# Phytochrome B regulates seed germination by integrating light and temperature signals in Nicotiana tabacum L.

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# Abstract

Background: Phytochrome is the most abundant photoreceptor in Arabidopsis thaliana ( Arabidopsis ), which integrates light and temperature signals, and in turn regulates plant development. However, the exact pattern of integrated signals during seed germination remains unknown. In this study, we analyzed the effect of NtPHYB1 genotype in response to ecological environments in Nicotiana tabacum L. Results: The germination frequencies of WT seeds showed at least no significant difference, and were significantly higher than that of NtPHYB1 - GFP and NtPHYB1 -RNAi seeds in some environments or. According to the maximum germination frequency , germination of NtPHYB1 - GFP seeds was mainly inhibited by continuous light exposure, while the germination of NtPHYB1- RNAi seeds was repressed by low temperature and no light (darkness) exposure. At 15ºC, the germinations of all three genotypic seeds were inhibited by the low-temperature, and the germination frequency of NtPHYB1 - GFP seeds was significantly lower than that of WT and NtPHYB1 -RNAi seeds; while light signal had no effect at 15ºC. At 20 and 25ºC, the temperature signal promoted germination, and the signal of light was dispensable. At this condition, the maximum germination frequencies were obtained for NtPHYB1 - GFP and WT seeds. At 30 and 35ºC, the light signal was indispensable to maintain seed germination for all three genotypic seeds. At this condition, NtPHYB1 -RNAi seeds reached the maximum germination frequency. Conclusion: Phytochrome B regulates seed germination by integrating light and temperature signals. The above results elucidate why warm spring and autumn (about 25ºC) are more suitable for sowing compared to cool winters (less than 15ºC) and hot summers (greater than 30ºC).


# Background

Seed germination is controlled by a number of environmental factors, such as temperature, light, pH, and soil moisture, which ensure that plants start a new life cycle under suitable conditions. For example, light regulates the early stage of germination, while temperature is crucial during all the germination processes. Phytochromes are types of photoreceptors that are fundamental for perception of the light environment and regulation of the germination processes. So far, multiple phytochrome photoreceptors have been discovered. For example, in the model species *Arabidopsis thaliana*, five genes (*PHYA, PHYB, PHYC, PHYD and PHYE*) encoding phytochrome apoproteins have been found [1-3]. Among them, *PHYA* and *PHYB,* which are located in the embryo and endosperm, respectively, have an essential role in regulating light-dependent germination responses [4, 5], which in turn control spatial and temporal patterns of seeds. In this particular specie, the germination is regulated by endosperm after seed imbibition and later on by the embryo [6, 7]. Other three genes, i.e. *PHYC*, *PHYD* and *PHYE* are involved in seed germination regulation under some special condition [8-11], and their functions are affected by light wavelength and irradiance.

Recently, a new function of phytochromes in temperature-dependent germination responses has been discovered, which is functionally diverse among different members [12]. Different phytochromes regulate seed germination response to temperature during seed imbibition and maturation in different ways. PHYB is a crucial contributor when seeds are not imbibed at low temperature; while PHYD is indispensable for

full germination when seeds are imbibed at high temperatures. PHYA and PHYE are manly activated at high temperatures [13]. Moreover, compared to matured seeds at a higher temperature, those matured at low temperature are in a state of strong dormancy. PHYB and PHYD are necessary to break cold-induced dormancy, and PHYA helps maintain cold-induced dormancy [14, 15].

During germination, the sensitivity of seed to light is associated with the temperature perception [16, 17], which might vary among species. Two hypotheses have been proposed to explain these phenomena: (a) sensitivity of seeds increases to the preexisting Pfr; (b) the Pfr level in seeds rapidly increases or decreases at certain temperatures. Studies have discovered that PHYB is the sensor of both light and temperature [1, 2], which integrates light and temperature cues in *Arabidopsis* seeds [18]. Nevertheless, the exact pattern of integrated signals during seed germination remains unknown.

The working model of phytochromes integrating light and temperature signal has been proposed in plants [19]. Red light drives the production of active Pfr-Pfr, while far-red favors inactive Pfr-Pr or Pr-Pr. In addition, the active Pfr-Pfr pool is governed by thermal reversion. Temperature affects the activity levels over a range of light conditions, while plants are more vulnerable to conditions such as warm conditions in darkness, low light, or FR rich. A recent study has indicated that Phytochrome B dynamics depart from photo equilibrium in the field. The PHYB activity mainly responds to the spectral distribution of the light under full sunlight environment; its activity departs from photo equilibrium and becomes affected by irradiance and temperature at the extremes of the photoperiod, or in the presence of rapid fluctuations of the light environment [20].

In this study, the seasonal variation of the natural environment was simulated and the difference between seeds sowed on the ground or in the soil was also considered in order to explore the signal (light and temperature) integration mechanism used by *PhyB* to regulate seed germination. In addition, we elucidated from biological point of view why sowing is more suitable in warm spring and autumn compared to cool winters and hot summers.

