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The Correlation between Maturity, Plant Architecture and Yield Related Traits of Soybean Cultivars in Northern China

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ABSTRACT

Background: The traits of the time to flowering and maturity and other important agronomic traits, *e.g.*, plant height, the numbers of nodes and branches are key factors for the adaptability of soybean cultivars to a certain photoperiodic length and ecological environment. Correlation analysis of these traits will facilitate fine-tuning of phenotyping procedure for each trait.

Methods: In this field investigation during 2017-2020 at two experimental stations in Heilongjiang Province, northern China, a total of 1133 cultivars or accessions were phenotyped for traits related to maturity, architecture, *e.g.* plant height, the numbers of nodes and branches and yield. Statistical analysis was performed to reveal the correlation between different traits, years and locations.

Result: R1 (days to the first flowering stage) and R2 (days to the fully flowering stage) are strongly stable traits among different years and different locations in this study. Significant correlations were identified between maturity trait, plant height, the numbers of nodes and branches. However, the traits of the height of the first effective node and the pod number per plant demonstrated some weak correlation among different years or with these maturity traits. Therefore, phenotyping procedures in this study can be directly applicable for accurate evaluation of maturity and plant architecture-related traits *e.g.* plant height, the numbers of nodes and branches.

Key words: Agronomic trait, Correlation analysis, Maturity, Phenotyping, Soybean.

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.] is a typical short-day crop. The time to flowering and maturity are ecologically and agronomically important traits for breeders to judge a soybean cultivar's adaptability to latitudinal region. Researchers have cloned numerous major flowering time-related genes, *e.g.*, *E1* to *E4*, *J* and *QNE1* through positional cloning and Genome-wide association study (GWAS) (Liu *et al*., 2008; Watanabe *et al*., 2009; Watanabe *et al*., 2011; Xia *et al*., 2012; Lu *et al*., 2017; Xia *et al*., 2022). The stem growth habits, *i.e.*, indeterminate, determinate, or semi-determinate, in soybeans generally shape plant architecture (Liu *et al*., 2010). These growth habit types are largely controlled by the *Dt1* and *Dt2* genes (Zhang *et al*., 2019). However, more minor effect genes might also affect the time to flowering, maturity and other agronomically important traits, *e.g.*, plant height, branch and pod number per plant. As more genes regulating the above traits have been cloned, more accurate phenotypic data are needed for functional studies as well as for the cloning of minor genes by removing the large effects of known master genes.

The classification of the different developmental stages was mainly developed by Fehr *et al*. (1971). Up to date, quantitative trait locus (QTL) or genome-wide association study (GWAS) analyses on natural or various genetic populations have been well documented and many QTLs or candidate loci have been mapped, some of which were cloned (Sayama *et al*., 2010; Xia *et al*., 2021). Analyses among maturity, plant architecture and yield-related traits of some cultivars or accessions have been reported to estimate the internal genetic relationship and response to environmental fluctuations (Kumar *et al*., 2015; Srivastava *et al*., 1976). Currently, the northern provinces are the main soybean production area in China. Accurate phenotyping of flowering time, maturity, plant architecture and yield-related traits can benefit soybean production through the latitudinal arrangement of soybean cultivars. In this study, we performed correlation analysis among traits of maturity, plant architecture (plant height, the numbers of nodes and branches, the height of the first effective node) and yield-related traits (the pod number per plant) from 2017 to 2020 at two geographic locations in the northern China towards the fine-tuning phenotyping procedure for agronomically important traits.

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MATERIALS AND METHODS

The cultivars or accessions were mainly obtained from the Gene Resource Center of Chinese Academy of Agricultural Sciences, China (Qiu *et al*., 2013). A total of 1133 different cultivars or accessions (Table 1) were phenotyped at Hailun experimental station (47°47′N, 126°97′E) (years of 2017 to 2020) and Harbin experimental station (45°61′N, 126°74′E) (year of 2017) of the Northeast Institute of Geography and Agroecology, Heilongjiang Province, China. The soil was derived from sedimentary materials of loamy loess and classified as Pachic Haploborolls with a texture lass of silty clay (Hao *et al*., 2023).

