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Full Length Article

Estimation of Base Temperature for Germination of Rapeseed (*Brassica napus*) using Different Models

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Abstract

Germination tests of 8 rapeseed varieties (*Brassica napus* L.) were performed in incubators at constant temperatures of 3, 5, 8, 10, 13, 18 and 23ºC. The germination speed of rapeseed declined as temperature decreased from 23 to 3ºC. The cold-resistant varieties still maintained higher final germination under low temperature despite the extension of germination process. Five conventional models (Linear regression of percent germination per day, germination rate index, percent germination per day and reciprocal time to median germination versus temperature, and repeated probit analysis) were evaluated to estimate the base temperature for seed germination. The repeated probit analysis model was found the most suitable for estimating the base temperature of rapeseed due to the lowest respective root mean square error, and the mean base temperatures estimated by this model were 1.4 and 4.1ºC for the cold-resistant and cold-sensitive varieties, respectively. The repeated probit analysis model was also able to predict the germination percentage at any given time when the base temperature was confirmed. This research provides useful information and has implication for evaluation of different models for the estimation of base temperature for rapeseed germination and the management of rapeseed production with respect to sowing time.

Keywords: *Brassica napus*; Germination; Base temperature; Germination models

Introduction

Good performance of seed germination, which determinates the distribution of plants per unit area, is a prerequisite for rapeseed yield stability in direct-seeding cultivation (Sierts *et al.,* 1987; Wang *et al.,* 2015). Many environmental factors interact to influence the germination process, and temperature is a dominant factor affecting the rate and speed of seed germination (Bellairs and Bell, 1990; Cony and Trione, 1996; Elliott *et al.,* 2011). Low temperature slowed down the enzymes activities and mobilization of total lipid and protein reserves, limiting the coordination of metabolic processes required for germination development (Nykiforuk and Johnson-Flanagan, 1993; Hoppe and Theimer, 1997). Knowledge of the base temperature (T_b) is essential to calculate growing degree day and develop management models (Vigil *et al.,* 1997). In non-dormant seeds, accumulated temperature above the base temperature regulates germination process (Batlla and Benech-Arnold, 2015). The hydrothermal time model can describe and quantify the effects of temperature on seed germination with a necessitation for accurate low temperature (Dahal and Bradford, 1994; Bradford, 2002). Thus, estimation of base temperature for seed germination can help predict germination and determine the suitable sowing time based on the local temperature.

The determination of an accurate base temperature is important whether using a simple heat unit model or a more complex thermal-driven computer model (Wiese and BIinning, 1987). Previous studies have presented many methods to estimate the base temperature for crops and weeds (Moot *et al*., 2000; Gardarin *et al*., 2010; Wang *et al*., 2011). Simple and sound mathematical formulae have been used to calculate the base temperatures for other crops, such as snap bean, cowpea and sweet corn (Yang *et al.,* 1995). For instance, percent germination per day, reciprocal median response time, and germination rate index were regressed against temperature with the x-intercept representing the low threshold temperature (Wiese and BIinning, 1987; Holt and Orcutt, 1996). Additionally, cumulative percent germination was probit transformed and then paired with the temperature-time (i.e., In [T-T_b] × time) to find the optimal T_b when the mean square residual caused by the changed T_b was minimized (Covell *et al.,* 1986; Steinmaus *et al.,* 2000).

Seed germination is a critical stage of the life cycle reflecting adaptation to local habitats and affecting seedling establishment and plant population (Qiu *et al*., 2010).

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Thus far, to our knowledge, few studies have been performed on the base temperature estimation for seed germination of rapeseed (*Brassica napus* L.) and the suitable model for estimation this stage also need a comprehensive and further research. A previous study reported that linear regressions of 1/time to germination of given percentiles indicate T_b is about 3ºC for all oilseed cultivars (Marshall and Squire, 1996). There was an obvious difference in germination speed of rapeseed varieties (*Brassica napus* L.) from an extensive global collection (Zhang *et al.*, 2015), implying the diversity of base temperature in germination process of varieties. Additionally, Steinmaus *et al.* (2000) tested several methods for estimating T_b of weeds in experiments and suggested that the most statistically robust and biological relevant method was the reciprocal time to median germination. Therefore, present study was conducted to estimate the base temperature of rapeseed varieties (*Brassica napus* L.) using a range of mathematic models, and to identify the most robust and realistic model for rapeseed.