# Results

### Photo- and thermal- control of seed germination

Batches of after-ripening *Nicotiana tabacum* L. *(N. tabacum)* seeds exposed to different environmental conditions displayed variation in germination, ranging from about zero to over 85% (**Figure 1**). As shown in **Table 1**, significant differences in mean germination frequency were observed when exposing seeds to different light period and/or temperatures. The germination frequencies of seed cultivated at 12h photoperiod (12h light/12h dark) were significantly higher ($p<0.001$) compared to plants exposed to 24h light (Light) or those placed in dark (Dark). In addition, the uniform seed germination under this photoperiod was also superior to other treatments (**Figure S1**). With reference to exposure to different temperatures, a temperature of 25ºC resulted as the most appropriate temperature for seed germination; the percentage of germination was significantly higher ($p<0.001$) at 25ºC compared to seed exposed to 15ºC, 20ºC, 30ºC and 35ºC (**Table1**). These results indicated that 12h photoperiod and a moderate

temperature (25 ºC) are favorable condition for seed germination in *N. tabacum*. Furthermore, the ANOVA results confirmed the significant effect (*p□0.001*) of light and temperature on seeds germination (**Table 2**).

### Interactions between light and temperature signal on seed germination

Light and temperature are two important environmental factors for regulation of seed germination in *N. tabacum*. Furthermore, in this study we found a connection between these factors when regulating seed germination (*p<0.001*, **Table 2**). Seeds cultured in all dark conditions whose germination frequency displayed Normal distribution with the temperature (**Figure 2** and **Table S1**). Briefly, the germination frequency was lower at low temperature (15ºC); it increased and reached a maximum value at 25ºC, then it showed a decreasing trend until reaching 35ºC, during which very low germination was observed. However, a logarithmic pattern was observed with temperature, both with the continuous or periodic light treatment (**Figure 2** and **Table S1**). These observations suggest that the sensibility of seed to temperature signal could be disturbed by illumination during germination in *N. tabacum*. When exposed to light, the temperature ranges allow significant germination of seeds; while in the absence of light, an appropriate lower temperature (<30ºC) is required for seeds to reach a higher germination potential.

At temperature of 25ºC, there was no significant difference in germination frequency either with or without illumination (**Table S2**). At low temperature (15 or 20 ºC), the germination frequency was higher without light; while at high temperature, appropriate light duration allowed seeds to maintain a higher germination frequency (**Figure 1**), especially at 35ºC (**Table S2**; **Figure 1-3**). These observations suggest that the sensibility of seed to light could be overturned by temperature during germination in *N. tabacum*. The temperature itself can promote and dominate seed germination within bounds (about 25ºC), and then the light signal is not required. However, beyond the proper temperature boundary, seed germination is regulated by the integrated signal of light and temperature, which is synergistic, which means that insufficient light with low temperature, or sufficient light with high temperature are beneficial for seed germination. On the contrary, sufficient light with low temperature or insufficient light with high temperature can restrain seed germination. Whenever, light regulation of seed germination depends on temperature signal, which can then affect other metabolisms and signals responsible for seed germination.

### NtPHYB1 integrates signal both light and temperature during seed germination

As shown in **Table 1**, different genotypes have different germination frequency (*p<0.001*). The mean germination frequency of WT seeds was significantly higher compared to *NtPHYB1*-RNAi seeds (*p<0.05*), which was higher compared to *NtPHYB1-GFP* genotype. The two-way interactions between genotype and photoperiod or temperature were not significant, respectively, while the three-way interaction was significant for seed germination (***p<0.001,*** **Table 3**). There was no difference in germination frequency among the three genotypes seeds that were incubated under some environmental conditions (**Table 3**, **Table S1** and **S2**). Subsequently, the environmental conditions of different genotypes seeds that achieve

maximum and minimum germination frequency were explored. According to the maximum germination frequency, germination of *NtPHYB1-GFP* seeds was mainly inhibited by continuous light treatment, while germination of *NtPHYB1*-RNAi seeds was inhibited by low-temperature and exposure to darkness (**Table 3**).

The above results imply that wild-type seeds can adapt to ecological environments in order to complete germination. In addition, WT seeds allow a wider range of environmental scales to reach the maximum germination frequency; this range is narrower in the other two types. High temperatures (>30ºC) are necessary for *NtPHYB1-RNAi* seeds and *NtPHYB1-GFP* seeds, which should not be exposed to a constant light.

# Discussion

### Role of phytochromes in light- and temperature- dependent germination responses

The involvement of phytochrome in the seed germination has been proven in *Arabidopsis* [4, 5, 8, 15, 21], *Lycopersicon esculentum* Mill. (*Lycopersicon esculentum*) [22], *Lactuca sativa* Linn (*Lactuca sativa*) [23, 24] and some other species. However, different roles of phytochromes in temperature-dependent and light-dependent germination responses still remain unexplored. Physiologically, photo-control and thermal-control of germination could have larger differences, and germination state of a seed could be reversed by a beam of light in duration of few minutes [16]. Our results revealed that seed germination could be inhibited or initiated by temperature signal; while light-regulating germination was strongly dependent on the temperature signal. Therefore, we assumed that temperature might be a long-lasting environmental factor in regulating seed germination, while the light was more like a high-efficiency one, possibly due to the different roles of these two signals on the phytochrome responses.

