number of nodes: 3 [†]
V2	Second node. Fully developed trifoliolate leaf at node above the unifoliolate node. Total number of nodes: 4 [†]
V(n)	nth node on the main stem with a fully developed leaves beginning with the unifoliolate node. Total number of nodes: (n + 2) [†]
R1	Blooming. One open flower at any node on the main stem.
R2	Full bloom. Open flower at one of the two uppermost nodes on the main stem with a fully developed leaf
R3	Beginning pod. Pod 5 mm long at one of the four uppermost nodes on the main stem with a fully developed leaf
R4	Full pod. Pod 2 cm long at one of the four uppermost nodes on the main stem with a fully developed leaf
R5	Beginning seed. Seed 3 mm long in a pod at one of the four uppermost nodes on the main stem with a fully developed leaf
R6	Full seed. Pod containing a seed that fills the pod cavity at one of the four uppermost nodes on the main stem with a fully developed leaf
R7	Physiological maturity. One normal pod on the main stem that has reached its mature pod color
R8	Full maturity. 95% of the pods have reached their mature color.

[†] Total number of nodes estimated using Equation 1, considering the cotyledonary as the first node.

Table 2. Main features of the four experiments conducted during 2015 to assess the influence of temperature on node appearance and initiation in soybean.

Experiment	Location (latitude and longitude)	Condition	Growing season	Soil type	Variety name (and MG)	Treatment
1	Lincoln, NE (40.80 N, 96.68 W)	Greenhouse	Spring (Feb -May)	Sandy loam	IA 2102 (MG 2.1) IA 3024 (MG 3.0)	HT MT IT LT
2	Lincoln, NE (40.80 N, 96.68 W)	Field experiment	Summer (April-Oct)	Silt loam	IA 2102 (MG 2.1) IA 3024 (MG 3.0)	SD1 SD2 SD3 SD4 SD5 SD6
3	Lincoln, NE (40.80 N, 96.68 W)	Greenhouse	Fall (Sept-Dec)	Sandy loam	IA 2102 (MG 2.1) IA 3024 (MG 3.0)	HT MT IT LT
4	Atkinson, NE (42.47 N, 98.75 W)	Field experiment	Summer (April -Oct)	Sandy and sandy loam	A2733 (MG 2.7)	F1
	Saronville, NE (40.57 N, 98.13 W)			Silty clay loam	A2431 (MG 2.4)	F2
	Smithfield, NE (40.58 N, 99.67 W)			Silt loam and silty clay loam	P24T19 (MG 2.4)	F3
	Mead, NE (41.15 N, 96.48 W)			Silty clay loam	P31T11 (MG 3.1)	F4

HT: high temperature; MT: moderate temperature; IT: increasing temperature; LT: low temperature; SD: sowing date; F: farmer; MG maturity group.

Table 3. Sowing date and days from sowing to emergence (VE), flowering (R1), beginning of pod setting (R3) and beginning of seed filling (R5) across the four experiments.

Experiment	Treatment[†]	Maturity group	Sowing date	Days after sowing to VE	R1	R3	R5
1	HT	2.1	Feb 21	6c	33g	41f	52g
		3.0		6c	37f	57e	70e
	MT	2.1		9b	49c	59e	67f
		3.0		9b	53e	67d	82d
	IT	2.1		17a	57d	67d	81d
		3.0		18a	67b	79b	95b
	LT	2.1		17a	61c	73c	89c
		3.0		18a	73a	85a	101a
2	SD1	2.1	April 23	12b	53a	66a	81a,b
		3.0		12b	55a	66a	84a
	SD2	2.1	April 30	15a	52a	65a	78b,c
		3.0		15a	54a	65a	84a
	SD3	2.1	May 13	12b	47b	56b	72d,e
		3.0		12b	45b	56b	79c,d
	SD4	2.1	May 26	7d	40c	47e	63f
		3.0		7d	40c	51c,d	71e
	SD5	2.1	June 8	8c	39c	50d,e	64f
		3.0		8c	39c	55c	66f
	SD6	2.1	June 19	5e	36d	48e	59g
		3.0		5e	39c	50d,e	61g

Experiment	Treatment[†]	Maturity group	Sowing date	Days after sowing to			
				VE	R1	R3	R5
3	HT	2.1	Sept 16	5d	33d	40d	51d
		3.0		5d	37c	55c	71c
	MT	2.1		9b	58b	64b	76b
		3.0		9c	66a	72a	83a
	IT	2.1		11b	nr	nr	nr
		3.0		11b	nr	nr	nr
	LT	2.1		13a	nr	nr	nr
		3.0		13a	nr	nr	nr
4	F1	2.7	April 25	13d	65a	79a	100a
	F2	2.4	May 2	19a	57b	67c	89c
	F3	2.4	May 13	16b	52c	71b	99b
	F4	3.1	May 18	14c	45d	63d	84d

nr: no record because experiments were terminated due to severe infestation of powdery mildew around 85 days after sowing.
Different letters indicate significant (P <0.05) differences between treatments within each experiment for the variables identified at the top of each column.
[†] See Table 2 for treatment codes and description.

3. RESULTS

3.1 Environmental conditions

Experiments conducted in this study were subjected to a wide range of weather, soil, and management practices, which influenced the duration of the time intervals amongst phenological stages (Fig. 1, Tables 2 & 3). For example, the time period between sowing to R5 ranged from 52 d (Expt. 3, MG 2.1, HT) to 101 d (Expt. 1, MG 3.0, LT). The soybean plants or crops were also exposed to variable of photo-thermal conditions during the period of node appearance (from VE to *ca.* R5); mean temperature and incident radiation ranged from 15 to 26ºC and 5.9 to 22.1 MJ m^{-2} d^{-1}, respectively (Fig. 1, Table 4). The mean temperature was purposely varied in the GH experiments (from 15 to 26ºC), and also varied by our choice of locations for the field experiments (20 to 26ºC). In contrast, range in mean photoperiod (considering a 0º solar angle) was very narrow (14.4 to 15 h) across treatments and experiments (Fig. 1, Table 4). Similar ranges in temperature, incident radiation, and photoperiod were explored during the period of node initiation, from sowing to *ca.* R3 (Table 4).
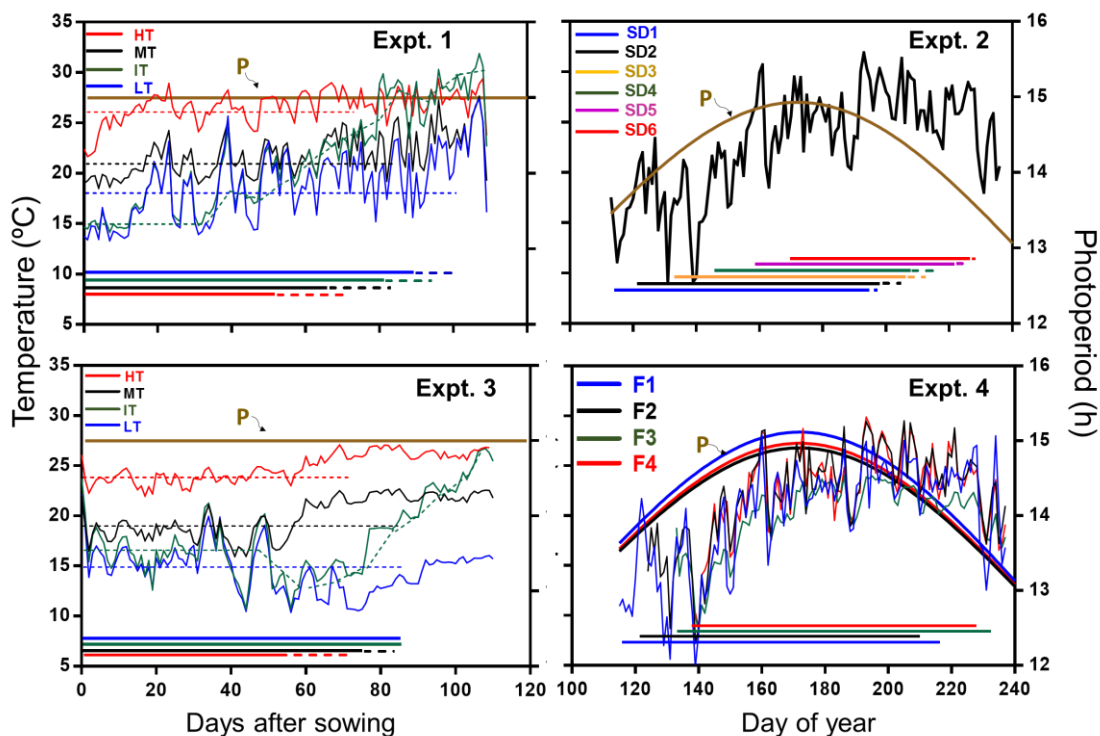
Figure 1. Seasonal patterns in average daily air temperature (ºC) and photoperiod (P; h) in greenhouse (Expt. 1 and 3) and field conditions (Expt. 2 and 4). Dotted lines indicate average temperature for each treatment in Expt. 1 and 3 (note that a moving average was calculated for IT). Horizontal lines at the bottom of each panel indicate duration from sowing to R5 for each treatment, with dashed lines indicating the longer duration of MG 3.0 in Expt. 1, 2, and 3. See Table 2 for treatment codes and description.

