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Modified logistic model to explore the relationship between DNA methylation and meteorological factors during tobacco (*Nicotiana tabacum*) cultivation*

FENG JIA[1], YUNPENG FU[2], HUANJU ZHEN[3], and WEIQUN LIU[4]

Henan Agricultural University, Zhengzhou Henan, China 450 002

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The flue-cured tobacco (*Nicotiana tabacum* L.) is one of the most commercially valued crops (Xiao *et al.* 2007). It is extremely sensitive to the environment changes in the process of growth and development. DNA methylation is a crucial strategy of plants for adapting to environmental changes (Steward *et al*. 2002). In contrast, the environmental stress factors (e g heat, drought) can have an influence on DNA methylation in plant (Finnegan *et al.* 1993). Although mechanisms that the meteorological factors affect DNA methylation are not well understood, there is increasing evidence that these factors are involved in the process of DNA methylation (Finnegan *et al.* 1993). For example, low temperature or cold treatment during growth season can lead to reduce level of DNA methylation of plant (Hashida *et al.* 2006). Drought stress and precipitation can change DNA methylation in the desert plant *Zygophyllum dumosum* Boiss (Granot *et al.* 2009). These results indicate that there is a certain correlation between meteorological factors and DNA methylation.

Logistic regression model can be used in many fields, as a tool for analyzing the relationship between a dependent variable and an independent variable over the last decade (Hosmer and Lemeshow 2002). The logistic model can also provide a set of modified styles that introduce more independent variables in one model. The modified logistic model had been applied to research the growth pattern of tomato successfully (Yang *et al.* 2008). Our previous study showed that the changes of level of DNA methylation are consistent with result of logistic regression model during leaf senescence in tobacco (Jia *et al.* 2010). In addition, the theory of GRA could assess the close or far relationship of biological systems from a lare number of factors in the case of less data. For instance, GRA had been successfully applied to analyze the pesticide pollution management (Jiang and Wan 2009) and the relationship between meteorological factors and chemical components of tobacco (Li and Tang 2008).

Prevoius study showed that meteorological factors could affect the flavour and aroma of tobacco (Pandeya *et al.* 1985). Numerous practices of tobacco production demonstrate that the tobacco of the same species could form different aroma styles in different meteorological areas. Although many previous studies are focused on the relationships between the meteorological factors and chemical contents of tobacco (Wang and Li 2005, Yu *et al.* 2009) or one meteorological factor and DNA methylation (Choi and Sano 2007; Christensen *et al.* 2009, Hashida *et al.* 2006), relatively little is known regarding the relationship between meteorological factors and level of DNA methylation in tobacco. The purpose of this work is to build a modified logistic model for analyzing the relationship between them, and might provide a useful tool for researchers to explore the relationship between one dependent variable and one or more independent variables in the future.

Field experiments were conducted during 2007–09 at Queshan County, Henan Province, China. Tobacco (*Nicotiana tabacum* L., cv. NC89) plants were grown on a medium clay loam with organic carbon content of 16.3 g/kg, *p*H ($CaCl_2$) 6.4, middle level fertility, alkali hydrolysable nitrogen of 80.08 mg /kg, available phosphorus of 6.61 mg/kg, available potassium of 154.03 mg/kg, and crop rotation. The applied fertilizer was tobacco-specific inorganic compound fertilizer (N: P: K=10: 10: 20), in which the nitrate nitrogen content was = 450 mg/g. A row space and planting distance of tobacco were 120 cm and 60 cm, respectively. Other cultivation and management measures were in accordance with the measures of high quality tobacco production and cultivation in Queshan County. Hundred plants of uniform

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*Short note

[1] PhD Scholar (e mail: mrjiafeng@163.com), College of Bsioengineering, Henan University of Technology; [2]Associate Professor (email: yunpengfu@yahoo.com.cn), College of Tobacco Science; [3]Associate Professor (email:zhenhuanju@163.com), Staff Development Institute of China National Tobacco Corporation; [4] Principal Scientist (email: liuweiqun2004@163.com), College of Life Sciences.

development were tagged in one block (20 m×5 m/block, 36 blocks totally) after eight weeks of transplanting. The 18th leaves (from bottom to top leaves) were obtained every 10 days when these leaves expanded about 1 cm. The mesophyll tissues of these leaves were frozen in liquid nitrogen and stored at –80°C for experiments. The levels of total DNA methylation were determined with the method of Jia *et al.* (2010). The meteorological data were offered by Queshan Meteorological Bureau. The data are divided into two major groups. The first group is the mean value of factors from transplanting to sampling, which comprised mean daily temperature (MDT), mean highest temperature (MHT), mean lowest temperature (MLT), mean temperature difference (MTD), mean relative humidity (MRH), and mean sunshine hours (MSH). The second is the sum value of factors, which include total precipitation (TP), total sunshine hours (TSH), ≥5°C accumulated temperature (5AT), ≥10°C accumulated temperature (10AT), ≥15°C accumulated temperature (15 AT), and ≥20°C accumulated temperature (20 AT).