Phytochromes have an important role in the germination of light-required seed [4, 5, 23-25]. The signal of light received by phytochromes is converted into internal cues, which then regulates the physiological processes in seeds. In *Arabidopsis*, phytochrome photoreceptors perceive red (R) and far-red (FR) light. PHYA and PHYB receptors are essential for promoting seed germination [4, 5]. PHYB appears to be solely responsible for LFR in *Lycopersicon esculentum*, while PHYA inhibits germination under continuous R or FR light [25]. Surprisingly, phytochromes of *Lycopersicon esculentum* and *N. tabacum* have higher homology compared to *Arabidopsis*; nevertheless, seeds of *Arabidopsis* and *N. tabacum* are light-required, while seeds of *Lycopersicon esculentum* are light-inhibited during germination.

So far, a number of studies have investigated the role of phytochromes in temperature-dependent germination responses. For example, *PHYB* is activated at different temperatures and regulates seed germination, while the effects of *PHYA* and *PHYE* are more significant at warmer and colder temperatures, respectively [12]. In this study, we noticed the temperature signal can dominate seed germination within a range; nevertheless, the germination beyond boundaries is regulated by the integrated signal of light and temperature. Recently, it has been found that the activity of phyB is mainly controlled by photoequilibrium when the light is sufficient enough. If the light is insufficient, it can

dynamically escape from photoequilibrium when it is mainly controlled by temperature [20]. Therefore, we proposed that in nature, both light and temperature signal could be sensed by the seeds phytochromes for initiation of germination. In addition, sometimes temperature signals are more important than light signals, since it is hard for seeds to sense the light signal in soil.

During germination, the thermal sensitivities of seeds among species are similar, i.e. the germination is the highest when seeds are exposed to moderate temperature. However, there are variations in photosensitivity, which could be light-required, light-neutral or light-inhibited. Even though these mechanisms are rarely discussed, the photo-thermal sensor phytochromes might be important factors. Moreover, phytochromes systems have been evolved in different species to adapt to the environment. For instance, the phytochrome family consists of five members (PHYA-E) in *Arabidopsis*, *Lycopersicon esculentum* and *N. tabacum* [26-28], whose seeds are photosensitive, while three members (PHYA-C) in gramineous crops have seeds that are photo-insensitive, to adapt to being sown in the soil. During domestication, crop seeds generally lose their sensitivity to light, while temperature sensitivity remains, because germination at the unsuitable temperature can be harmful, or even fatal.

Phytochromes regulate both light-dependent and temperature-dependent germination [5, 12] and permit plants to adapt the variable environment. Phytochrome B (PHYB) is the main receptor found in *Arabidopsis* [1, 2, 18]. In this study, we discovered that PHYB regulates seed germination by integrating light and temperature signals in *N. tabacum*. However, it is still unclear whether other phytochromes (expect *PHYB*) participate in the integration of the light and temperature signal during seed germination. According to this study, we estimated that their integration within the temperature range is limited compared with that of PHYB. The *PHYB* regulates seed germination across a larger range of temperatures, while *PHYA* and *PHYE* are activated only when exposed to warm or cold temperatures, respectively [12].

Seeds germination can be interrupted by imbibition in continuous darkness or at supraoptimal temperatures in lettuce. In both cases, germinations are impaired when seeds are subsequently incubated under favorable conditions. Interestingly, both can be broken by a combination of red light with hormones [29, 30]. In this study, we noticed that seed germination was significantly inhibited when exposed to high temperature and dark condition. These suggested that three types of environmental inhibition might be controlled by a similar mechanism, and that photothermal sensor phytochromes might have a key role in this process. Dark and/or high-temperature imbibition induces the conversion of Pfr- Pfr to Pfr-Pr, which inhibits the seeds germination. This conversion could be reversed by a red or white light, while reversed Pfr- Pfr was able to support germination. These results confirmed the working model proposed by Halliday and Davis [19], according to which phytochromes integrated light and temperature signals in plants could be applied to seed during germination.

The photothermalsensitivity of seeds is attributed to the natural adaptation of their progenitor species to a physio-climate as cool winters, warm spring and autumn and hot summer. Seeds shed in the winter will not germinate during unseasonable rainfalls while temperatures are still low (cool-season); when rainfall

coincides with warm climate in the spring, the seeds will germinate and then flower in autumn (warm-season). Seeds shed in the summer will not germinate during unseasonable rain falls while the temperatures are still high (hot-season); when rainfall coincides with cooler temperatures in the autumn, the seeds will germinate and then flower in the following spring (warm-season). Temperature and light are two important environmental regulators for seed germination. Signaling information of season matches the germination potential of the seeds, including periods favorable for seedling emergence and survival, and subsequent phases of the plant life cycle.

