

Leaf phenology influence the growth of rubber seedlings

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ABSTRACT: Rubber seedling rootstock cultivation is the first step of rubber budding propagation. However, there is still a lack of systematic studies investigating leaf phenology effect on rubber seedling nursery. In this study, leaf phenology, stem diameter, plant height and the chlorophyll parameters were observed at six periods. The results showed that bronze stage was the rapid growth and regulation stage of plant height, bronze stage I was the rapid growth and regulation stage of stem diameter, and light green leaf stage was sensitive to environmental temperature. Taken together, leaf phenology affects the growth of rubber seedlings, and understanding phenological effects helps to regulate the phenological process to raise rootstock seedlings.

KEYWORDS: *Hevea brasiliensis*, growth index, leaf phenology, seedlings.

1 INTRODUCTION

Rubber propagation in China is mainly by the bud-grafting method and this technique requires an appropriately sized rootstock. Rootstock size affects rubber latex yield [1], rubber budding growth potential [2] and start time of rubber bud grafting [3]. The speed of rootstock growth is related to the change of phenological process. Leaf phenology is related to bud-grafting survival rate, disease control, and rubber tapping [4]. Ambient temperature [5], light [6-7], water and nutrients [8-9] affect the phenological process. At various developmental stages of leaf phenology, there are fluctuations in leaf color and leaf shape, accompanied with the changes of leaf SPAD [10], leaf nutrients [11], leaf photosynthesis [12], the laticifer number [13], leaf protein expression [14] and leaf gene expression [15]. However, the detailed changes of rubber seedling growth index was still not clear, which make it confused to precisely regulate the propagation process. Here we investigated growth index of seedlings at different leaf phenology to further extend current knowledge of rubber rootstock seedling cultivation.

2 PLANT MATERIALS AND TREATMENTS

The experiment was conducted from November 2019 to October 2020 at the nursery base of natural rubber of Rubber Research Institute of Chinese Academy of Tropical Agricultural Sciences, Danzhou City, Hainan Province, China. Clone GT1 seeds were sown in sand bed for germination and about 20-25 days later the GT1 seedlings were transplanted in polybags (8cm width*33cm length) for seedling nursery. Leaf phenology was investigated from July 7 to September 30, which was best growth period for rubber seedling with 9mm average rainfall daily and good temperature (24.6°C-32.6°C). At different leaf phenology of new leaf whorl (Figure1), stem diameter was measured with vernier caliper (0.01 mm) and plant height were recorded with plastic ruler (0.1 mm). A Portable Chlorophyll Meter (CY-YL04, China) was used for detecting chlorophyll content (SPAD value), nitrogen content, leaf temperature and leaf humidity of plants during 9: 30-10: 30. Each leaf phenology of new leaf whorl 100 plants and 30 plants were investigated for the growth index (stem diameter and plant height) and for the chlorophyll parameters, respectively. Correlation heatmap analysis among the growth index and the chlorophyll parameters was evaluated on Tutools platform (<http://www.cloudtutu.com>), a free online data analysis website. Statistical analyses were performed with data processing system (DPS) statistical software package version 16.5 using student's t-test and one-way ANOVA followed by

the Duncan's Multiple Range Test (SSR) to evaluate significant difference among different leaf phenology of seedlings at $P < 0.05$. All data were shown in the mean \pm SE.

3 RESULTS

3.1 EFFECT OF LEAF PHENOLOGY ON PLANT HEIGHT

As shown in Figure 2A, plant height of seedlings new leaf whorl at bronze stage II, coloring stage, light green leaf stage, stable leaf stage and mature leaf stage were higher 35.09% ($P < 0.01$), 62.67% ($P < 0.01$), 75.27% ($P < 0.01$), 78.78% ($P < 0.01$), and 89.09% ($P < 0.01$) than that at bronze stage I, respectively. Plant height of seedlings at coloring stage, light green leaf stage, stable leaf stage and mature leaf stage were higher 20.42% ($P < 0.05$), 29.75% ($P < 0.01$), 32.35% ($P < 0.01$), 39.98% ($P < 0.01$) than that at bronze stage II, respectively. There was no significant difference in plant height of seedlings among coloring stage, light green leaf stage, stable leaf stage and mature leaf stage. These results suggest that bronze stage was the rapid growth and regulation stage of plant height for rubber seedlings.