The method of gray relational analysis (GRA) was described by Liu *et al.* (2004). According to GRA, the relationship between DNA methylation and meteorological factors was an ambiguous system. Where $y_0(k)$ stands for the k^{th} level of DNA methylation (a dependent variable), k stands for the number of sampling (k = 1,2,3,4,5), and $x_i(k)$ stands for the k^{th} value of the ith meteorological factors (independent variables). The sequence of the level of DNA methylation is denoted with $y_0 = (y_0(1), y_0(2), \ldots, y_0(k))$. Sequences of the values of meteorological factors are denoted with $x_i = (x_i(1), x_i(2), \ldots, x_i(k))$, where i stands for the ith factor among the twelve meteorological factors as shown in the section *2.3*. The grey relational coefficient between y_0 and x_i at the kth item is defined as follows:

$$\gamma(y0(k),x_1(k)) = \frac{\Delta_{min} + \xi\cdot\Delta_{max}}{\Delta 01(k) + \xi\cdot\Delta_{max}}$$

Where $D_{0l}(k)=|y_0(k)-x_1(k)|$, $\Delta_{min} = \underset{\forall l}{\min}\ \underset{\forall k}{\min}\ \Delta_{01}(k)$

$\gamma(y_0, x_1) = \frac{1}{n}\sum_{k=1}^{n}\gamma(y_0(k),x_1(k))$ is a distinguishable coefficient controlling the resolution between D_{min} and D_{max} [îx¡Ê(0,1)]. It has been proved that the value of î could change the magnitude of GRA, but it can not change the result of ranking. In this study, it was set at 0.5. Aggregating the grey relational coefficient calculated at each item, we can obtain the grey relational grade for an entire sequence as follows: $\gamma(y_0, x_1) = \frac{1}{n}\sum_{k=1}^{n}\gamma(y_0(k),x_1(k))$. Here, γã ($y_0$, x_i) represents the degree of similarity between the sequences x_i and the sequence y_0. The larger the value of ã (y_0, x_i) is, the closer the relationship between x_i and y_0 is.

The modified logistic model is expressed by an equation, namely $Dm = \frac{100}{(1 + e^{53.834-1.653x-0.102y-0.005z+0.016m})}$. Where Dm is the level of DNA methylation, K is the maximum value of DNA methylation. The x, y, z, and m are four independent variables (meteorological factors) in this model. The a, b, c, d, and e are five parameters of logistic model, in which one (a) is constant and the others (b, c, d, and e) are coefficients of independent variables.

MHT, MAT, TP, and TSH are four factors that are the most closely related with DNA methylation using GRA (Table 1). The top two of them MHT and MAT that have autocorrelation with each other could not meet the requirement of the factors that should be independent in logistic model. Factors following them are not suitable for the requirement of model until MRH. In the light of GRA and the requirement of logistic model, MHT, TP, TSH, and MRH were selected to construct the modified logistic model and stand for a temperature factor, a precipitation factor, a sunshine factor, and a humidity factor, respectively.

The four independent variables of x, y, z, and m in modified logistic model are denoted by MHT, TSH, MRH, and TP respectively. This model is solved by five observation values of four meteorological factors and level of DNA methylation during tobacco cultivation. As a result, the modified logistic model is $Dm = \frac{100}{(1 + e^{53.834-1.653x-0.102y-0.005z+0.016m})}$. The coefficient of determination (R) of the model was 0.913, indicating a good fit. The results of the partial correlation coefficient test show they are significant above 95% confidence level. On the basis of standard partial regression coefficient, the order of four factors affecting DNA methylation is MHT (1.653)>TSH (0.102)>TP (0.016)>MRH (0.005). This result shows that MHT (temperature) and TSH (sunshine time) have greater influence on DNA methylation and should be valued in cultivation and management practices. The result of Durbin-Watson test (d=2.38) illuminates that there is negligible self-correlation among the four meteorological factors, which it can satisfy the requirement of the model.