### Phytochromes regulates seed germination response to the environment during seed maturation, and maternal effects can enhance the adaptability of seeds to environments in future

The presence of Pfr in dark-germinating seeds has been found in *Lactuca sativa*, *Lycopersicon esculentum*, and *Cucumis sativus* [31], suggesting that the active form of phytochrome stimulates germination in darkness. In this study, we noticed that the temperature signal itself could initiate and maintain germination at 25ºC, with or without the presence of light. These results suggest that active phytochromes stored in seed sense the temperature signal, thus promoting temperature-dependent germination at proper environmental conditions.

Maternal environment can influence the seasonal germination. For example, the ratio of dark germination of *Arabidopsis* seeds is significantly affected by the light quality under which the parent plant is grown [32]. Recently, Donohue *et al.* have confirmed that phytochromes differently regulate seed germination response to the temperature during seed maturation [14, 15]. Compared with the matured seeds at a higher temperature, those at low temperature are in a state of strong dormancy. *PHYB* and *PHYD* are required to break cool-induced dormancy, while *PHYA* contributes to the maintenance of cool-induced dormancy [14, 15]. The natural selection of phytochromes occurs through their effects on seed germination, while the maternal effects change the contribution of phytochromes to germination [10]. However, whether these maternal environmental effects are imposed by the maternal genotype, the endosperm genotype or the embryonic genotype needs to be further investigated [33]. Understanding this would enable us to comprehend how the maternal-plants predict climate changes under the natural environment and properly regulate the dormancy and germination state of their seeds, so that their offspring can better adapt to the complex environment.

## Conclusion

Phytochrome B regulates seed germination by integrating light and temperature signals in *N.tabacum*. At cool environment, low-temperature signal perceived by phytochrome B restrains germination of seeds, regardless of the presence or absence of light; in the warm environment, temperature signal initiates seed germination when the light signal is dispensable; while in hot environment, high-temperature signal restrains germination unless the light signal is involved. Temperature and light are two critical environmental regulators for seed germination. In order to appropriately match the germination and to seasonally start a new life cycle, signaling of these two regulators is sensed by phytochromes of seeds.

# Materials And Methods

### Plant material

The WT seeds of *Nicotiana tabacum* L. “K326” and its transgenic seeds were obtained from Tobacco Institute in Guizhou province. To study the effects of *NtPHYB1* in mediating the seed germination under multiple interactions of light and temperature cues, we compared germination of seed form Wild-type genotypes k326 and its mutants that exhibit loss or strengthen of function in *PHYB* genes. The gene isolation, plasmid construction, production of transgenic *N. tabacum* plants, molecular identification of *NtPHYB1*$^{K326}$*-GFP* and *NtPHYB1*$^{K326}$-RNAi transgenic *N. tabacum,* as well as phylogenetic analysis of PHYB homologous proteins were performed according to a previously described approach, which shares 75% identity with AtPHYB [34]. To control the effects of seed maturity and photodormancy on experimental results, ten capsules at the same maturity level (DAP40) were collected from the mother plant. All seeds were stored at room temperature for more than six months until after-ripening and then were used for germination test [35].

### Seed germination test

Germination tests were performed on three replicates of 100 seeds. Seeds were grown in artificial climate chambers with the temperature of interactive environment (15, 20, 25, 30, and 35ºC) and photoperiod (dark, 12h light/12h dark, light). Seeds were checked for germination every day for a total of 7 d. Germination was notarized as the length of observed radicle approximately equal to the length of the seed [36].

### Data analysis

A multivariate analysis model was exploited to assess the genotype effect on the environment variations of light and temperature during seed germination. Seed germination frequencies were compared among the three genotypes under fifteen environmental conditions. This model included genotype, temperature, and photoperiod during seed germination, and all possible interactions as fixed effects.

# Abbreviations

WT: Wide type; PHY: Phytochrome; T: Temperature; G: Genotype; L: Light; H: hour

# Declarations

### Acknowledgements

Not applicable

### Author contributions

WXY, LYL and ZM performed all the experiments. LZH analyzed the data and drafted the manuscript. LZH conceived the study and participated in the design and coordination. All authors have read and approved the final manuscript.


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### Availability of data and materials

Not applicable

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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# Tables

**Table 1** Comparisons of mean difference within the genotype, light and temperature treatment. Values are shown as means of observed values for each genotype