Materials and Methods

Materials and Experimental Design

Seeds of eight varieties of *Brassica napus* L. (Ganyouza No.5, Wenyou 99, HN3, Qinyou No.7, Huayouza No.9, Fengyou 737, Shengguang 77, Fengyou 730) were freshly harvested in 2013. The plump and consistent-size seeds were picked out and saturated in 1% sodium hypochlorite for 15 min to sterilize pathogen, then washed four times in distilled water. After this treatment, seeds were naturally dried and then stored in a seed low humidity storage cabinet with temperature controlled at 23 ± 1ºC and humidity controlled at 15 ± 2%.

Germination experiments were carried out in temperature-controlled incubators under constant temperatures of 3, 5, 8, 10, 13, 18 and 23ºC. The temperature fluctuation was controlled within ± 1ºC. For each variety, one hundred seeds (arranged in 10×10) were sown in a germination box (12 cm×12 cm×6 cm) and covered with 3-layer sterilized filter paper in three replications. The filter paper was saturated with 10 mL Hoagland's solution (Li, 2000; Ping *et al.*, 2015), and 1 mL nutrient solution was added every day during the experimental period to provide adequate water for seed germination. Seeds were subjected to the temperature treatment for 20 days. The number of germinated seeds was counted every day based on the criterion that the radicle had extended more than 2 mm beyond the seed coat (Schopfer and Plachy, 1984; Zhang *et al.*, 2015).

Germination Models

Model 1. Linear regression of germination index (*GI*) versus temperature. Germination index was calculated by the following equation (ISTA, 1999; Tan *et al.*, 2017):

$$GI = \sum_{i=0}^{n} \frac{g_i}{t_i}$$

Where, n is the last day of germination during the 20-d experiment and g_i is the number of germinated seeds on the *i*th day (t_i).

Model 2. Linear regression of germination rate index (*GRI*) versus temperature. Germination rate index was calculated by the following equation:

$$GRI = \frac{1}{n}\sum_{i=0}^{n} \frac{G_i}{t_i}$$

Where, $G_i$is the cumulative number of germinated seeds by the day t_i.

Model 3. Linear regression of percent germination per day (*PGD*) versus temperature. The percent germination per day was calculated by the following equation:

$$PGD = \frac{Gn}{p \times n} \times 100$$

Where, *Gn* is the final cumulative number of germinated seeds during the entire experimental period; p is the number of seeds sown in the germination box.

Model 4. Linear regression of reciprocal time to median germination versus temperature.

The 1/time to median germination was assessed at each constant temperature for each variety. Cumulative percent germination at a given temperature was probit transformed to normal equivalent deviate value and regressed against the germination time; the reciprocal time to median germination was estimated when normal equivalent deviate was 0 (Holt and Orcutt, 1996; Steinmaus *et al.*, 2000).

Model 5. Repeated probit analysis.

Cumulative percentage germination at each time point was transformed to a normal equivalent deviate (NED) value, then these NED values from all time points and suboptimal temperature were pooled and regressed against a logarithmic function of time (t_i) and temperature (*T*) by the following equation:

$$NED(i) = a + b[In((T - T_b) \times t_i)]$$

The intercept and slope were estimated by ordinary least squares methods and base temperature (T_b) was estimated by an iterative method, which varied T_b until the mean square residual term of the regression was minimized (Ellis *et al.*, 1986; Dahal *et al.*, 1990; Steinmaus *et al.*, 2000).

Goodness of Fit for Models

The traditional way to test the goodness of fit for models is based on root mean square error (RMSE) (Vigil *et al.*, 1997; White *et al.*, 2015). A modified and improved method, respective root mean square error (RRMSE), was used to eliminate the effects of dimensional difference of parameters in these models. The RRMSE value was calculated by the following equation:

$$\text{RRSME} = \sqrt{\sum_{i}^{n}\left(y_{obs-}y_{pred}\right)^{2}/\text{n}}\Big/\overline{y_{obs}}$$

Where, y_{obs} and y_{pred} are the observed and predicted values, n is the number of observations, and $\overline{y_{obs}}$ is the average across the observed values.