Table 4. Mean photoperiod, temperature, and incident solar radiation across treatments in the four experiments. Mean values were calculated separately for the period of node initiation (from sowing [S] to beginning of pod setting [R3]) and appearance (from S to beginning of seed filling [R5]). For photoperiod and solar radiation, means were calculated for the period beginning at emergence (VE). Soil temperature at seed depth was used for the period between S and VE while air temperature at stem apex height was used for the rest of the growing season.

Experiment	Treatment [‡]	Maturity group	Photoperiod (h)		Temperature (ºC)		Solar radiation (MJ m^{-2}d^{-1})	
			VE to R3	VE to R5	S to R3	S to R5	VE to R3	VE to R5
1	HT	2.1	15.0[§]	15.0[§]	25.9 (±0.3)	26.0 (±0.2)	9.0 (±0.4)	8.8 (±0.4)
		3.0	15.0[§]	15.0[§]	26.0 (±0.2)	26.3 (±0.2)	8.5 (±0.4)	9.0 (±0.4)
	MT	2.1	15.0[§]	15.0[§]	20.7 (±0.2)	20.9 (±0.2)	8.7 (±0.4)	9.0 (±0.4)
		3.0	15.0[§]	15.0[§]	20.9 (±0.2)	21.2 (±0.2)	9.0 (±0.4)	8.8 (±0.4)
	IT	2.1	15.0[§]	15.0[§]	18.3 (±0.4)	19.3 (±0.4)	9.1 (±0.5)	8.9 (±0.4)
		3.0	15.0[§]	15.0[§]	19.1 (±0.4)	20.5 (±0.4)	8.7 (±0.4)	8.8 (±0.4)
	LT	2.1	15.0[§]	15.0[§]	17.8 (±0.4)	18.1 (±0.3)	9.0 (±0.5)	8.9 (±0.4)
		3.0	15.0[§]	15.0[§]	18.1 (±0.3)	18.4 (±0.3)	8.8 (±0.4)	9.0 (±0.4)
2	SD1	2.1	14.6 (±0.04)	14.7 (±0.03)	20.4 (±0.6)	21.5 (±0.6)	15.9 (±1.0)	16.4 (±0.8)
		3.0	14.6 (±0.04)	14.7 (±0.03)	20.4 (±0.6)	21.6 (±0.6)	15.9 (±1.0)	16.4 (±0.8)
	SD2	2.1	14.8 (±0.03)	14.8 (±0.02)	21.3 (±0.6)	22.1 (±0.6)	17.0 (±1.0)	17.4 (±0.9)
		3.0	14.8 (±0.03)	14.7 (±0.02)	21.3 (±0.6)	22.5 (±0.6)	17.0 (±1.0)	17.4 (±0.8)
	SD3	2.1	14.8 (±0.01)	14.8 (±0.02)	22.3 (±0.6)	23.6 (±0.6)	17.6 (±1.0)	18.3 (±0.8)
		3.0	14.8 (±0.01)	14.7 (±0.02)	22.3 (±0.6)	23.9 (±0.5)	17.6 (±1.0)	18.2 (±0.7)
	SD4	2.1	14.9 (±0.01)	14.8 (±0.02)	24.3 (±0.5)	25.3 (±0.5)	17.7 (±1.0)	18.1 (±0.8)
		3.0	14.8 (±0.01)	14.7 (±0.03)	24.6 (±0.5)	25.3 (±0.4)	17.8 (±1.0)	18.0 (±0.7)
	SD5	2.1	14.8 (±0.03)	14.6 (±0.03)	26.1 (±0.4)	26.0 (±0.3)	19.0 (±0.8)	18.5 (±0.7)
		3.0	14.7 (±0.04)	14.6 (±0.05)	26.1 (±0.4)	26.1 (±0.3)	19.0 (±0.7)	18.6 (±0.7)
	SD6	2.1	14.6 (±0.04)	14.4 (±0.05)	26.1 (±0.4)	26.3 (±0.3)	18. (±0.8)1	18.1 (±0.7)
		3.0	14.5 (±0.03)	14.4 (±0.06)	26.2 (±0.4)	26.0 (±0.4)	18.1 (±0.8)	17.9 (±0.7)

Experiment	Treatment[‡]	Maturity group	Photoperiod (h)		Temperature (ºC)		Solar radiation (MJ m^{-2}d^{-1})	
			VE to R3	VE to R5	S to R3	S to R5	VE to R3	VE to R5
3	HT	2.1	15.0[§]	15.0[§]	23.7 (±0.1)	23.8 (±0.1)	7.3 (±0.3)	7.0 (±0.3)
		3.0	15.0[§]	15.0[§]	23.8 (±0.1)	24.2 (±0.1)	6.9 (±0.3)	6.3 (±0.3)
	MT	2.1	15.0[§]	15.0[§]	18.7 (±0.2)	19.1 (±0.2)	6.6 (±0.3)	6.0 (±0.3)
		3.0	15.0[§]	15.0[§]	19.0 (±0.2)	19.4 (±0.2)	6.3 (±0.3)	5.9 (±0.3)
	IT	2.1	15.0[§]	15.0[§]	16.2 (±0.3)[†]	16.2 (±0.3)[†]	5.9 (±0.3)[†]	5.9 (±0.3)[†]
		3.0	15.0[§]	15.0[§]	16.2 (±0.3)[†]	16.2 (±0.3)[†]	5.9 (±0.3)[†]	5.9 (±0.3)[†]
	LT	2.1	15.0[§]	15.0[§]	14.6 (±0.3)[†]	14.6 (±0.3)[†]	5.8 (±0.3)[†]	5.8 (±0.3)[†]
		3.0	15.0[§]	15.0[§]	14.6 (±0.3)[†]	14.6 (±0.3)[†]	5.8 (±0.3)[†]	5.8 (±0.3)[†]
4	F1	2.7	14.9 (±0.03)	14.8 (±0.06)	18.5 (±0.6)	19.7 (±0.5)	20.1 (±0.9)	21.0 (±0.7)
	F2	2.4	14.8 (±0.02)	14.7 (±0.03)	20.2 (±0.6)	21.7 (±0.5)	20.0 (±1.0)	20.5 (±0.7)
	F3	2.4	14.8 (±0.02)	14.5 (±0.05)	19.9 (±0.4)	20.2 (±0.3)	22.6 (±0.7)	22.1 (±0.6)
	F4	3.1	14.9 (±0.02)	14.6 (±0.04)	22.1 (±0.6)	22.9 (±0.5)	19.2 (±0.9)	18.6 (±0.7)

[‡] See Table 2 for treatment codes and description.

[†] Mean values calculated from sowing until 85 days after sowing due to severe infestation of powdery mildew afterwards

[§] No standard error values since photoperiod was set constant in greenhouse experiments.

3.2. Dynamics of node appearance and node initiation

A tri-segment linear model provided the best fit ($R^2 \geq 0.99$; $P < 0.001$) for the observed

patterns of node appearance (Fig. 2, Table 5). NAR1 encompassed the time period

between VE and the first breakpoint (X1), and for the various treatments ranged from 13

to 49 d after sowing (DAS) (Table 5). The duration of the sowing-VE period (range: 5-19

d) was observed to be strongly associated with soil temperature ($y = 32.2 - 1.12x$; $r^2 =$

0.81; $P < 0.001$); however, there was no statistically significant relationship between

temperature and the duration of the VE-X1 period (range: 6-46 d) ($r^2 = 0.01$; $P = 0.66$).

Actually, it was not possible to associate the breakpoint X1 with any specific vegetative

stage (Fig. 2) between VC to V4 (*i.e.,* 2 to 6 appeared nodes). For the same temperature,

or same sowing date treatment in Expt. 1, 2, and 3, the MGs 2.1 and 3.0 cultivars

exhibited almost identical NAR1 and X1 values (Table 5). Thus, while sowing-VE

duration and NAR1 were controlled by temperature, the duration of VE-X1 was

apparently independent of temperature, even when the X1 breakpoint was as early as VC

or as late as V4 (Fig. 2).

The NAR2 period commenced at breakpoint X1 and continued until node appearance

ceased (Fig. 2). NAR2 encompassed exclusively the appearance of trifoliolate nodes. The

date at which node appearance ended (X2) ranged from 45 to 101 DAS, depending on the

treatment, but consistently ended on the date of R5 (Fig. 2, Tables 3 & 5). This was a

gradual decline in node appearance as crop approached R5 (Fig. 2), which was consistent

with the results of Setiyono *et al.* (2007), whereas Sinclair (1984a) reported that node

appearance abruptly stopped at R5. The final number of appeared nodes (Y_F) at R5

ranged from six (Expt. 3, HT & MT, MG 2.1) to 19 (Expt. 2, SD3, MG 3.0 & Expt. 4, F4) across treatments (Table 5). For a given temperature, or given sowing date treatment in Expt. 1, 2, and 3, the MGs 2.1 and 3.0 cultivars exhibited almost identical NAR2 values (except for Expt. 1, HT), but the MG 3.0 cultivar had longer X1 to X2 duration because of its later R5 date, and thus it finished the season with a higher number of final nodes (Fig. 2, Table 5).