| Source | (I) | (J) | Mean Difference (I-J) |
|---|---|---|---|
| Genotype | *NtPHYB1-GFP* | *NtPHYB1-RNAi* | -6.2963*** |
| | | WT | -10.9407*** |
| | *NtPHYB1-RNAi* | *NtPHYB1-GFP* | 6.2963*** |
| | | WT | -4.6444* |
| | Wild Type | *NtPHYB1-GFP* | 10.9407*** |
| | | *NtPHYB1-RNAi* | 4.6444* |
| Light | Dark | 12h Light/Dark | -14.7000*** |
| | | Light | -5.0444** |
| | 12h Light/Dark | Dark | 14.7000*** |
| | | Light | 9.6556*** |
| | Light | Dark | 5.0444** |
| | | 12h Light/Dark | -9.6556*** |
| Temperature | 15ºC | 20°C | -36.3148*** |
| | | 25°C | -48.7407*** |
| | | 30°C | -41.0000*** |
| | | 35°C | -30.3889*** |
| | 20ºC | 15°C | 36.3148*** |
| | | 25°C | -12.4259*** |
| | | 30°C | -4.6852* |
| | | 35°C | 5.9259** |
| | 25ºC | 15°C | 48.7407*** |
| | | 20°C | 12.4259*** |
| | | 30°C | 7.7407*** |
| | | 35°C | 18.3519*** |
| | 30ºC | 15°C | 41.0000*** |
| | | 20°C | 4.6852* |
| | | 25°C | -7.7407*** |
| | | 35°C | 10.6111*** |
| | 35ºC | 15°C | 30.3889*** |
| | | 20°C | -5.9259** |
| | | 25°C | -18.3519*** |
| | | 30°C | -10.6111*** |

**Table 2** Test of significance and summary statistics for the interaction between light and temperature affects the germination of *NtPHYB1* transgenic seeds in *N tabacum* Analysis of variance tests for significant effects of temperature and photoperiod on seed germination of three genotypes of NtPHYB1. F ratios and degrees of freedom (d.f.) are given for each test. Three genotypes (G) are wild-type and its mutants that exhibit loss or strengthen of function of *NtPHYB1*. Seed germination photoperiod treatments (L) are all dark, 12h light/12h dark, all light. Seed germination temperature treatments (T) are 15, 20, 25, 30 and 35ºC. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

| Source | III.sum of squares | d.f. | MS | *F* | Sig. |
|---|---|---|---|---|---|
| Genotype (G) | 4800.759 | 2 | 2400.380 | 22.199*** | .000 |
| Light (L) | 7936.154 | 2 | 3968.077 | 36.697*** | .000 |
| Temperature (T) | 63436.466 | 4 | 15859.116 | 146.666*** | .000 |
| G*L | 975.230 | 4 | 243.807 | 2.255 | .064 |
| G*T | 1206.704 | 8 | 150.838 | 1.395 | .200 |
| L*T | 61711.869 | 8 | 7713.984 | 71.339*** | .000 |
| G* L*T | 3070.141 | 16 | 191.884 | 1.775* | 0.036 |

**Table 3** Germination frequencies (G) of *NtPHYB1* in repose to interactive environmental factors of light (L) and temperature (T). The three-way interaction was significant. The subscript letters of light (L) and temperature (T) represent the photoperiod and temperature, respectively.

| Germination radio among differ Genotype (G) |
|---|

| | Environmental combinations of light and temperature |
|---|---|
| No significant difference | *NtPHYB1-GFP*, WT and *NtPHYB1*-RNAi: $T_{15}L_{12}$, $T_{15}L_{24}$, $T_{20}L_{24}$, $T_{25}L_{0,}$ $T_{25}L_{12}$, $T_{30}L_{12}$, $T_{35}L_{0}$, $T_{35}L_{12,}$ and $T_{35}L_{24.}$ |
| Significant difference | WT*NtPHYB1-GFP*: $T_{15}L_{0}$, $T_{20}L_{12}$, $T_{25}L_{24,}$ $T_{30}L_{0}$, $T_{30}L_{24}$; WT*NtPHYB1*-RNAi: $T_{20}L_{0}$ and $T_{30}L_{0}$; *NtPHYB1*-RNAi*NtPHYB1-GFP*: $T_{25}L_{24}$. |
| Maximum | *NtPHYB1-GFP*: $T_{20}L_{0}$, $T_{25}L_{0}$, $T_{30}L_{12}$, $T_{35}L_{12}$; WT: $T_{20}L_{0}$, $T_{25}L_{0}$, $T_{25}L_{12}$, $T_{30}L_{12}$, $T_{30}L_{24}$, $T_{35}L_{12}$; *NtPHYB1-RNAi*: $T_{30}L_{12}$, $T_{30}L_{24}$, $T_{35}L_{12}$. |
| Minimum | *NtPHYB1-GFP*: $T_{15}L_{0}$, $T_{15}L_{12}$, $T_{15}L_{24}$, T35$L_{0}$; WT: $T_{15}L_{24}$, $T_{35}L_{0}$ ; *NtPHYB1-RNAi*:$T_{35}L_{0}$ |

# Supplementary Materials

**Figure S1** Germination kinetic curve for NtPHYB1-RNAi and NtPHYB1-GFP mutants relative to wild-type (WT) N tabacum exposed to 7 d dark (dark), 12h light/dark and 24h photoperiod (light).