Plots were 5-m long with 0.6-m row spaces. Plants were generally 20 cm apart. Each cultivar or accession was arranged in a row and at least 15 plants per cultivar or accession were grown. Seeds were generally sown into the field around May 5th-10th. We followed standard local agricultural practices to control insects and weeds. Flowering time (R1) of each plant is defined as days from emergence (DAE) to one open flower at any node on the main stem (Fehr *et al*., 1971). Also in full bloom (R2) stands DAE to one open flower at one of the two uppermost nodes on the main stem with a fully developed trifoliate leaf node. R6 represents one pod containing a green seed that fills the pod cavity at one of the four uppermost nodes on the main stem with a fully developed trifoliate leaf node. Beginning maturity (R7) stands for DAE to one normal pod on the main stem that has reached its mature pod color (normally brown or tan), while full maturity (R8) is defined as DAE to 95 per cent of the pods that have reached their mature pod color.

For a given cultivar, each specific maturity stage is defined only when at least 50% of individual plants reach that stage. Generally, 10 plants per cultivar or accession were harvested individually to measure or account for plant height (PH), branch number (BN), node number on the main stem and height from the ground to the first pod located on the main stem (height of the first effective node), as well as pod number per plant (Sayama *et al*., 2010; Zhai *et al*., 2014).

The correlation analyses were performed using GraphPad Prism Version 5.0 for Windows, GraphPad Software (San Diego California USA, www.graphpad.com). All correlation plots were generated using 'ggplot2' package (Ginestet, 2011) in R. The heatmap was generated based on the matrix of correlation coefficients for all traits using TBtools II v2.096 with dist method of Euclidean, Cluster method of Complete and Branch Form of Cladogram (Chen *et al*., 2023).

RESULTS AND DISCUSSION

The total cultivars or accessions investigated from 2017 to 2020 in Harbin and Hailun locations in this study is shown in Table 1.

Correlations among R1, R2, R6, R7 and R8 within the same year

At first, R1 and R2 were highly correlated in all years and locations at p <0.001(Fig 1, Fig 2A). In Hailun (HL) location, the correlation coefficients (r) were 0.9795***, 0.9867***, 0.9759*** and 0.9869*** for the years of 2017, 2018, 2019 and 2020, respectively (Fig 1). The correlations between R1 and R6 or R7 or R8 were also statistically significant at p<0.001, although not as higher as that between R1 and R2. In 2018, the r values were 0.5815*** between R1 and R6; 0.5630*** between R1 and R7; and 0.5581*** between R1 and R8 (Fig 2 B-D). Similarly, in 2019, the r values were 0.7141*** between R1 and R6; 0.6914*** between R1 and R7; and 0.6649*** between R1 and R8 (Fig 2 F-H). A similar trend was observed in 2020 (Fig 2 J-L). The r values in 2018 between R2 and R6 (Fig 1) or R7 or R8 were 0.6679***, 0.6582*** and 0.6440***, respectively. In 2019, the r values between R2 and R6 (Fig 1) or R7 and R8 were 0.7820***, 0.7587*** and 0.7308***, respectively. Similar correlations were observed in 2020 in Hailun (Fig 1). At the late maturity stage, R6 was highly correlated with R7 or R8 with the r values of 0.8493*** and 0.8150*** in 2018; 0.8875*** and 0.8320*** in 2019; and 0.9883*** and 0.9679*** (Fig 1) in 2020. A very high correlation was observed between R7 and R8 in all years, with the r values of 0.9264*** in 2018, 0.9288*** in 2019 and 0.9840*** in 2020. Also in Harbin, the r values between R1 and R2 were 0.8843*** in 2017; the r values between R1 and R6 or R7 or R8 were 0.7974***, 0.6885*** and 0.5088***, respectively. Furthermore, the r values between R6 and R7 or R8 were 0.8644*** and 0.8141***, respectively.

The correlations of the same R1, R2, R6, R7, R8 between different years in Hailun

The correlations of DAE to the same maturity stages between 2017 and 2018 or 2019 and 2020 were shown in Hailun (Fig 1). At the early reproductive stage, the r values were 0.9377*** for R1 between 2018 and 2019 and 0.9672***for R1 between 2018 and 2020. The r value of 0.9500*** was for R2 between 2018 and 2019 and 0.9683*** for R2 between 2018 and 2020 (Fig 1). Meanwhile, at the late reproductive stage, the r values of 0.6560*** and 0.5801*** were for R6 between 2018 and 2019 (Fig 1) and 0.5801*** for R6 between 2018 and 2020. Similarly, the r value of 0.6365*** was for R7 between 2018 and 2019 and 0.5902*** for R7 between 2018 and 2020. Furthermore, the r value of 0.5932*** was R8 between 2018 and 2019 and 0.5729*** for R8 between 2018 and 2020 (Fig 1). Apparently, R2 was a consistently stable trait among different years.