In contrast to node appearance, there were no observable breakpoints in the seasonal node initiation trends, and thus, a simple linear regression model ($r^2 > 0.95$; $P < 0.001$) provided the best fit (Fig 3, Table 6). There were two exceptions and these were for the MG 3.0 cultivar in IT, where a two-segment linear model was a better fit than a one-segment model in Expt. 1 (F-test, $P < 0.001$) and in Expt. 3 (F-test, $P = 0.006$). Three common features were notable in the observed node initiation patterns across all experiments. First, three initiated nodes (*i.e.,* cotyledonary, unifoliolate, and first trifoliolate) were already present in dormant seeds (Figs 3 & 4). Second, node initiation invariably ceased around R3 (Figs 3 & 6b). Third, the final number of initiated nodes always exceeded the final number of appeared nodes in each treatment (Figs 2 & 3, Tables 5 & 6), with the difference ranging from 2 (Expt. 4, F3) to 11 (Expt. 1, MG 3.0, IT & MG 2.1, LT). In other words, not all initiated main stem nodes show up as observable appeared nodes before the crop matures.
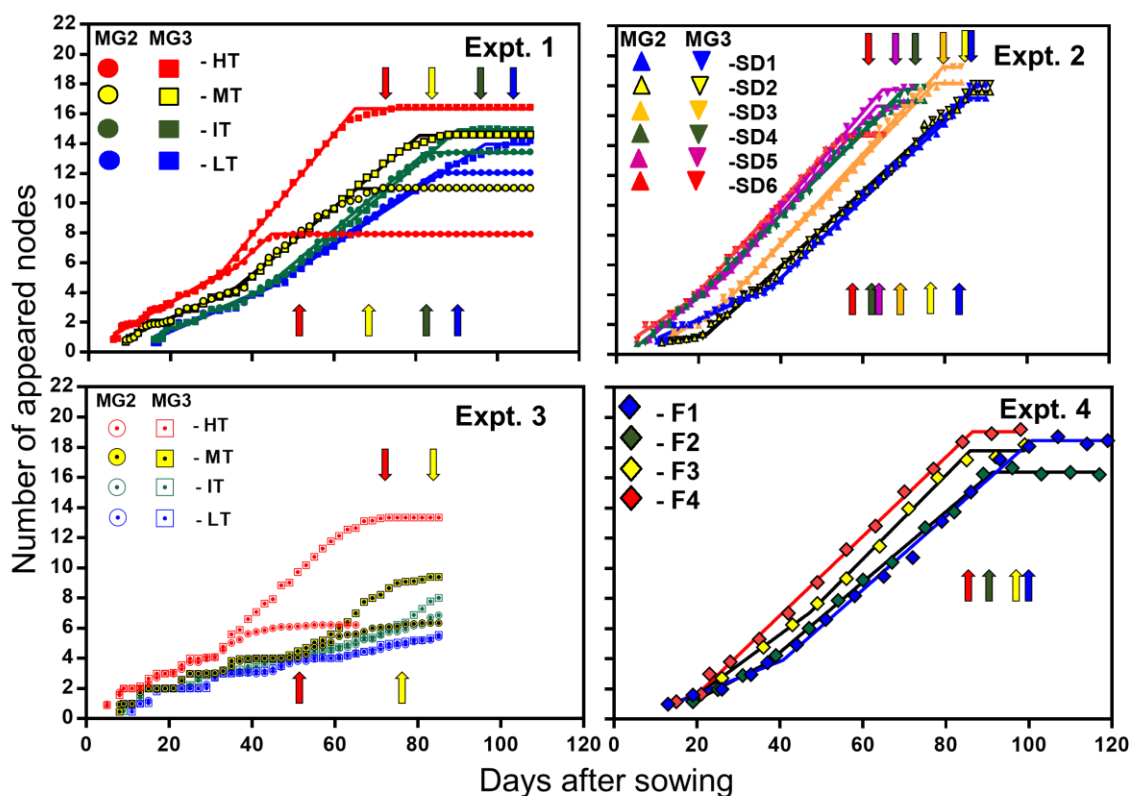
Figure 2. Seasonal patterns in node appearance for all treatments across the four experiments. Symbols indicate the maturity group in Expt. 1, 2, and 3. Arrows indicate date of beginning of seed filling (R5) for MG 2.1 (↑) and MG 3.0 (↓) in Expt. 1, 2, and 3. Tri-segment linear regression models were fitted to all treatments from emergence until node appearance ceased, following equation 3, except for Expt. 3 (see Section 2.4.1). Appeared node = 0 was arbitrarily used to denote the sowing stage. Estimates of model parameters are shown in Table 5. See Table 2 for treatment codes and description.
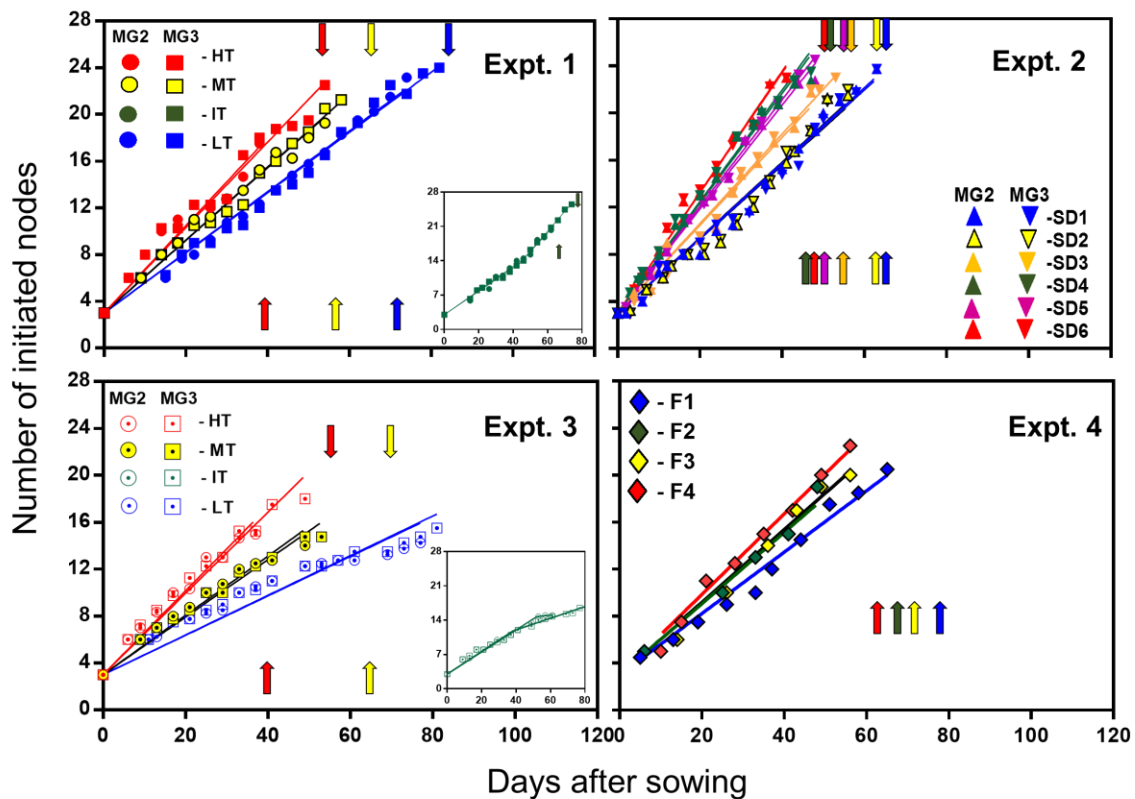
Figure 3. Seasonal patterns in node initiation for all treatments across the four experiments. Insets show node initiation patterns for IT treatments in Expt. 1 and 3. Symbols indicate maturity group in Expt. 1, 2, and 3. Arrows indicate date of beginning of pod setting (R3) for MG 2.1 (↑) and MG 3.0 (↓) in Expt. 1, 2, and 3. Linear regression lines were fitted following equation 4. Estimates of model parameters are shown in Table 6. See Table 2 for treatment codes and description.

Table 5. Estimates of the parameters of fitted tri-segment linear model (Equation 3) to the seasonal trends in node appearance in Expt. 1-4 (see Figure 2). The final number of appeared nodes (A_F) is also shown. Parenthetic values indicate the standard error. All fitted regressions were highly significant ($R^2 > 0.99$; $P < 0.001$).