Table 1 Results based on the relation between meteorological factors and DNA methylation using GRA

Meteorological factor	Correlation degree γ	Weight (%)	Rank
MHT	0.926	10.0	1
MAT	0.885	9.6	2
TP	0.855	9.3	3
TSH	0.843	9.1	4
MLT	0.832	9.0	5
20 AT	0.788	8.5	6
5 AT	0.788	8.5	7
10 AT	0.788	8.5	8
15 AT	0.788	8.5	9
MRH	0.772	8.4	10
MSH	0.492	5.3	11
ATD	0.464	5.0	12

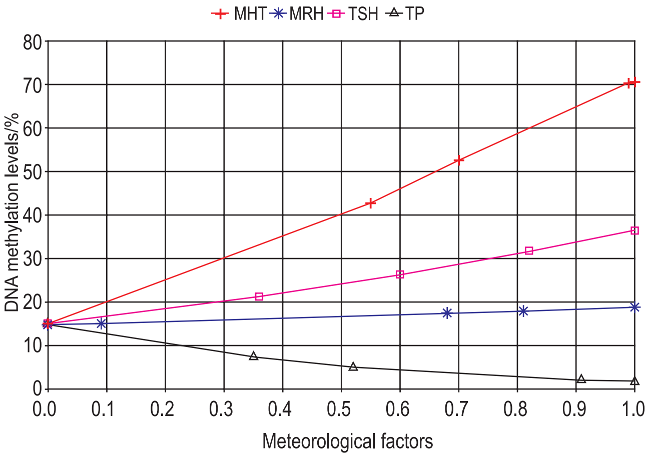

Fig 1 Response chart of one factor affecting DNA methylation in logistic model, while the other factors were the level of the first sampling. The values of vertical axis denote level of DNA methylation, and the values of the horizontal axis show meteorological factors with normalized. MHT, mean highest temperature; MRH, mean relative humidity; TSH, total sunshine hours; TP, total precipitation.

The results of single meteorological factor analyses (Fig 1) indicate that levels of DNA methylation are changed by each of four meteorological factors. The level of DNA methylation could be increased 4- and 2-fold by MHT and TSH respectively, while MRH could affect level of DNA methylation slightly. By contrast, there is a negative effect between TP and DNA methylation. TP has a greater influence on DNA methylation than MRH, because it has larger values.

The effects of two-interaction factors on DNA methylation are not only possible simple additive effects, but also possible synergistic or antagonistic effects (Fig 2). It is observed from Fig 2A that higher temperature make level of DNA methylation increase rapidly at the same humidity, and higher humidity could increase level of DNA methylation subtly at the same temperature. Therefore, the effect of MHT and MRH on DNA methylation has a synergistic effect. The effect of TSH and MHT interactions (Fig 2B) is similar to, however, greater than the effect of MRH and MHT on DNA methylation. Moreover, the effect of TSH and MRH interactions (Fig 2C) has also a small synergistic effect on DNA methylation. In contrast, Fig 2D show that abundant rainfall makes level of DNA methylation decrease rapidly at the same temperature, and higher temperature increase level of DNA methylation rapidly at the same rainfall. The effect of TP and MHT on level of DNA methylation has an antagonistic effect to a certain extent. Finally, the effects of two-interaction factors of TP and TSH or MRH on level of DNA methylation (Fig 2E, 2F) also have antagonistic effects, which are similar to the effect of TP and MHT on level of DNA methylation.

This paper is the first time describing the quantitative relationship between the meteorological factors and DNA methylation using the modified logistic model. Although logistic regression model has used in many fields to analyze the relationship between a dependent variable and one or more independent variables (Hosmer and Lemeshow 2002), there are few studies to analyze the relationship between meteorological factors and DNA methylation with the logistic regression model. Certainly, many previous studies are focused on the relationship either between meteorological factors and chemical contents of tobacco (Wang and Li 2005, Yu *et al.* 2009) or between one meteorological factor and DNA methylation (Choi and Sano 2007, Christensen *et al.* 2009, Hashida *et al.* 2006). In our study, we have introduced five parameters (*a, b, c, d*, and *e*) in model, in which one is constant and the others are coefficients of independent variables. Moreover, these factors could completely and really reflect local meteorological environment during plant growth period.