Table 1: The number of cultivars or accessions were phenotyped in this study.

Years	Hailun	Harbin
2017	789	756
2018	1122	-
2019	771	-
2020	676	-

The correlations of R1, R2, R6, R7, R8 between two latitudinal locations

The phenotypic data of flowering time and maturity between Hailun and Harbin in 2017 were analyzed. The r values of 0.8204***, 0.9327***, 0.7697***, 0.6130*** and 0.6205*** were respectively for R1, R2, R6, R7 and R8 between Hailun and Harbin in 2017 (Fig 1). Apparently, the R2 was the most stable trait between the two locations.

Correlation among agronomic traits and maturity traits Plant height

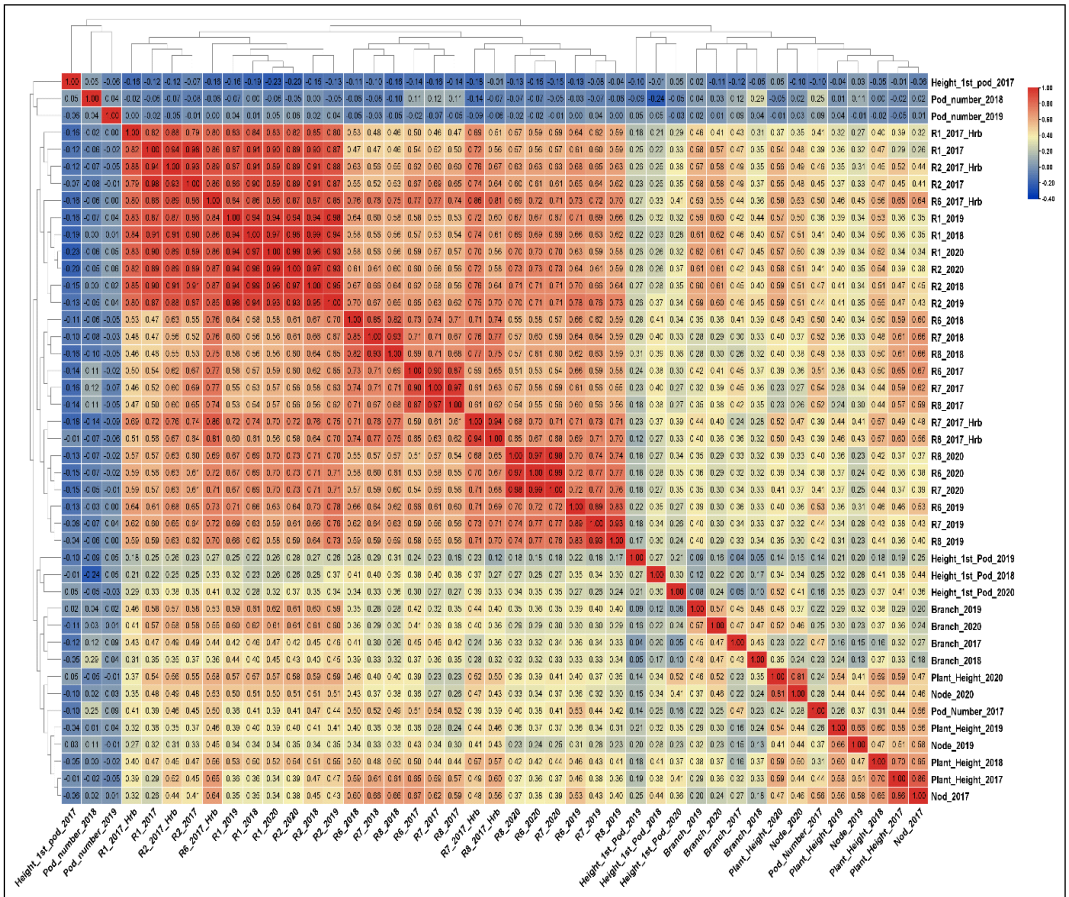
The traits of plant height (PH) were highly correlated between different years. The r values of 0.6981*** were for PH between 2017 and 2018, 0.5994*** between 2018 and 2019 and 0.5875*** between 2018 and 2020. Also, PH was significantly correlated to R1, R2, R6 and R8. The r values between PH and R1, R2, R6, R7, or R8 were 0.5019***, 0.5109***, 0.5017***, 0.4787*** and 0.4956***, respectively (Fig 1; Fig 3A-D) in 2018 at Hailun. Similarly, r values between PH and R1, R2, R6, R7, or R8 were 0.3876***, 0.4066***, 0.3626***, 0.3370*** and 0.3083***, respectively, in 2019 at Hailun (Fig 1; Fig 3E-H). A similar correlation trend was observed in 2020 at Hailun.