# Figures

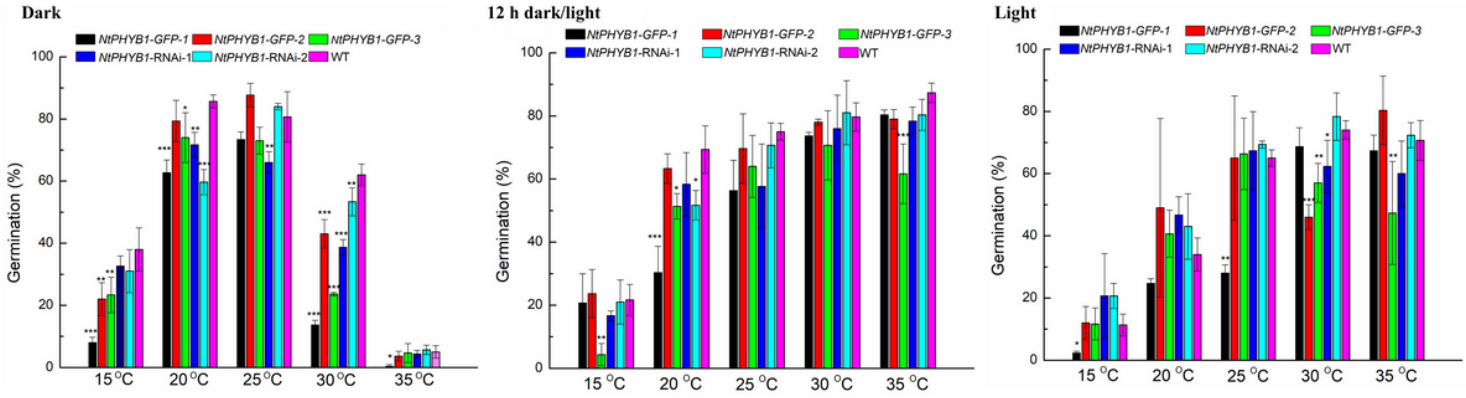


Figure 1

Germination frequency of NtPHYB1-RNAi, NtPHYB1-GFP and wild-type (WT) Nicotiana tabacum L seeds after 7 d exposure to dark (dark), 12h photoperiod (12h light/dark) and 24h photoperiod (light). ***, ** and * represent P < 0.001, P < 0.01, P < 0.05 level, respectively.