Experiment	Treatment[‡]	Maturity group	B1 (node d^{-1})	X1 (d)	B2 (node d^{-1})	X2 (d)	Y_F (nodes)
	HT	2.1	0.15 (±0 .003)a	36 (±1.1)c,d	0.25 (±0.019)b	45 (±0.4)e	8 (±0.2)e
		3.0	0.16 (±0.005)a	32 (±0.7)d	0.34 (±0.005)a	65 (±0.3)d	16 (±0.6)a
	MT	2.1	0.13 (±0.006)b	36 (±1.6)c,d	0.22 (±0.006)b,c	66 (±0.5)d	11 (±0.3)b
1		3.0	0.12 (±0.004)b	36 (±0.9)b,c,d	0.23 (±0.003)b,c	81 (±0.3)c	14 (±0.4)a,b
	IT	2.1	0.12 (±0.005)b	43 (±1.1)a,b	0.23 (±0.003)b,c	83 (±0.4)c	13 (±0.6)b,c
		3.0	0.12 (±0.004)b	47 (±1.0)a	0.23 (±0.003)b,c	90 (±0.4)a,b	15 (±0.2)a,b
	LT	2.1	0.11 (±0.005)b	42 (±1.5)a,b,c	0.19 (±0.003)b,c	85 (±0.4)b,c	12 (±0.6)c,d
		3.0	0.12 (±0.006)b	44 (±2.3)a	0.18 (±0.003)c	97 (±0.7)a	14 (±0.2)b,c
	SD1	2.1	0.13 (±0.005)d	38 (±0.9)a	0.26 (±0.003)d	86 (±0.5)a	17 (±0.5)a,b,c
		3.0	0.12 (±0.006)d	38 (±0.9)a	0.27 (±0.003)c,d	87 (±0.6)a	18 (±0.4)a,b
	SD2	2.1	0.05 (±0.033)e	22 (±1.2)c	0.25 (±0.003)d	87 (±0.8)a	18 (±1.2)a,b
		3.0	0.04 (±0.003)e	21 (±1.0)c	0.25 (±0.002)d	88 (±0.8)a	18 (±0.4)a,b
	SD3	2.1	0.15 (±0.021)c,d	24 (±1.3)b,c	0.29 (±0.003)a,b,c,d	78 (±0.5)b	18 (±0.4)a,b
2		3.0	0.18 (±0.019)b,c,d	27 (±1.7)a,b,c	0.30 (±0.003)a,b,c,d	80 (±0.6)a,b	19 (±0.3)a
	SD4	2.1	0.22 (±0.016)a,b	20 (±3.6)c,d	0.27 (±0.003)c,d	68 (±0.5)c,d	17 (±0.2)a,b,c
		3.0	0.23 (±0.017)a	25 (±4.4)b,c	0.28 (±0.004)b,c,d	70 (±0.7)c	18 (±0.5)a,b
	SD5	2.1	0.22 (±0.011)a,b	27 (±1.7)a,b,c	0.31 (±0.004)b,a	63 (±0.4)d	17 (±0.2)b,c
		3.0	0.24 (±0.011)a	26 (±1.9)b,c	0.32 (±0.004)a	65 (±0.4)c,d	18 (±0.2)a,b
	SD6	2.1	0.18 (±0.017)a,b,c	18 (±1.4)d	0.30 (±0.004)a,b,c	55 (±0.4)e	15 (±0.2)c
		3.0	0.19 (±0.016)a,b,c,d	18 (±1.4)d	0.30 (±0.004)a,b,c,d	55 (±0.4)e	15 (±0.2)c
	HT	2.1	0.10 a[†]	29 e[†]	§	46 e[†]	6 (±0.2)c
3		3.0	0.12 a[†]	26 e[†]	0.22 a[†]	66 d[†]	13 (±0.4)a
	MT	2.1	0.08 b[†]	39 d[†]	§	65 c[†]	6 (±0.2)c
		3.0	0.07 b[†]	41 d[†]	0.15 b[†]	76 b[†]	9 (±0.3)b

Experiment	Treatment[‡]	Maturity group	B1 (node d^{-1})	X1 (d)	B2 (node d^{-1})	X2 (d)	Y_F (nodes)
	IT	2.1	0.06 c[†]	49 b,c[†]	0.08 c,d[†]	85 a[†]	nr
3		3.0	0.06 c[†]	47 c,d[†]	0.10 b,c[†]	85 a[†]	nr
	LT	2.1	0.05 c[†]	59 a[†]	0.05 e[†]	85 a[†]	nr
		3.0	0.05 c[†]	51 a,b[†]	0.05 e[†]	85 a[†]	nr
	F1	2.7	0.11 (±0.019)b	41 (±3.1)a	0.24 (±0.006)a	101 (±1.3)a	18 (±0.4)a
4	F2	2.4	0.14 (±0.031)a,b	37 (±4.9)a	0.23 (±0.006)a	91 (±0.95)b	16 (±0.4)b
	F3	2.4	0.20 (±0.019)a	47 (±5.8)a	0.28 (±0.011)a	86 (±1.3)c	18 (±0.4)a
	F4	3.1	0.13 (±0.119)b	21 (±5.4)a	0.26 (±0.005)a	86 (±1.1)c	19 (±0.2)a

nr: no record because experiments were terminated due to severe infestation of powdery mildew around 85 days after sowing.

Different letters indicate significant (P<0.05) differences between treatments within each experiment for the variables identified at the top of each column

[‡] See Table 2 for treatment codes and description.

[†] Values were manually computed (see Section 2.4.1).

[§] Values not possible to establish due to short time period between X1 and X2.

Table 6. Estimates of the parameters of the fitted linear regression models (Equation 4) describing the seasonal pattern of node initiation in Expt. 1-4 (see Figure 3). The final number of initiated nodes (I_F) is also shown. Parenthetic values indicate the standard error. All fitted regressions were highly significant ($r^2 > 0.95$; $P < 0.001$).

Experiment	Treatment ‡	Maturity group	B1 (node d^{-1})	I_F
1	HT	2.1	0.37 (±0.036)a	18 (±0.5)d
		3.0	0.37 (±0.200)a	23 (±0.6)a,b
	MT	2.1	0.30 (±0.011)a,b,c	19 (±0.5)c,d
		3.0	0.31 (±0.009)a,b	21 (±0.4)b,c
	IT	2.1	0.28 (±0.018)b,c,d†	20 (±0.6)b,c
		3.0	0.30 (±0.008)a,b,c†	26 (±0.8)a
	LT	2.1	0.26 (±0.005)d	23 (±0.4)a,b
		3.0	0.26 (±0.007)d	24 (±0.5)a
2	SD1	2.1	0.32 (±0.011)d	24 (±0.3)a,b
		3.0	0.32 (±0.012)d	24 (±0.3)a,b
	SD2	2.1	0.31 (±0.018)d	22 (±0.3)d
		3.0	0.32 (±0.016)d	22 (±0.0)d
	SD3	2.1	0.37 (±0.020)c	22 (±0.0)d
		3.0	0.38 (±0.014)c	23 (±0.0)c,d
	SD4	2.1	0.47 (±0.019)b	23 (±0.0)c,d
		3.0	0.47 (±0.017)b	24 (±0.3)a,b
	SD5	2.1	0.44 (±0.013)b	23 (±0.4)c,d
		3.0	0.45 (±0.007)b	25 (±0.3)a
	SD6	2.1	0.51 (±0.019)a	23 (±0.0)c,d
		3.0	0.51 (±0.020)a	23 (±0.4)c,d

Experiment	Treatment ‡	Maturity group	B1 (node d^{-1})	I_F
	HT	2.1	0.36 (±0.025)a	15 (±0.0)b,c
		3.0	0.35 (±0.022)a	18 (±0.5)a
	MT	2.1	0.25 (±0.017)b	14 (±0.0)c
3		3.0	0.25 (±0.013)b	15 (±0.5)b,c
	IT	2.1	0.20 (±0.020)c†	15 (±0.0)b,c
		3.0	0.17 (±0.011)d†	17 (±0.5)a
	LT	2.1	0.17 (±0.014)d†	14 (±0.0)c
		3.0	0.17 (±0.012)d†	16 (±0.5)b
	F1	2.7	0.28 (±0.029)d	21 (±0.3)b
4	F2	2.4	0.34 (±0.057)c	19 (±0.0)c
	F3	2.4	0.35 (±0.053)b	20 (±0.0)b
	F4	3.1	0.36 (±0.035)a	23 (±0.3)a

Different letters indicate significant (P <0.05) differences between treatments within each experiment for the variables identified at the top of each column

‡ See Table 2 for treatment codes and description.