Node numbers

The trait of node number was correlated with different years. The r value of 0.5825*** was for node number between 2017 and 2019, 0.4630*** between 2017 and 2020 and 0.4418*** between 2019 and 2020. The r between node number and time for different reproductive stages (R1, R2, R6, R7 and R8) was statistically significantly correlated with a range from 0.2333*** to 0.5882*** (Fig 1). The node number showed a higher correlation with plant height at r = 0.8573*** in 2017, 0.6618*** in 2019 and 0.8121*** in 2020 (Fig 1).

Branch number (BN)

Based on the phenotypic data of BN in 2017, 2018, 2019 and 2020, the r value between branch numbers among different years was statistically significant, with a range from 0.4512*** (2019 vs. 2017) to 0.5727*** (2019 vs. 2020). Also, the trait of branch number was statistically significantly correlated with the R1, R2, R6 and R8. In 2018, the r values between BN and R1, R2, R6, R7, or R8 were 0.4001***, 0.3958***, 0.3875***, 0.3254*** and 0.3159***, respectively (Fig 1). In 2019, the corresponding r values were 0.5862***, 0.5891***, 0.3931***, 0.4023*** and 0.3991***, respectively (Fig 1). Furthermore, a similar correlation trend was



The heatmap was generated using TBtools II v2.096. The number in each cell represents the correlation coefficient (r).

Fig 1: The correlation heatmap of all traits in soybean.

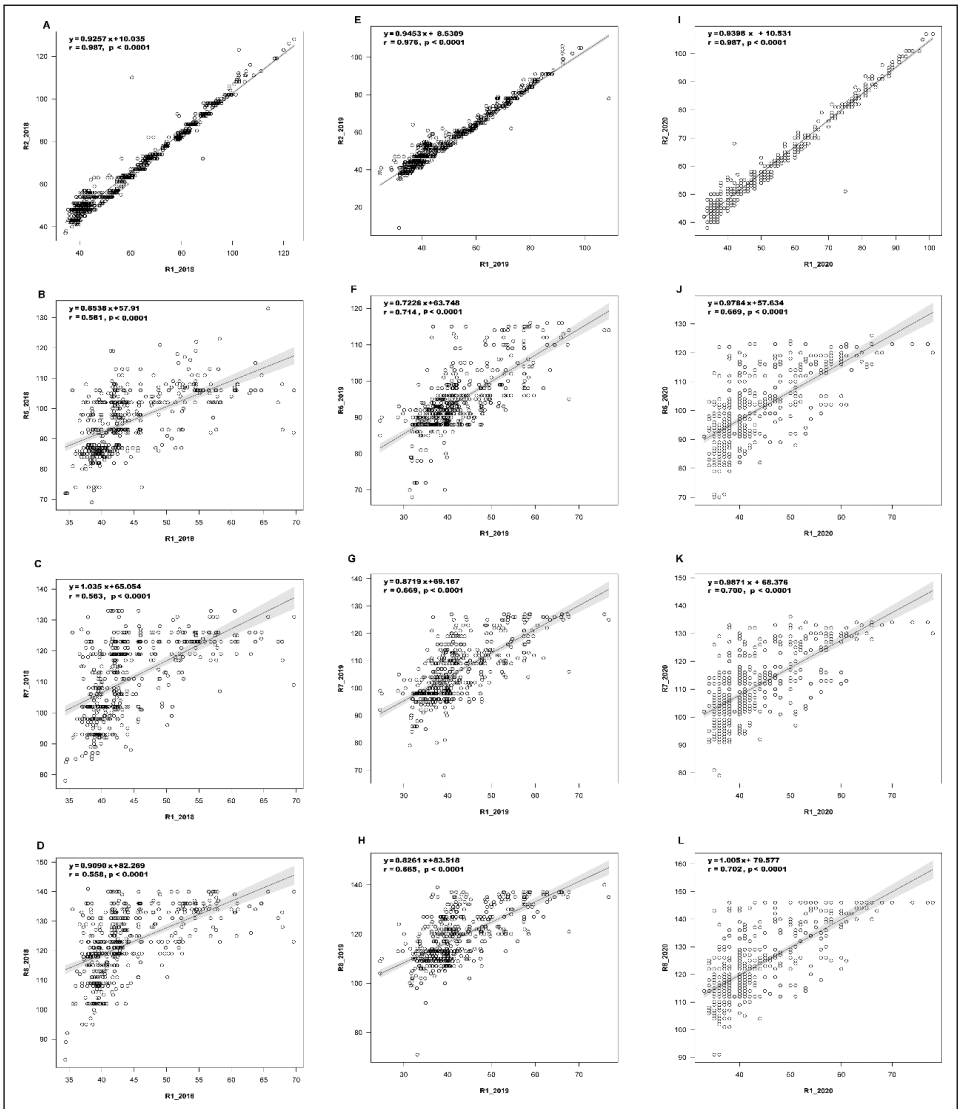
observed in 2020 at Hailun (Fig 1). The trait of BN was significantly correlated with plant height, with r = 0.3704*** in 2018, 0.2899*** in 2019 and 0.5244*** in 2020. Also, BN was significantly correlated with the node number, with the r values of 0.3204***in 2019 and 0.4560*** in 2020.