In order to analyze relationship between these factors and DNA methylation, we hypothesize that antagonistic factors that may interfere with each other or have competitive inhibition might share the same pathways to regulate DNA methylation, whereas synergistic factors might have different pathways to regulate DNA methylation. In accordance with this hypothesis, synergistic or antagonistic effects of the two among four meteorological factors on DNA methylation are shown in Fig 3. Therefore, we hypothesized that four meteorological factors may affect DNA methylation by four different pathways. Furthermore, it is attractive to advance a model of DNA methylation pathways regulated by the meteorological factors (Fig 3). Previous investigations on *E. coli.* demonstrated that a methylation blocking factor (MBF) could form complexes with DNA and then prevent DNA methylation at low temperature, while MBF would separate from DNA and then increase the level of DNA methylation at high temperature (White-Ziegler *et al.* 2002). Therefore, we inferred that temperature might have a key role in the pathway of affecting DNA methylation.

On the one hand, another early work showed that desert plants, from wet to dry season, regulated gene expression using the post-transcriptional modification of histone H3 N-terminal tail (Granot *et al.* 2009). On the other hand, many studies demonstrated that histone modification may be coupled with DNA methylation (Jackson *et al.* 2002, Malagnac *et al.* 2002, Tamaru and Selker 2001). Taken together, we suggested that rainfall might regulate DNA methylation by modifications of histone (Fig 3).

The pathways of TSH (light) and MRH (humidity) have not been enunciated clearly so far. However, results of our study indicated that both TSH and MRH could affect DNA methylation, but their pathways of affecting DNA methylation may be different from that of the formers (MHT and TP). Although UV light treatment could change the dynamics of the genome to enhance the rate of somatic homologous