† Values were manually computed as average of the two slopes from the bilinear regression model, weighted by the relative duration of each phase.
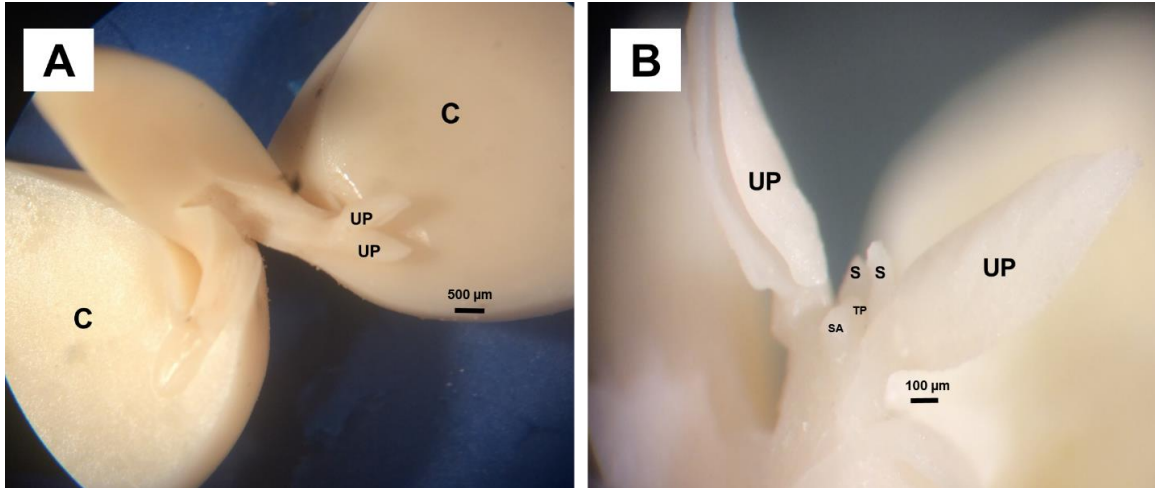
Figure 4. A germinated soybean seed. (A) The initiated cotyledon (*C*) and unifoliolate primordia (*UP*) under 15X magnification. (B) The stem apex (*SA*) and the initiated first trifoliolate primordia (*TP*) at higher magnification (60X). *S* = stipule.
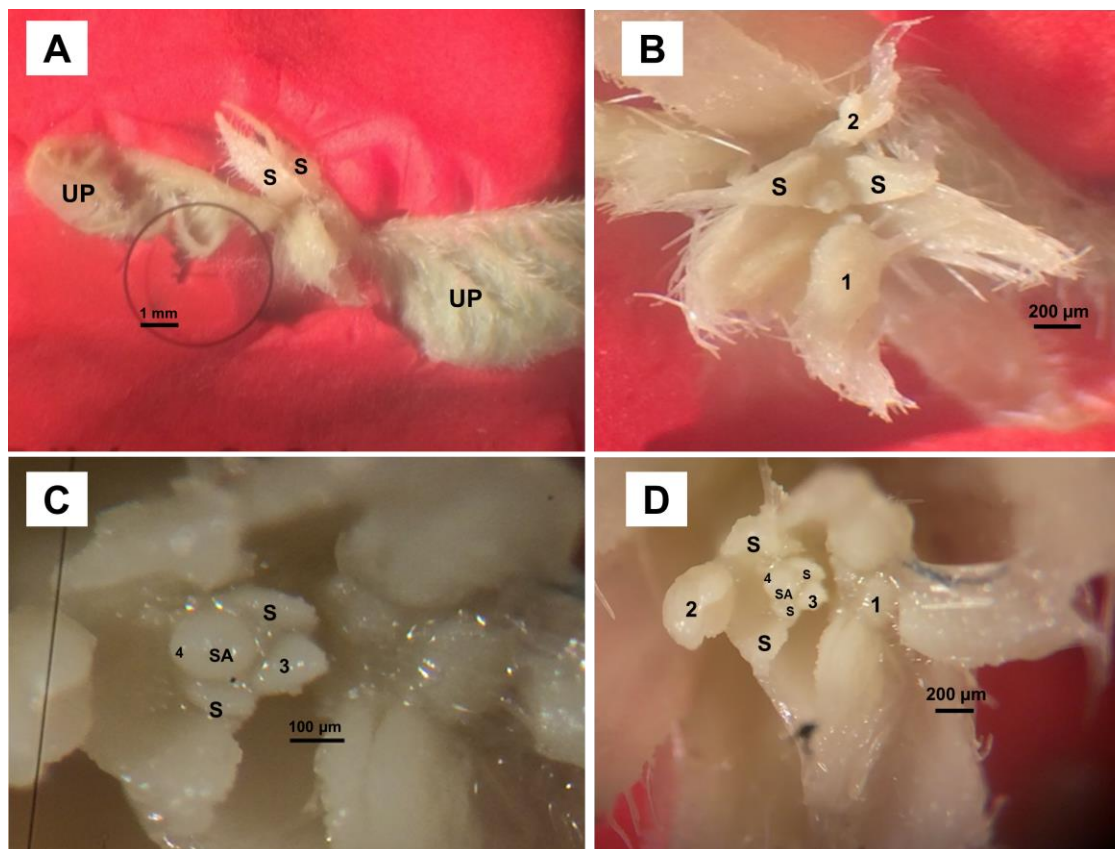
Figure 5. A soybean terminal bud at emergence (with cotyledons removed). (A) The unifoliolate primordia (*UP*) under 10X magnification. (B) The first (*1*) and second (*2*) initiated trifoliolate primordia under 45X magnification. (C) The stem apex (*SA*), with the third (*3*) and fourth (*4*) initiated trifoliolate primordium under 60X magnification. (D) The four trifoliolate primordium alternately initiated at the SA under 45X magnification. *S* = stipule.
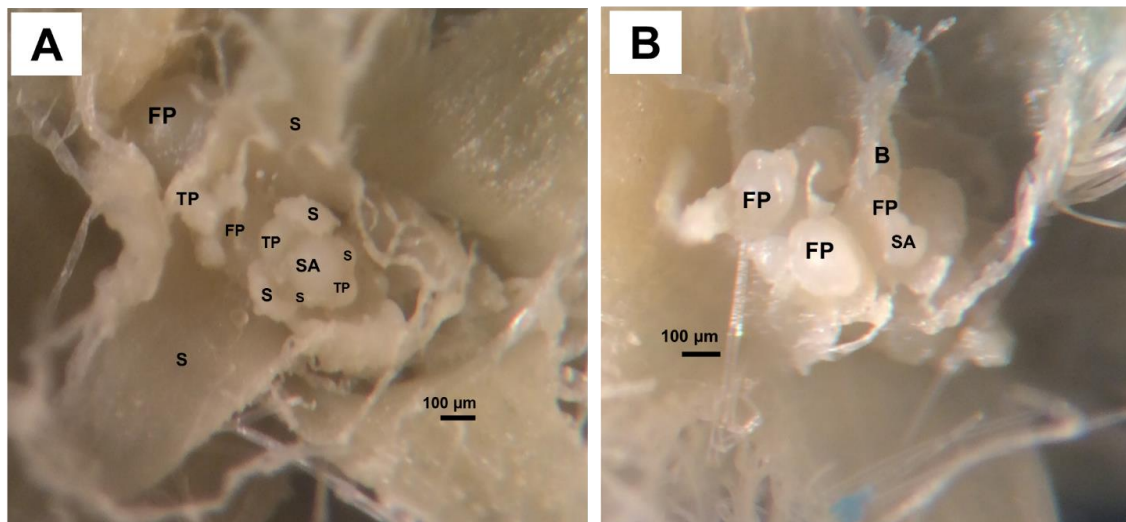
Figure 6. (A) Soybean terminal bud before beginning of pod setting (R3) showing the initiation of a trifoliolate primordia (*TP*) at the flank of stem apex (*SA*). Axillary buds have already initiated a floral primordia (*FP*). (B) Soybean terminal bud after R3 showing differentiation of FP and bracts (*B*) in the axillary bud adjacent to the SA, coincident with the end of node initiation. *S* = stipule.

3.3. Temperature influence on node appearance and initiation rates

Temperature had a strong influence on NAR and NIR (Table S1, S2 & S3), with faster rates of each occurring with increasing temperature in all experiments (Figs 2 & 3, Tables 5 & 6). Fitted linear regression models to the relationships between mean temperature and NAR2 and NIR, using the pooled data, had a very high predictive power ($r^2 \geq 0.92$; $P <0.001$), despite the wide range in weather, soil, and management across treatments and experiments (Fig. 7). Moreover, there was also a significant relationship between mean temperature and NAR1 (*i.e.,* the VE to X1 rate) ($r^2 = 0.64$; $P <0.001$) (Fig. 7, inset). Based on this relationship between NAR1 and air temperature, a phyllochron of 83ºCd and T_b of 9.5 ºC can be derived from the tri-segment model for node appearance in the VE and X1 timeframe. The fitted regression models indicated that NAR2 increased from 0.09 node d^{-1} (*i.e.*, 11 d node^{-1}) at 15ºC to 0.26 node d^{-1} (*i.e.*, 3.8 d node^{-1}) at 25ºC (Fig.

7). The latter value is almost identical to the 3.7 d node^{-1} value reported by Bastidas *et al.* (2008) for the 22-26ºC range of mean temperatures in the four sowing dates. Values of NIR increased from 0.18 node d^{-1} (5.6 d node^{-1}) to 0.46 nodes d^{-1} (*i.e.*, 2.2 d node^{-1}) along the same 15 to 25 ºC temperature range (Fig. 7). Finally, there was no evidence of a photoperiod effect on rates of node appearance and initiation. Patterns of node initiation and appearance in Expt, 2, plotted on a thermal time scale, indicated that NAR and NIR were almost identical across sowing dates, despite differences in photoperiod among treatments and within the season (Fig. S1).