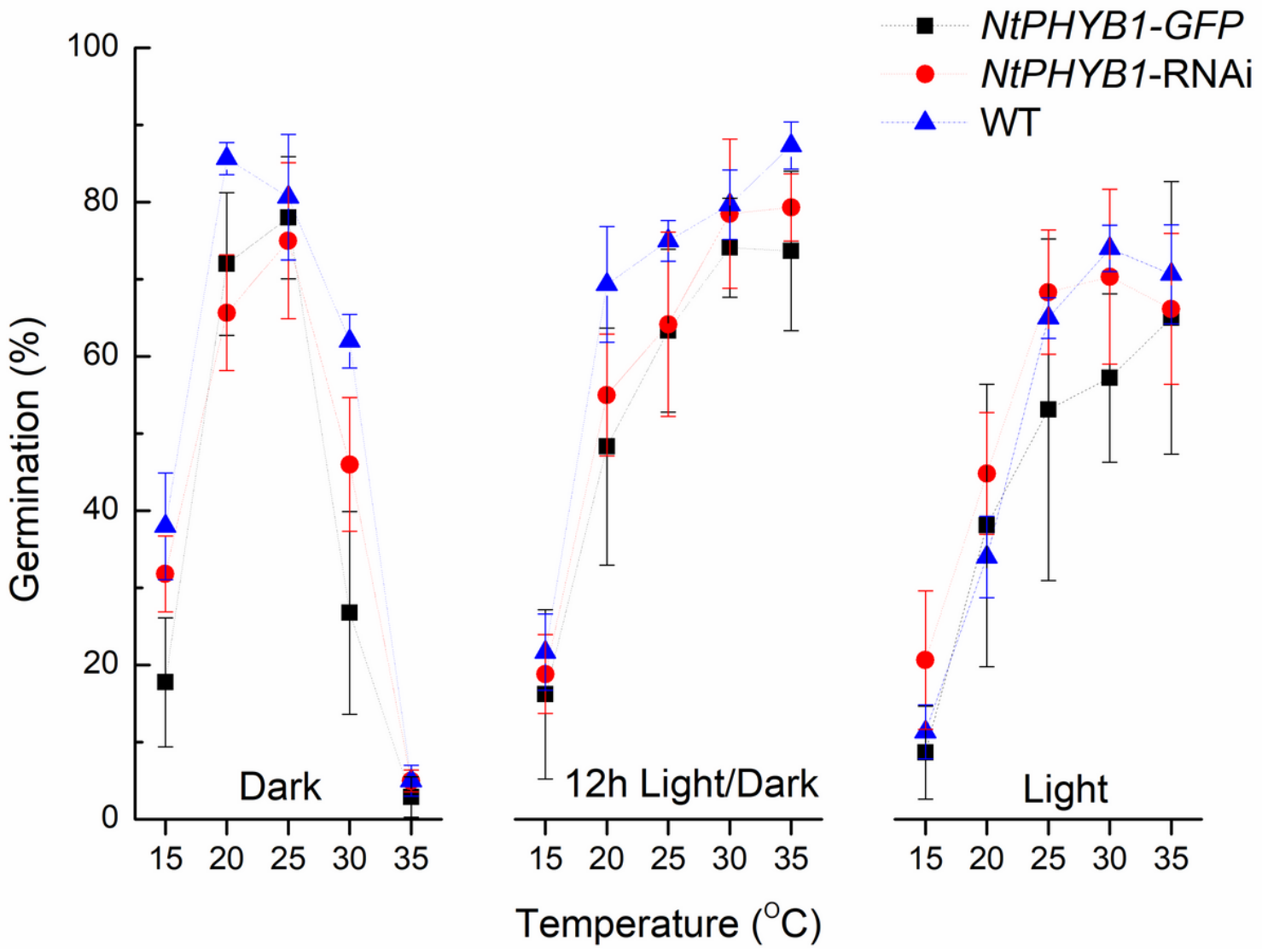


Figure 2

Germination frequency of NtPHYB1-RNAi, NtPHYB1-GFP and wild-type (WT) Nicotiana tabacum L seeds after exposure to different temperature and constant photoperiod (dark, 12h light/dark, light).

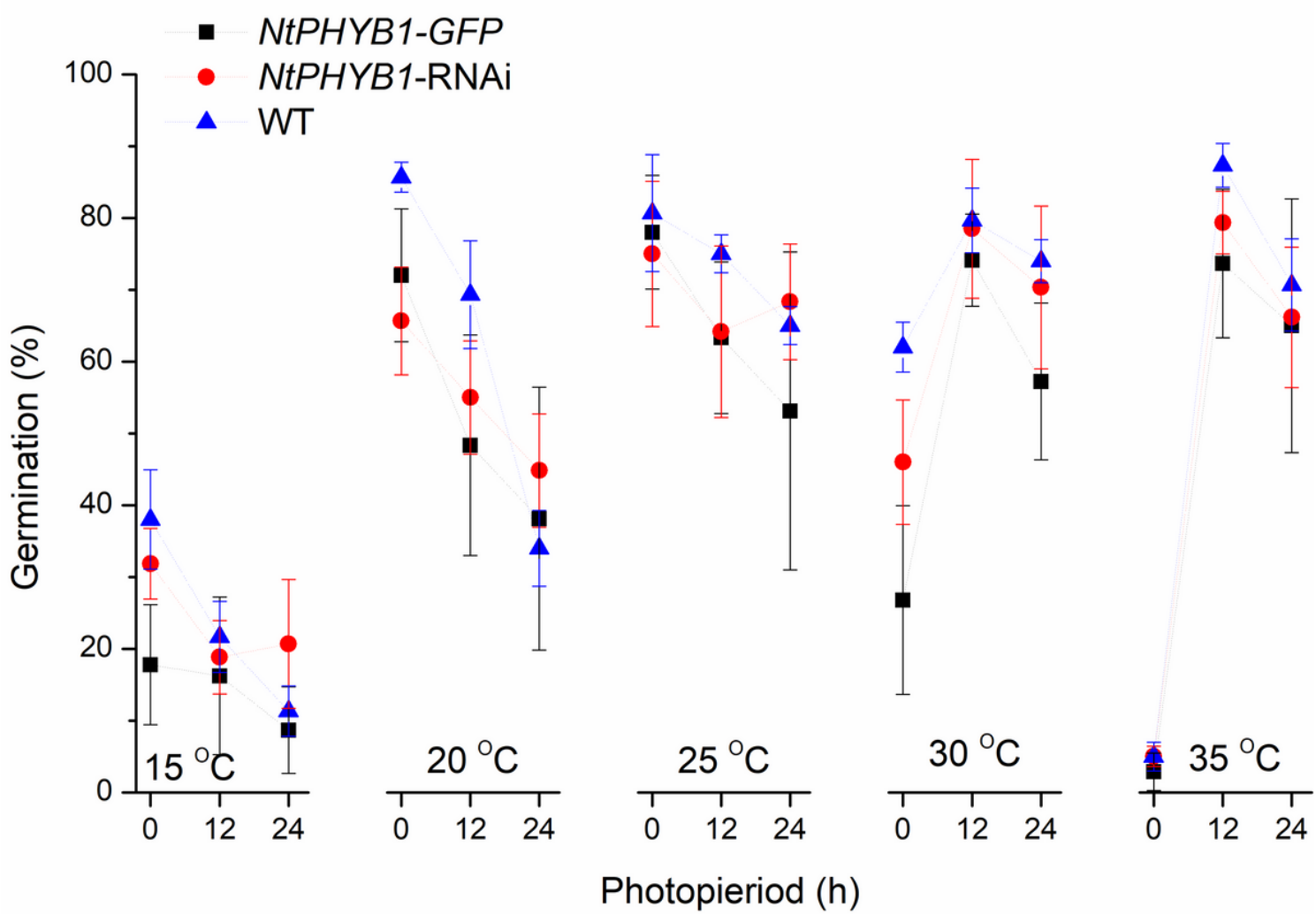


**Figure 3**

Germination frequency of NtPHYB1-RNAi, NtPHYB1-GFP and wild-type (WT) Nicotiana tabacum L seeds exposed to different photoperiod and constant temperature (15, 20, 25, 30, and 35ºC).