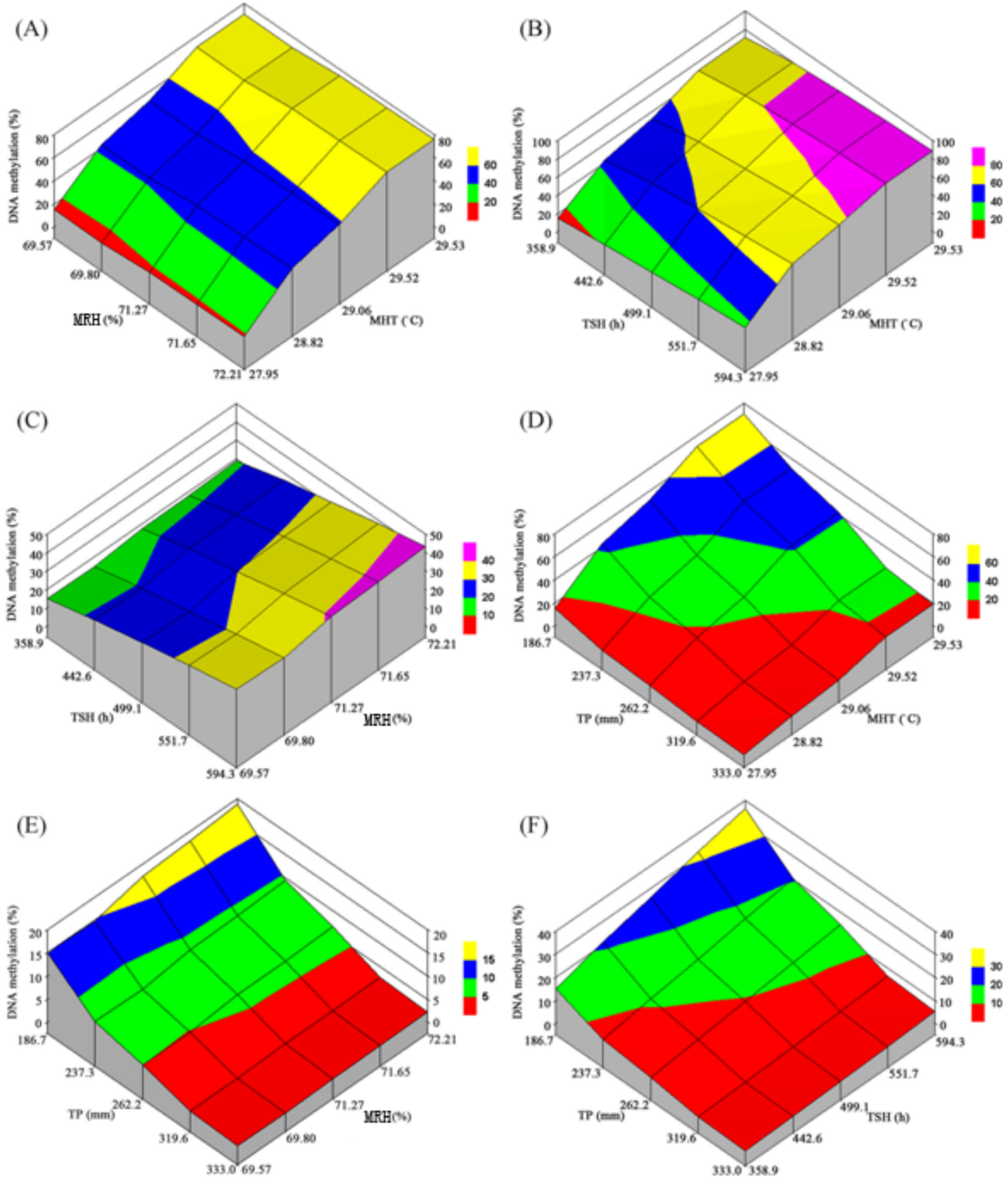

Fig 2 Three dimension (3D) graphs of two-interaction factors affecting DNA methylation with the logistic model
(A) a synergistic effect of MHT and MRH on level of DNA methylation; *(B)* a synergistic effect of MHT and TSH on level of DNA methylation; *(C)* a little synergistic effect of MRH and TSH on level of DNA methylation; *(D)* an antagonistic effect of TP and MHT on level of DNA methylation; *(E)* an antagonistic effect of TP and MRH on level of DNA methylation; *(F)* an antagonistic effect of TP and TSH on level of DNA methylation

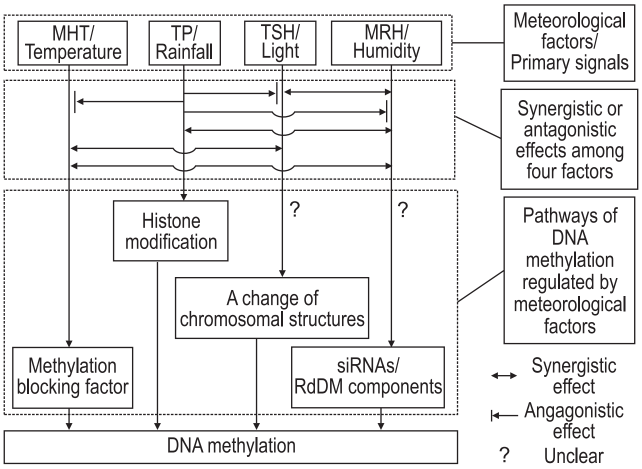

Fig 3 The pathways model of DNA methylation regulated by meteorological factors (Chinnusamy and Zhu 2009; Granot *et al.* 2009; Pan *et al.* 2009; White-Ziegler *et al.* 2002). Arrows indicate synergistic effects on DNA methylation and bars indicate antagonistic effects on DNA methylation.

recombination (Molinier *et al.* 2006), there is not satisfactory evidence to support that light intensity is relevant to DNA methylation. Taken together, we think TSH might affect DNA methylation in the way of changing chromosomal structures of plants. In addition, another study showed that small interfering RNAs (siRNAs) are involved in the methylation of at least one-third of methylated loci (Lister *et al.* 2008). We guess that MRH might be relevant to the RNA-directed DNA methylation (RdDM) or small RNAs. In short, we came up with a four-pathway model of meteorological factors affecting DNA methylation (Fig 3).

SUMMARY

The meteorological factors play a critical role in the process of growth and development of plant. These factors can change DNA methylation for adapting to environmental changes, but the mechanisms are not clear. Here four major meteorological factors, mean highest temperature (MHT), total sunshine hours (TSH), mean relative humidity (MRH) and total precipitation (TP), are selected to construct the modified logistic model by the grey relational analysis (GRA). The model is to depict the quantitative relationship between DNA methylation and these factors in tobacco. The result of model is $Dm = \frac{100}{(1+e^{53.834-1.653x-0.102y-0.005z+0.016m})}$. The coefficient of determination (R) of the model is 0.913, and the model passed the partial correlation coefficient test and Durbin-Watson test. The results of single factor analysis show that there are positive effects between DNA methylation and MHT, TSH, or MRH, respectively. The order of the factors of affecting DNA methylation is MHT > TSH > MRH. However, TP has a negative effect on DNA methylation. Furthermore, interactions of two factors indicate that there are synergistic effects between MHT and MRH, MHT and TSH, and MRH and TSH. Moreover, there are antagonistic effects between TP and MHT, TP and TSH, and TP and MRH, respectively. According to these results, a four-pathway model of the meteorological factors affecting DNA methylation is suggested.

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