The relationship between NAR2 and NIR with temperature indicated no statistically significant ($P \geq 0.15$) difference in their two respective T_b values (9.6ºC *versus* 8.5ºC) (Fig. 7). These values were also almost identical to the estimated T_b for NAR1 (9.5ºC). Note however that the slope for NIR *versus* temperature was *ca.* 65% higher than the slope for NAR2 (0.028 *versus* 0.017 node d^{-1} ºC^{-1}), indicating that node initiation occurred at much faster rate than node appearance even though both were operating at the same temperature (Fig. 7). Plastochron and phyllochron, obtained from the inverse of the NIR and NAR2 slopes, were 36 and 58ºCd, respectively. The estimated phyllochron for NAR2 (58ºCd) represented *ca.* 70% of the phyllochron estimated for NAR1 (83ºCd). In other words, when expressed on a thermal time basis, NAR2 is *ca.* 30% faster than NAR1 (a rate that applies before the X1 breakpoint). And, as a result of this change in phyllochron with ontogeny, the phyllochron-to-plastochron ratio changed from 2.3 to 1.6 before and after the breakpoint.
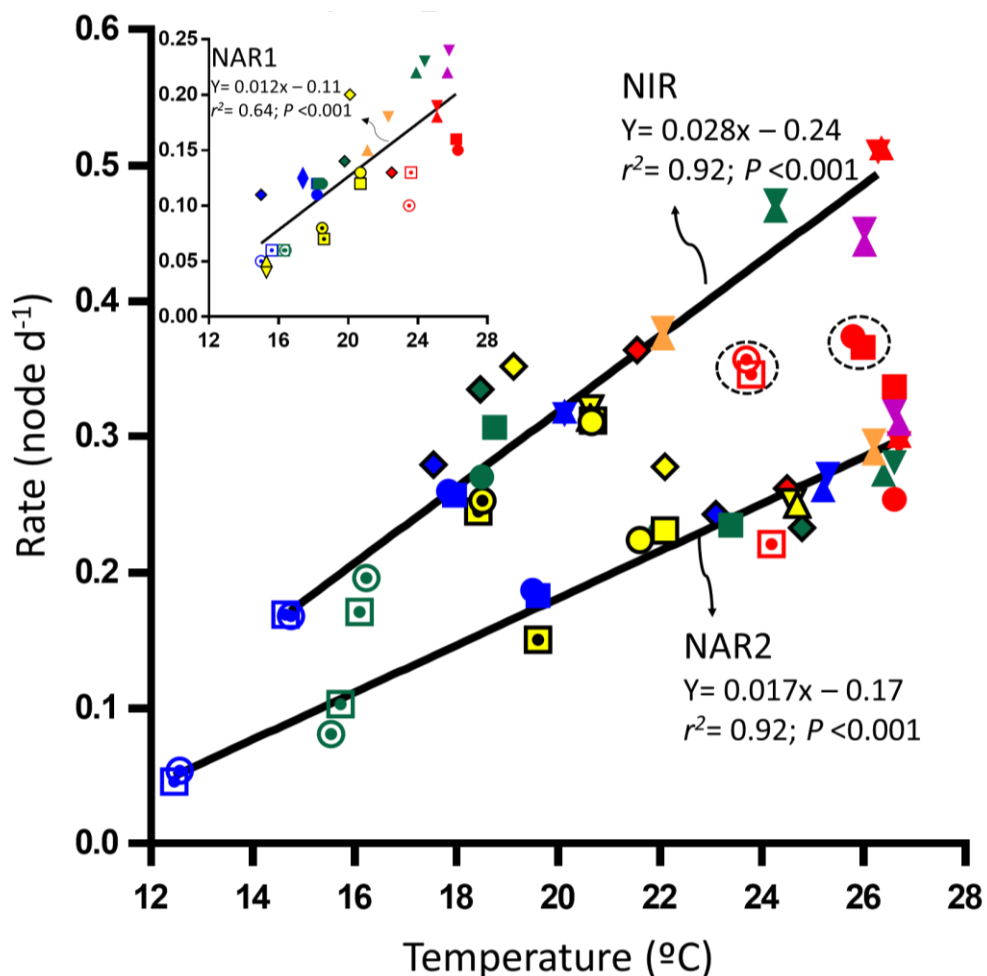
Figure 7. Node initiation rate (NIR) and the second phase of node appearance rate (NAR2, B2) *versus* mean temperature from sowing to beginning of pod setting (NIR) and between X1 and X2 (NAR, see equation 3), respectively. Data from the four experiments were pooled and each line represents the fitted linear regression models. Circled data points were excluded from the NIR regression analysis (see Section 2.4.2). See Fig. 2 and 3 for symbol code and Table 2 for treatment description. Inset: first phase of node appearance rate (NAR, B1) versus mean temperature between emergence and X1.

3.4. Co-ordination between node appearance and initiation

A robust relationship was observed between the number of initiated nodes and the number of appeared nodes using the pooled data collected across the four experiments (R^2= 0.98; P <0.001). Clearly, the two mechanisms are functionally co-ordinated (Fig. 8). This is remarkable given the wide range of weather, soil, and management explored across the four experiments and the different MGs used in the study. The model was also robust at reproducing the observations inferred by Johnson *et al*. (1960) for determinate and indeterminate varieties, suggesting that the co-ordination model has remained unchanged despite a half-century difference between cultivars released and used in the 1960s and those used in the 2010s. Based on the fitted model, soybean had already six initiated nodes by VE, including the cotyledonary and unifoliolate nodes plus four trifoliolate nodes (Figs 5 & 8).

The fitted two-segment relationship between the number of initiated nodes and number of appeared nodes summarizes the dynamics of node appearance and initiation in soybean. First, dormant seeds already have three initiated nodes (cotyledon, unifoliolate, first trifoliolate). Second, the ratio between new initiated and appeared nodes abruptly changed from 2.6 to 1.5 when the appeared node was 4 and the initiated was 12, which was consistent with the change in phyllochron-to-plastochron ratio from 2.3 to 1.6 (see previous section). This abrupt change in phyllochron-to-plastochron ratio occurred when plants had *ca.* 4 appeared nodes (V2 stage), which fit within the 2 to 6 range of appeared node number at which X1 occurred across experiments. Finally, initiated nodes continued to appear despite the fact that the number of final appeared nodes was always less, which

indicated that initiated nodes near the end of plant development do not necessarily

advance past the primordial stage (Fig. 8). The difference in final number of initiated

nodes *versus* final appeared nodes was inversely related to the length of the R3-R5 phase

(R^2 =0.46; P <0.001) (Fig. 8, inset). This phase matched well the time period, measured

in thermal time, between end of node initiation and earlier end of node appearance (Figs

2 & 3). Hence, a longer duration of the R3-R5 phase allowed a greater proportion of the

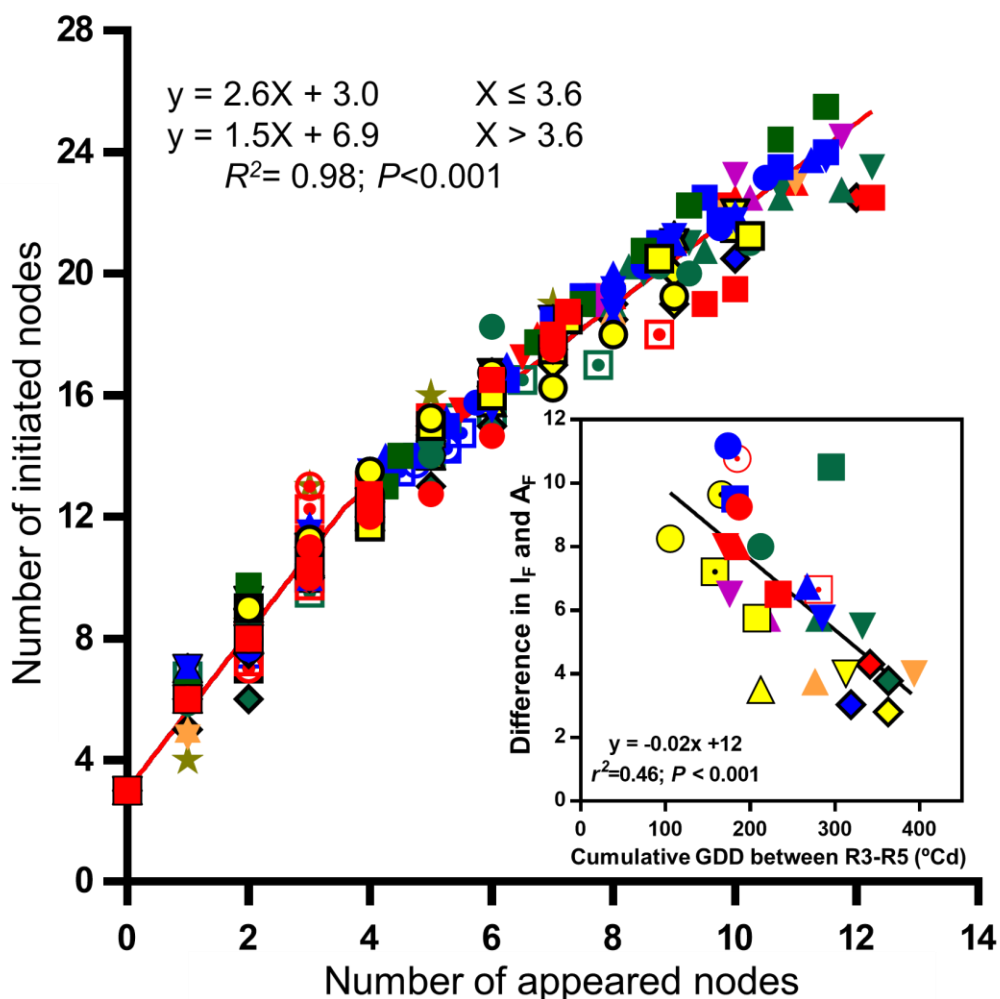initiated nodes that had not appeared before R3 to subsequently appear.