Results

Final germination and germination speed of each variety varied under different temperature regimes (Fig. 1). A period of 20 days was sufficient to complete germination for all rapeseed varieties at varying temperatures and germination speed was declined as temperature decreased from 23 to 3°C. Final germination of all these varieties was more than 98% and time to final germination was less than 3 days at 23°C. For variety HN3, final germination under all temperature regimes exceeded 96%, and time to final germination increased from 3 to 20 d as temperature decreased from 23 to 3°C. For Shengguang 77, final germination declined from 100 to 4% when the temperature decreased from 23 to 3°C. Additionally, HN3, Qinyou No.7, Wenyou 99 and Ganyouza No.5 germinated rapidly when the incubation temperature was above 8°C. Based on their performance at different constant temperatures, HN3, Qinyou No.7, Wenyou 99 and Ganyouza No.5 were categorized as resistant varieties, while Fengyou 730, Huayouza No.9, Fengyou 737 and Shengguang 77 were categorized as sensitive varieties to low temperature at germination phase (Fig. 2).

The relationships between the germination indices and temperature for all varieties used in model 1 to model 4 could be described well by linearity function (Fig. 3). The normal equivalent deviate used in model 5 also showed a linear response to In [*T-Tb*] × time (Fig. 4). Despite some difference in the base temperatures of the eight varieties estimated by these models, the determination coefficient (R^2) of these models revealed a strong linear relationship (Table 1). For the eight varieties, the base temperatures estimated by model 1 to model 5 ranged from 0.2 to 5.0°C, 2.3 to 5.9°C, 0.1 to 4.9°C, 0.8 to 4.6°C and 0.8 to 3.8°C, respectively (Table 1). The lowest base temperature was obtained by model 3, which was 0.1°C for Qinyou No.7. The highest base temperature was estimated up to 5.9°C for Shengguang 77 by model 2. Despite the difference in the estimated *Tb* values of these models, there was a consistent tendency that the base temperatures of HN3, Qinyou No.7, Wenyou 99 and Ganyouza No.5 were lower than those of Fengyou 730, Huayouza No.9, Fengyou 737 and Shengguang 77, except for model 2.

In order to identify the most robust and realistic model for predicting the germination progress and guiding

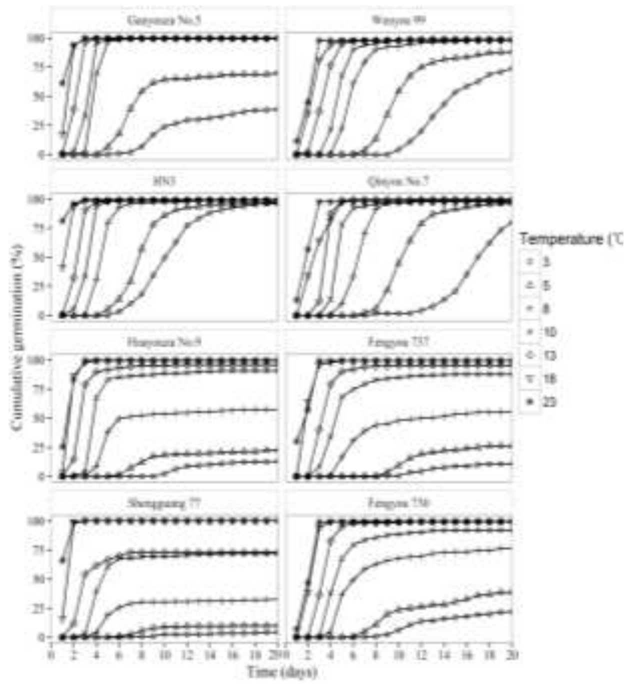

Fig. 1: Effect of different constant temperatures on cumulative germination processes of eight rapeseed varieties