Height of the 1st effective node

The trait of height of the first effective node was statistically significant between different years, with the r values of 0.2745*** between 2018 and 2019, 0.2959*** between 2018 and 2020 and 0.2109*** between 2019 and 2020. Also, the trait of height of the first effective node was significantly correlated to R1, R2, R6 and R8 (Fig 1). At Hailun in 2018, the r values between the height of the 1st effective node and R1, R2, R6, R7, or R8 were 0.2327***, 0.2831***, 0.4083***, 0.4022*** and 0.3909***, respectively (Fig 1). Correspondingly, the r values were 0.2518***, 0.2610***, 0.2246***, 0.1847*** and 0.1672***, respectively, in 2019 at Hailun. Furthermore, a similar correlation trend was observed in 2020 at Hailun, with a range of r values from 0.3194*** to 0.3664*** (Fig 1). In 2020, the first effective node was significantly correlated with plant height at the value of 0.5196***, with the node number at r = 0.41169*** and with branch number at r = 0.2384*** (Fig 1).

Pod numbers per plant

This yield-related trait, pod number per plant, in 2017 was positively correlated with R1, R2, R6, R7, or R8 with the



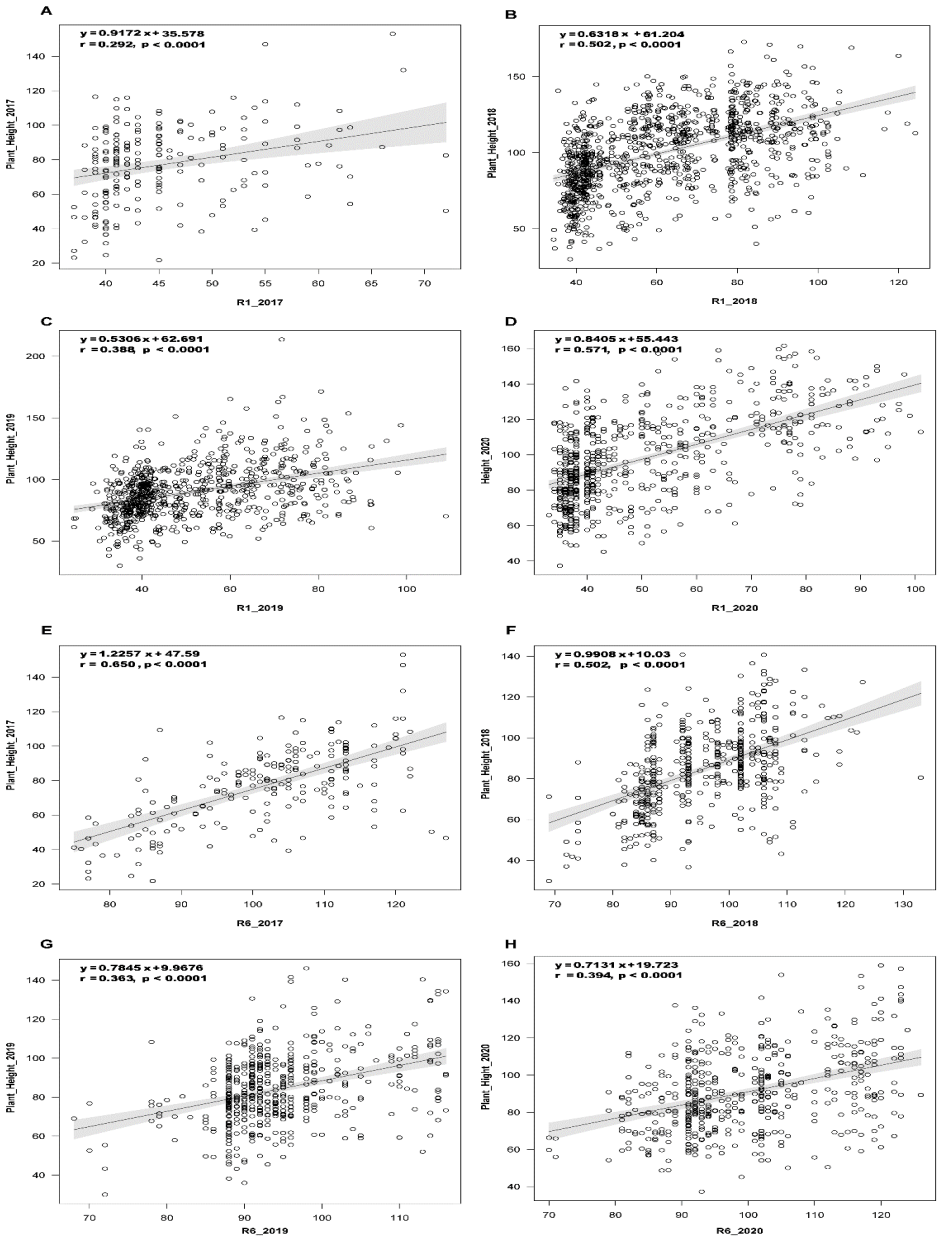
A-D correlation in 2018. A: between R1 and R2. B: between R1 and R6. C: between R1 and R7. D: between R1 and R8. E-H correlation in 2019. E, between R1 and R2. F: between R1 and R6. G: between R1 and R7. H: between R1 and R8. I-L correlation in 2020. I: between R1 and R2. J: between R1 and R6. K: between R1 and R7. L: between R1 and R8.

Fig 2: Correlation among the times for different reproductive stages within the same year in Hailun from 2018 to 2020.