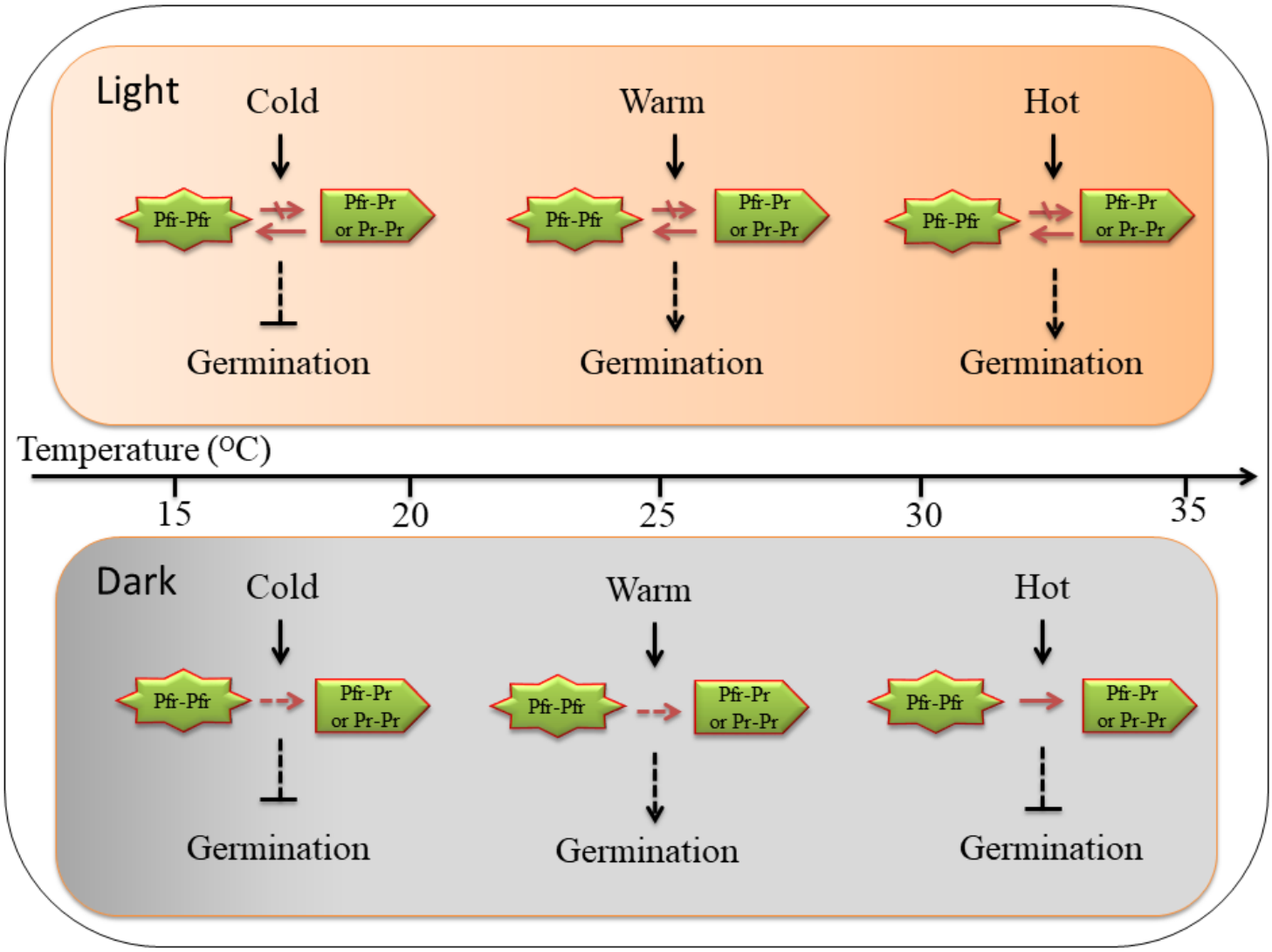


**Figure 4**

Diagram of phytochrome B integrates light and temperature signal to regulate seed germination in different seasons. The pink block are seeds exposed to the ground, while the black blocks are seeds buried underground. and represent inactive and active phytochrome. , and represents direct, indirect promotion, and indirect inhibition of germination. , and represents quick, slow, and obstructive conversions. In winter and early spring (≤15°C), the low-temperature signal sensed by phytochrome B inhibited seeds germination, and the effect of the light signal was almost ineffective. During late spring and autumn (about 20°C), temperature signal initiated germination with the combination of the light. In summer and early autumn (≥30°C), light is indispensable to maintain seed germination by persistently activating the phytochrome B.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TablesS1andS2andFig.S1.pdf