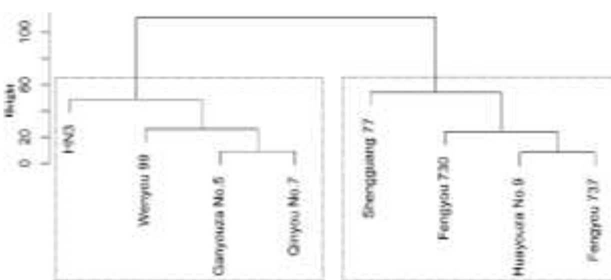

Fig. 2: Cluster dendrogram of eight rapeseed varieties by euclidean distance. These varieties were divided as cold-resistant and sensitive categories based on their cumulative germination at different temperatures

cultivation practice of rapeseed, the respective root mean square error (RRSEM) was used to estimate these models by comparing the observed values and predicted values. The data of eight varieties from every model were pooled, and model 5 using repeated probit analysis was found to be the most suitable method for estimating the base temperature of rapeseed due to the lowest RRSME. Model 2 using the regression of germination rate index (*GRI*) versus temperature provided the worst prediction (Fig. 5). Model 5 was based on the relationship of cumulative temperature and germination rate, with an advantage of being able to predict germination percentage at any given time when the base temperature was confirmed.

Table 1: Seed germination characteristics of five models for eight rapeseed varieties

Varieties		Model 1		Model 2		Model 3		Model 4		Model 5	
		Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
Ganyouza No.5	T_b	2.1		5.6		1.3		2.1		2.1	
	b	3.72	0.08	2.61	0.16	2.43	0.19	0.044	0.002	3.87	0.25
	a	-7.82	1.09	-12.12	2.12	-3.17	1.62	-0.092	0.021	-7.05	0.77
	R^2	0.99		0.93		0.89		0.98		0.83	
Wenyou 99	T_b	0.2		2.3		0.4		0.8		1.3	
	b	2.04	0.08	1.07	0.07	1.51	0.06	0.0238	0	3.68	0.19
	a	-0.48	1.08	-3.2	0.88	-0.53	0.89	-0.018	0.001	-8.45	0.7
	R^2	0.97		0.93		0.96		0.98		0.85	
HN3	T_b	2.3		4.9		0.7		1.2		0.8	
	b	4.19	0.16	3.22	0.22	2.33	0.13	0.0368	0.002	3.29	0.2
	a	-9.78	2.13	-15.61	2.91	-1.6	0.76	-0.0429	0.018	-5.08	0.64
	R^2	0.97		0.92		0.95		0.97		0.82	
Qinyou No.7	T_b	1.0		4.0		0.1		1.4		1.5	
	b	2.25	0.06	1.24	0.07	1.57	0.09	0.0265	0.001	4.28	0.27
	a	-2.2	0.81	-4.94	0.96	-0.15	0.19	-0.0398	0.012	-10.55	0.97
	R^2	0.98		0.94		0.95		0.98		0.81	
Huayouza No.9	T_b	3.6		4.9		3.7		2.5		2.6	
	b	3.24	0.12	1.92	0.11	2.37	0.06	0.0365	0.001	3.44	0.19
	a	-11.62	1.53	-9.45	1.44	-8.7	0.89	-0.0907	0.019	-6.37	0.62
	R^2	0.98		0.94		0.99		0.97		0.85	
Fengyou 737	T_b	3.9		4.9		3.7		3.2		3.2	
	b	2.98	0.08	1.59	0.07	1.88	0.1	0.034	0.001	2.51	0.14
	a	-11.5	1.12	-7.76	0.99	-7.01	1.31	-0.109	0.018	-3.59	0.46
	R^2	0.98		0.96		0.95		0.97		0.82	
Shengguang 77	T_b	5.0		5.9		4.9		4.6		3.8	
	b	4.36	0.2	2.9	0.22	2.98	0.2	0.0569	0.004	2.16	0.25
	a	-21.67	2.69	-17.02	2.92	-14.5	2.71	-0.262	0.047	-1.83	0.74
	R^2	0.96		0.9		0.92		0.94		0.6	
Fengyou 730	T_b	2.4		3.8		3.1		2.8		2.7	
	b	2.38	0.07	1.18	0.04	1.83	0.09	0.0303	0.001	2.94	0.16
	a	-5.8	0.89	-4.51	0.51	-5.7	1.15	-0.0828	0.015	-5.28	0.56
	R^2	0.98		0.98		0.96		0.98		0.81	

Model 1 to model 4 were based on the linear regression of germination index, germination rate index, percent germination per day and reciprocal time to median germination versus temperature; The base temperatures ($T_{b)}$ were estimated as the x-intercept for the linear regression. Model 5 was based on the regression of NED (normal equivalent deviate) against $\ln$ (degree − days). The description of the regression included y-intercept (a), slope (b) and coefficient of determination (R^2)