Figure 8. Relationship between number of initiated nodes and number of appeared nodes based on the pooled data from the four experiments. See Fig. 2 and 3 for symbol code and Table 2 for treatment description. A two-segment linear regression model was fitted following equation 5. Inset: relationship between the difference in final number of initiated and appeared nodes (I_F and A_F, respectively) and cumulative thermal time (GDD; T_b= 10.0°C) from R3 to R5. Data on node appearance collected after reaching the final number of initiated nodes are not shown here and were not used for fitting the two-segment model. Data from Johnson *et al* (1960) were also plotted for comparison (stars).

DISCUSSION

This is the first study to document the influence of temperature on the concurrent rates of node appearance and node initiation in soybean, when examined over a wide range of weather conditions, soil types, and management practices. The experiments were conducted in both field and GH settings using indeterminate soybean cultivars differing in maturity. Frequent seasonal observations and sampling provided the precision needed to document the node appearance and node initiation rate patterns during the crop season. Our results indicated that both NAR and NIR were temperature-sensitive. Moreover, we detected a two-phase phyllochron during crop ontogeny (*i.e.,* a rate changed from 83 to 58ºCd), but only a single phase plastochron (36ºCd), all with almost an identical T_b (*ca.* 10ºC), for the both indeterminate cultivars. Similarity between our experimentally estimated T_b values for both phyllochron and plastochron were consistent with findings for other crop species such as maize (Hesketh & Warrington 1989; Padilla & Otegui 2005), wheat (Kirby 1990), and sunflower (Sadras & Villalobos 1993).

The lack of difference in phyllochron and plastochron between the MG 2.1 and MG 3.0 cultivars grown in this study was consistent with what Setiyono *et al.* (2007) and Kumudini (2010) reported for cultivars that spanned a wide range of MGs. However, a much wider range of soybean MGs (eg. MGs 0 to 4) should be screened to fully understand the genotypic variation for phyllochron and plastochron in the indeterminate varieties grown in the USA as has been done in other crop species such as quinoa (Bertero *et al*. 2000) and maize (Padilla & Otegui 2005). Indeed, genotypic variation in phyllochron was previously reported by Sinclair (2004) for determinate soybean varieties

although it not clear if the reported variation in NAR is associated with differences in Tb,

phyllochron, or both among soybean varieties evaluated in this study.

The phyllochron reported here for trifoliolate node appearance (58ºCd) is almost identical

to the phyllochron (56ºCd) reported by Hesketh *et al*. (1973) and Thomas & Raper

(1976), although there is a critical three degree difference in T_b between studies (9ºC

versus 6ºC). Estimated T_b in earlier studies should be treated with caution since air

temperature at SA height was not likely measured. The linear relationship between NAR

and temperature described by Hesketh *et al*. (1973) when compared with the relationship

detected in this study (Fig. 7) revealed that the regression coefficients were nearly

identical (*i.e.,* 0.018 *vs* 0.017, respectively), but the intercepts were not (*i.e.,* -0.11 *vs.* -

0.17). The two trend lines were thus parallel, but the former was more elevated than the

latter. In our calculations, we used the temperatures measured at the height of the SA,

rather than the general GH air temperature. Findings of the present study does not support

the inference made by Bastidas *et al.* (2008) about lack of influence of temperature on

NAR. The discrepancy is probably due to the very narrow range of average temperature

during V1-R5 (22-26ºC) explored across treatments in the latter study. This finding

stresses the importance of evaluating response of physiological processes to temperature

across a wide range of temperature through a combination of experiments in both field

and controlled conditions. It is interesting, however, that the seasonal pattern for node

appearance in all treatments was remarkably linear, including those treatments where

temperature increase during the growing season (IT in Expt. 2 and 3 and all treatments in

Expt. 2 and 4). Based on the relationship between temperature and NAR, we would have

expected a curvilinear pattern of node appearance as temperature increased during the growing season; however, there was no observational evidence of increase in NAR over time in our experiments.

This study provided the first detailed description about node initiation dynamics in indeterminate soybean. While the number of initiated nodes we observed in dormant seed confirmed the findings of Miksche (1961), the present study extended the knowledge on node initiation dynamics in soybean by documenting a definitive linear pattern in node initiation that began at sowing, but ceased near the beginning of the pod set stage (R3). In previous studies, NIR was reported to be 0.50 to 0.52 node d^{-1} (*i.e.*, 1.9- 2.0 d node^{-1}) in determinate soybean cultivars grown at a mean temperature ranging from 23 to 24ºC in controlled environments (Thomas & Kanchanapoom 1991; Chiera *et al*. 2002). This NIR range is higher than the NIR we calculated (0.4 to 0.43 node d^{-1}) using linear model fitted to our data (Fig. 7) for the same temperature range. Possible explanations for this difference include (1) NIR from these previous studies were based on SA samples collected only in early vegetative development (*i.e.,* 10 days after VE or 28 DAS), a much shorter time period than in the present study (*i.e.,* 40 to 85 DAS); (2) the temperatures reported in these earlier studies may not correspond to the actual temperature at SA height, as reported in the present study; (3) previous studies do not account for difference between soil and air temperature on NIR before VE, but this was accounted for in the present study; and (4) only determinate cultivars were used in prior studies whereas indeterminate cultivars were used here.

Radiation intensity did not affect NAR and NIR except for treatments with both low

radiation and high temperature. In the present study, *ca.* 30% lower NIR was observed

when soybean was grown in controlled environments with high temperature (24-26ºC)

together with low radiation (*ca.* 50% of full sunlight) relative to full-sun field-grown

plants exposed to the same range of temperature. A lower NIR in high temperature and

low radiation conditions has been reported for other crop species, including sunflower

(Sadras & Villalobos, 1993), wheat (Rawson 1993), and maize (Padilla & Otegui, 2005).

Rawson (1993) postulated a source-limiting hypothesis to explain this phenomenon, in

which plants exposed to low radiation are source-limited while, at the same time,

potential sink activity is maximized due to high temperature. Interestingly, NAR has not

been found to be depressed by the combination of high temperature and low radiation. To

explain this contrasting response, Rawson and Zajac (1993) proposed that with a faster

NIR in relation to NAR, node primordia would be naturally in excess in the SA. Range of

mean photoperiod was very narrow in our treatments, which precluded a detailed

assessment of daylegth on NAR and NIR. Longer daylengths will increase the final

number of appeared nodes due to longer time period between VE and R5 (Johnson *et al.*

1960). Previous studies reported photoperiod influence on NAR to be negligible (Thomas

& Raper 1983; Nico *et al.* 2015). In the present study, almost identical slopes were found

across sowing date treatments in Expt. 2 when number of appeared node and initiated

nodes was plotted against thermal time (T_b= 10ºC), suggesting lack of photoperiod effect

(Fig. S1). Still, more research is needed to fully understand the influence of photoperiod

on NAR and NIR in soybean, which may be more relevant given the context of soybean

production expansion into low- and high-latitude environments (Sinclair *et al.* 2013, Specht *et al.* 2014).

This is the first study reporting that node initiation ceased by R3 while node appearance continues until R5 in indeterminate soybean, which coincided with beginning of pod setting and seed filling, respectively. Node cessation occurrence around R5 in indeterminate soybean due to photosynthate diversion to developing seeds, at expense of node primordia, was theorized as the cessation causal factor by several authors (Sinclair 1984a; Egli *et al.* 1985; Pedersen & Lauer 2004; Bastidas *et al.*2008). Relative to the main SA in the two indeterminate cultivars, we were unable to conclusively observe the development of a terminal floral inflorescence, in agreement with current understanding of the genetic control of growth habits in plants (Shannon & Meeks-Wagner 1991; Liu *et al.* 2010; Benlloch *et al*. 2015). We do note here that Caffaro *et al.* (1988) reported observing a terminal inflorescence in indeterminate soybean. This apparent discrepancy can be explained by Carlson & Lersten (2004), who reported that a terminal SA may sometimes appear to be a terminal floral inflorescence, but in reality, such an inflorescence is a series of small one- or two-flowered axillary inflorescences crowded together because of the short internodes near the main stem tip. That explanation is consistent with our observation of differentiated floral primordium in the axillary apex adjacent to the main terminal SA (Fig. 6b).