r values of 0.3913***, 0.4526***, 0.5075***, 0.5395*** and 0.5179***, respectively. Also, this trait was significantly related to plant height at r = 0.4422***, nod number at r = 0.5615*** and branch number at r = 0.4740*** (Fig 1). Surprisingly, this trait was not or marginally correlated with flowering time and maturity traits in 2018 and 2019, with a range of r values from -0.0970 (R8 in 2018, p = 0.0297) to 0.0436 (R1 in 2019, p = 0.3702***) (Fig 1). In 2018, the pod number per plant was not significantly correlated with plant height (r = 0.0029) but significantly correlated with branch number (r = 0.2905***) and significantly negatively correlated with the height of the first effective node (r = -0.2355***). However, in 2019, the pod number per plant was not significantly correlated with either plant height (r = 0.0410), or branch number (r = 0.0167), or height of the first effective node (r = 0.0497), or node number (r = -0.0069) (Fig 1).

The overall correlation among all traits

From the heatmap of correlation coefficients matrix, all maturity related traits were highly correlated and classified



A-D: Correlation between plant height and R1. A: in 2017. B: in 2018, C: in 2019 and D: in 2020.
E-H: Correlation between plant height and R6. E: in 2017. F: in 2018, G: in 2019 and H: in 2020.

Fig 3: Correlation between plant height and the time of different reproductive stages in Hailun from 2017 to 2020.

into a group, especially for the traits of R1 and R2 (Fig 1). The three architecture traits, *e.g.* branch number, plant height and node, were not only correlated each other, but also significantly related with maturity traits. Furthermore, the height of 1st effective nodes and pod number per plant were least or inconsistently correlated to above maturity and architecture traits (Fig 1).