Discussion

Temperature is a main factor affecting seed germination of rapeseed. Present study indicated that germination speed of rapeseed was declined as temperature decreased from 23 to 3°C, irrespective of genotypes. However, reduction in germination speed was different in all genotypes (Achary *et al.*, 1983). Under optimal conditions of 18 to 23°C, seed lots of these rapeseed varieties exhibited similar germination rates and short germination time, which was less than three days. It was considered beneficial if time to obtain 80% germination was less than four days (Russo *et al*., 2010). Under low temperature, the cold resistant varieties still maintained a high level of final germination despite extension of the germination process, while final germination of cold sensitive varieties decreased significantly. The diversity of seed germination behavior in response to temperature provided a potential approach for selecting genotypes with improved germination at low temperature (Kondra *et al.*, 1983; Squire *et al.*, 1997). It is encouraging that selection for rapid germination at a constant 10°C temperature resulted in the identification of plants with improved emergence and growth at variable temperature regimes from 7 to 22°C (Achary *et al.*, 1983). The variability of *Brassica* seed germination behavior in response to temperature indicates the potential flexibility of the sowing time of these crops (King *et al.*, 1986; Russo *et al.* 2010). Nykiforuk and Johnson-Flanagan (1993, 1999) showed that the better performance of germination under low temperature was associated with high isocitrate lyase activities and rapid mobilization of total lipid and protein reserves. The genotypes with lower base temperature exhibited a higher respiration rate, indicating its higher metabolic activity under low temperature stress (Leviatov *et al.*, 1994). The failure of seeds to germinate under below base temperature condition mainly attributed to the denaturation of proteins and the preventing the orderly association of proteins into organelles (Simon *et al.*, 1976).

Base temperature is an important parameter in crop development and the selection of an appropriate base temperature is critical to any heat unit model (Alvarado and Bradford, 2002; Orru *et al.*, 2012). Thus, many methods have been provided to estimate the base temperature in a variety of crops and weeds.

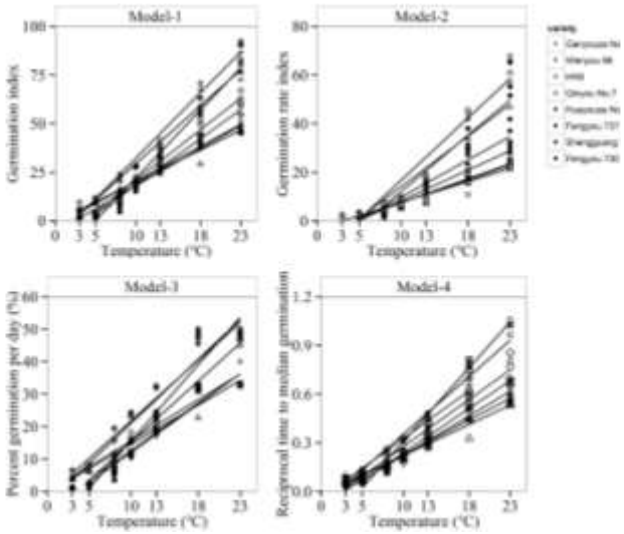

Fig. 3: The linear estimation of base temperatures of eight varieties by using model 1, model 2, model 3, and model 4 (Linear regressions of germination index (*GI*), germination rate index (*GRI*), percent germination per day (*PGD*) and reciprocal time to median germination versus temperature)

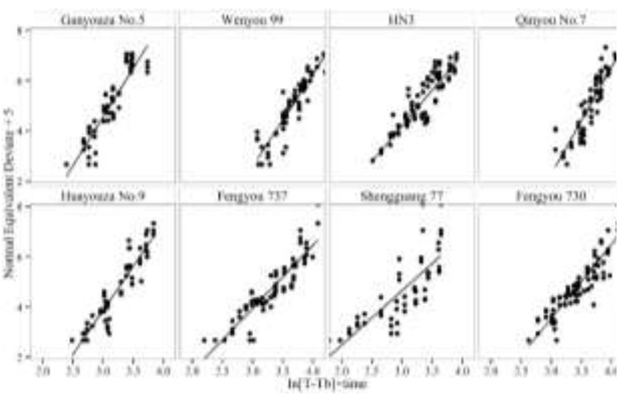

Fig. 4: The linear estimation of base temperatures of eight rapeseed varieties by using model 5 (Repeated probit analysis)