An original finding from this study is the contrasting behavior between node appearance and node initiation in relation to crop ontogeny. While plastochron was constant from

sowing to end of node initiation (R3), the phyllochron was 30% faster after the first

breakpoint (X1) in the two-phase regression (58 *versus* 83ºCd). Hesketh *et al*. (1973) and

Fehr & Caviness (1977) also noted that NAR was lower during the early vegetative

development. The estimate of phyllochron from VE-X1 in this study was not much

different from the phyllochron of 106 ºCd that we derived from our re-analysis of

Hesketh *et al*. (1973) data. In contrast to Bastidas *et al.* (2008), who reported that X1

corresponded closely to V1 (3 appeared nodes), our study indicated that X1 occurred at

stages VC to V4 (2 to 6 appeared nodes) in the various treatments conducted in the

present study. This may be due to not making more temporally frequent observations

during physiological phase shifts as was noted by Bastidas *et al.* (2008). Thus, we could

not definitely associate the breakpoint with specific leaf number, or with the transition

from seeding growth heterotrophic (dependence on cotyledonary reserves) to autotrophic

(photosynthesis-driven) phase as reported in other crop species (Sadras & Villalobos

1993; Miralles *et al.* 2001; Padilla & Otegui 2005). More research is needed to better

understand the mechanisms or environmental conditions that regulate the occurrence of

the first breakpoint in the soybean phyllochron.

Another novel finding from this study is that indeterminate soybean initiated nodes in

excess relative to that eventually appeared, with the magnitude of this 'surplus'

depending upon the length of the R3-R5 stage period expressed in thermal time. These

two stages match closely the time when node initiation and node appearance respectively

ceased. It was evident in the data obtained in our study that increases in thermal time

between R3 and R5 were associated with decreases in the difference between initiated

and appeared node totals. As noted by Board & Tan (1995), Board *et al*. (1999), and Bastidas *et al*. (2008), number of nodes per main stem is a key yield component. Therefore, extension of the duration of the R3-R5 phase (in thermal time) may be a route to maximize the total number of appeared nodes by R5 in relation to the total number of initiated nodes by R3, which, in turn, can contribute to increase seed number and seed yield. Consistent with this hypothesis, Kantolic & Slafer (2001) and Nico *et al*. (2015) reported that increase of the time period between R3-R5 through artificial photoperiod extension resulted in greater node number, seed number and final seed yield.

Relative to the co-ordinated seasonal correspondence between node initiation and node appearance, we were able to show that a bi-phasic linear model explained 98% of the variation in the correspondence relationship. Clearly, there is a strong co-ordination between node appearance and initiation in indeterminate soybean, which is consistent with the relationships reported in other crops species (Sadras & Villalobos 1993; Miralles *et al.* 2001; Padilla & Otegui 2005). In soybean, however, the co-ordination model exhibited two linear phases with different slopes, which reflected a change in the phyllochron-to-plastochron ratio before and after the breakpoint (X1). The regression equation we computed here for co-ordination between initiated nodes and appeared nodes provides an easy means of predicting the number of initiated nodes for any given observed number of appeared nodes, without the need for SA dissection, which is very laborious and time consuming. The co-ordination model presented here also can be embedded into crop simulation models for a more mechanistic simulation of both node appearance and initiation in indeterminate soybean.

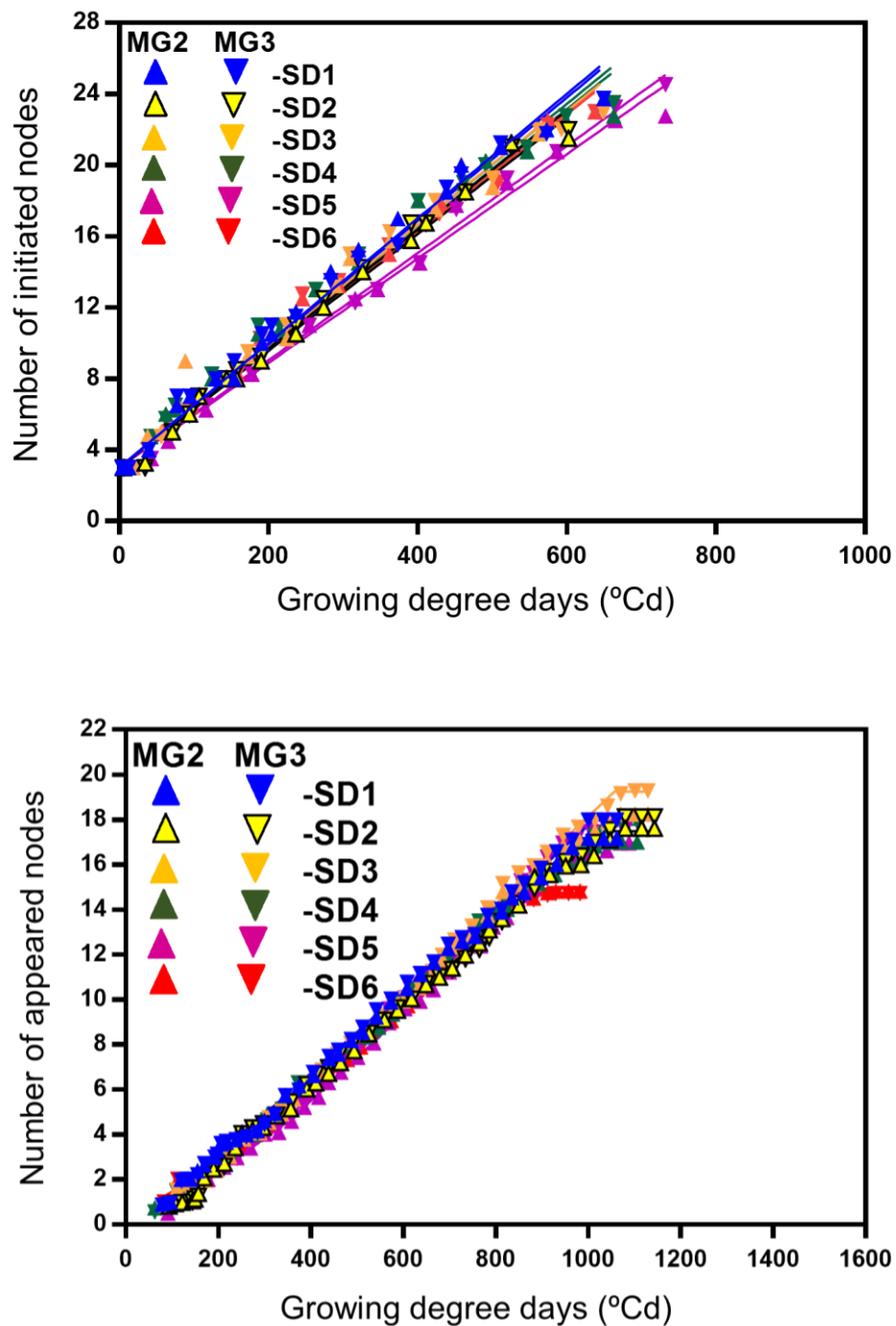**ACKNOWLEDGEMENTS**

SUPPLEMENTARY INFORMATION

Figure S1. Number of initiated (upper panel) and appeared nodes (bottom panel) in Expt. 2 as a function of cumulative thermal time after sowing. Thermal time was calculated using T_b= 10ºC.

Table S1. Analysis of variance (ANOVA) for significant effects of temperature (temp), sowing date (sd), maturity group (MG), and their interactions, on the first phase of node appearance rate (NAR1) for the four experiments.

Experiment	Type III Tests of Fixed Effects				
	Source	DF	Sum of Squares	F Value	Pr > F
1	temp	3	0.0070	32.8	<.001
	MG	1	0.0001	0.7	0.409
	temp*MG	3	0.0005	2.2	0.110
2	sd	5	0.0668	23.9	<.001
	MG	1	0.0003	0.5	0.506
	sd*MG	5	0.0007	0.3	0.930
3	temp	3	0.0065	188.6	<.001
	MG	1	0.0003	22.1	<.001
	temp*MG	3	0.0001	1.7	0.188
4	field	3	0.0132	7.5	0.004

Table S2. Analysis of variance (ANOVA) for significant effects of temperature (temp), sowing date (sd), maturity group (MG), and their interactions, on the second phase of node appearance rate (NAR2) for the four experiments.

Experiment	Type III Tests of Fixed Effects				
	Source	DF	Sum of Squares	F Value	Pr > F
1	temp	3	0.0596	34.4	<.001
	MG	1	0.0021	3.7	0.068
	temp*MG	3	0.0060	3.4	0.033
2	sd	5	0.0158	14.1	<.001
	MG	1	0.0003	1.1	0.296
	sd*MG	5	0.0011	1.0	0.425
3[†]	temp	3	0.0397	103.9	<.001
4	field	3	0.0040	1.5	0.261

[†] MG and temp x MG terms were not tested because of the very short duration of the NAR2 phase of MG2 cultivar in two treatments (HT and MT), which did not allow to establish a reliable estimate of NAR2.

Table S3. Analysis of variance (ANOVA) for significant effects of temperature (temp), sowing date (sd), maturity group (MG), and their interactions, on node initiation rate (NIR) for the four experiments.

Experiment	Type III Tests of Fixed Effects				
	Source	**DF**	**Sum of Squares**	**F Value**	**Pr > F**
1	temp	3	0.0137	26.5	<.001
	MG	1	0.0006	3.5	0.074
	temp*MG	3	0.0007	1.3	0.310
2	sd	5	0.1566	275.1	<.001
	MG	1	0.0001	1.2	0.286
	sd*MG	5	0.0009	1.6	0.182
3	temp	3	0.1373	646.2	<.001
	MG	1	0.0008	11.3	0.003
	temp*MG	3	0.0016	7.4	0.001
4	field	3	0.0140	231.9	<.001

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