The domestication of soybean occurred in China about 5000 years ago and there are a large number of germplasms, including landrace and modern cultivars, in China (Qiu *et al*., 2013). The characterization of the effects of different planting dates on flowering time and maturity in soybeans and other plant species triggered the discovery of photoperiodism (Garner and Allard, 1920). These reproductive-related traits are crucial for soybean production, breeding, soybean functional studies on gene regulatory networks controlling maturity and other related agronomic traits, *e.g.*, plant height and node numbers (Goyal *et al*., 2015). As progress is made on the various omics and accomplishments of the T2T genomes of many soybean cultivars (Zhang *et al*., 2023), accurate phenotypic data will greatly facilitate the cloning of new or minor genes as well as functional study. Currently, the main soybean production area is located in the northern China with higher latitude. Accurate phenotyping of flowering time, maturity and important agronomic traits will enable us to judge the ecological or latitudinal adaptation and yield potential of a given cultivar or accession (Ige *et al*., 2021).

In this study, Fehr's classification of reproductive stage was basically used for the phenotyping of traits of flowering time and maturity (Fehr *et al*., 1971). Consistent correlation results among different reproductive stages, different years and different locations indicated that the phenotyping of these traits is accurate and repeatable, reflecting the ecological or latitudinal adaptation of these accessions or cultivars. Especially, the R2 was the most stable trait based on the correlation analysis. Higher correlations were observed between R1 and R2, or between R6 and R8, but moderate correlations were revealed between R1 or R2 and R6 or R7 or R8. This result is in coincidence with many reports that, although many maturity genes can control flowering and maturity, some of them may mainly function in the late reproductive stages, *e.g. Gmfulb* might function most on the maturity time and reproductive length other than flowering time (R1) (Kumar *et al*., 2015; Escamilla *et al*., 2024).

As to the three architecture traits, branch number, plant height and node, were classified into a group, which showing a significant correlation each other, as well as a higher correlation with maturity traits (Fig 1). This relationship between these traits disclosed in this study is consistent with previous reports (Jain *et al*., 2018; Sayama *et al*., 2010). Variations in genes underlying photoperiod sensitivity and growth habit can have pleiotropic effects on other agronomic characteristics other than the major effects on flowering time and maturity (Cober *et al*., 2000).

The trait of the height of the first effective node approximately corresponds to the height of bottom pods (Cober *et al*., 2010). In this study, the correlation of this trait were significantly correlated with maturity traits, however, some inconsistences were shown with the same trait between different years or the correlation between this trait with maturity traits in some years. This phenomenon might be ascribed to the influence of the environmental changes in temperature, planting density, flooding and agricultural practices, *e.g.*, intertilling. At the molecular level, the interaction between *E1*, *DT1* and other genes, *e.g.*, *GmHY2a*, can determine the height of bottom pods (Cober and Tanner, 1995; Zhang *et al*., 2022). In the past, harvesting losses could be greatly resulted from low bottom pods (Curtis *et al*., 2000). The "high-bottom pods" might not be so demanding in that resent technical advances have been made in the harvesting machine to harvest soybean cultivars having low-bottom pods. Similarly, the yield-related trait, the pod number per plant, showed some consistent correlation with maturity, plant height and node number in some years, but not all the years. Yield-related traits are quantitatively inherited and easily subject to the influence of environmental changes, *e.g.* drought, flooding and agricultural practices such as intertilling, irrigation and the application of herbicides and fertilizer.

CONCLUSION

The maturity traits were classified into one group showing consistent correlation between different years and different locations in this study. The architecture traits, plant height, the numbers of nodes and branches displayed significant correlation within traits, also showed significantly correlated with the maturity traits. However, traits of the height of the first effective node and the pod number per plant showed consistent correlation within the same trait in some years, but some inconsistency also occurred. Accurate phenotyping of the yield-related traits or other environmental-sensitive traits, such as the height of the first effective node, needs a relatively large scale to get rid of the marginal effects and/or controlled density or agricultural management, *e.g.*, intertilling. Although the phenomics of soybeans have emerged, accurate traditional phenotyping is still demanding in soybean production, breeding and functional studies since it can be serve as a standard reference for calibrating automation systems.

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Conflict of interest

All authors declare that they no conflict of interest.

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