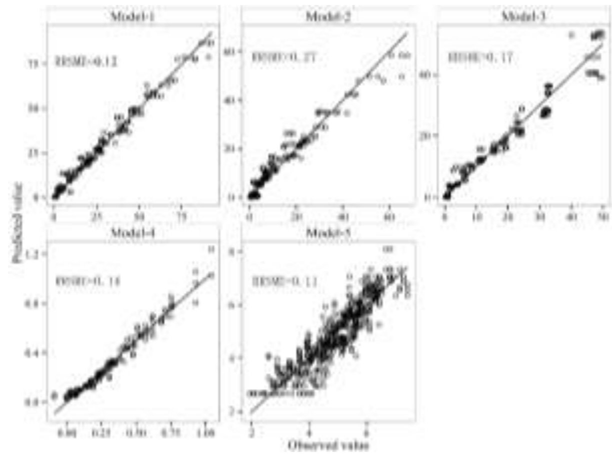

Fig. 5: The scatter plot of observed values and predicted values for the five models. Goodness of model fit was evaluated based on low respective root mean square error (RRMSE). The values for model 1 to model 5 were germination index (*GI*), germination rate index (*GRI*), percent germination per day (*PGD*), reciprocal time to median germination and $NED(i)$

For instance, Yang et al. (1995) obtained an accurate base temperature using four mathematical formulae without a cumbersome calculation process based on the least standard deviation in growing degree days (GDD), the least standard deviation in days, coefficients of variation in GDD, and regression coefficient methods. Most of the models for estimating the base temperatures are based on the linear relationship between germination related index and constant temperatures. Non-linear relationship is also transformed mathematically to linear relationship to simplify the model. Previously, it was reported that base temperature of seedling growth for rapeseed was 3°C (Hu and Ding, 2008; Ya-Jie *et al.*, 2015). In present study, the base temperatures estimated by model 1 to model 5 ranged from 0.2 to 5.0, 2.3 to 5.9°C, 0.1 to 4.9°C, 0.8 to 4.6°C and 0.8 to 3.8°C, respectively, indicating that the base temperatures estimated by these five models differed for any given variety in our experiment. Despite the difference in the estimated *Tb* values of these models, the base temperatures of HN3, Qinyou No.7, Wenyou 99 and Ganyouza No.5 were lower than those of Fengyou 730, Huayouza No.9, Fengyou 737 and Shengguang 77, which is entirely consistent with the cluster analysis result for all varieties.

Our results showed that the repeated probit analysis model was the most suitable for estimating the base temperature of rapeseed germination due to the lowest RRSME, and the mean base temperatures estimated by this model were 1.4°C and 4.1°C for cold resistant and sensitive varieties, respectively. Germination responses across a range of suboptimal temperature and water potential can be described by a general hydrothermal time model that combines the temperature and water potential components (Alvarado and Bradford, 2002; Boddy *et al.*, 2012). Steinmaus *et al*. (2000) found that the reciprocal time to median germination provided the most statistically robust and biologically relevant method for estimating the base temperature of weed species. Of the five models tested in the present study, model 2 using the linear regression of germination rate index against temperature provided the worst prediction for rapeseed with the highest RRSME in our experiment. Holt and Orcutt (1996) found that germination rate index was more suitable for estimating the base temperature of cotton seed germination through comparing the mean percentage per day, reciprocal median response time and two versions of germination rate index, implying that the suitability of a model for calculating the base temperature varies with crops or varieties.

Conclusion

The response of germination process to temperature was diverse among varieties of rapeseed. Under low temperature, the resistant varieties still maintained a high level of final germination despite the extension of germination process, while final germination of sensitive varieties decreased significantly. The repeated probit analysis method was found the most suitable for estimating the rapeseed base temperature due to the lowest RRSME and the average base temperatures for cold-resistant and sensitive varieties were estimated to be1.3 and 4.1ºC by this model, respectively. This study may contribute to the evaluation of different models for estimating base temperatures of rapeseed germination and facilitate the decision of appropriate sowing time and selection of rapeseed varieties.

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