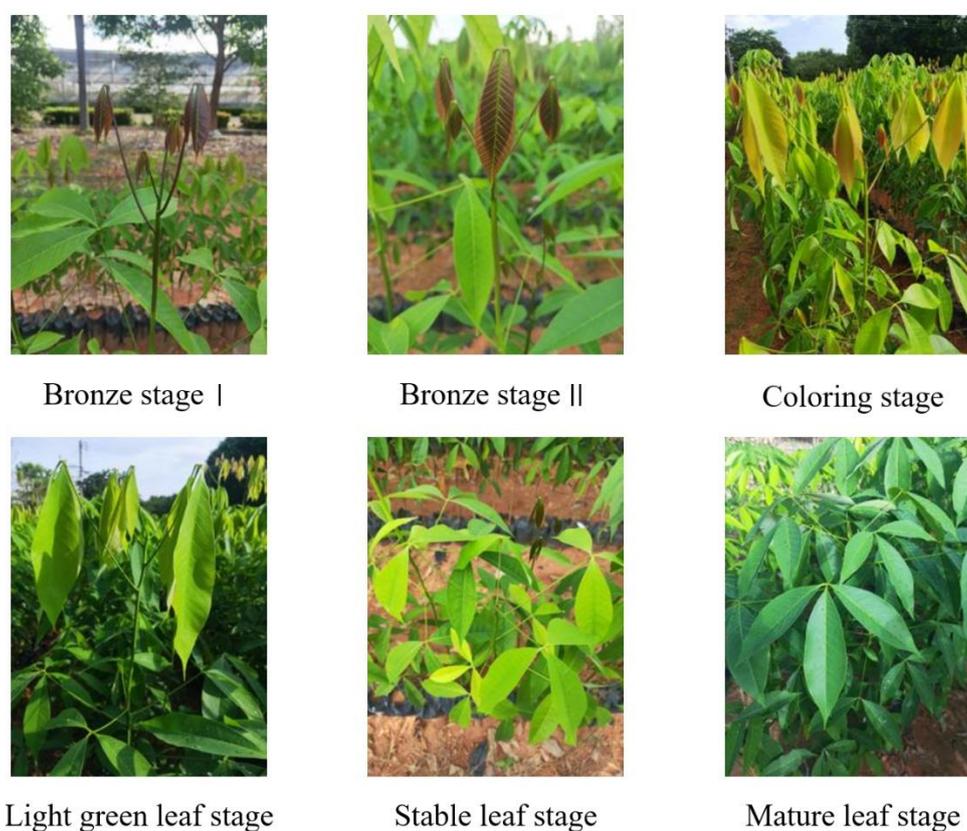


Fig. 1. Leaf phenology of rubber seedlings

Notes: Bronze stage I, bronze stage II, coloring stage, light green leaf stage, stable leaf stage, mature leaf stage took 2 days, 2 days, 3 days, 3 days and 4 days, respectively.

3.2 EFFECT OF LEAF PHENOLOGY ON STEM DIAMETER

As shown in Figure 2B, stem diameter of seedlings new leaf whorl at coloring stage, light green leaf stage, stable leaf stage and mature leaf stage were more 10.65% ($P < 0.05$), 17% ($P < 0.01$), 18.04% ($P < 0.01$), and 20.08% ($P < 0.01$) than that at bronze stage I, respectively. Stem diameter of seedlings new leaf whorl at light green leaf stage, stable leaf stage and mature leaf stage were more 11.12% ($P < 0.05$), 12.12% ($P < 0.01$), and as well as 14.05% ($P < 0.01$) than that at bronze stage, respectively. Stem diameter of seedlings new leaf whorl at mature leaf stage was more 8.52% ($P < 0.05$) than that at coloring

stage. These results suggest that bronze stage I was the rapid growth and regulation stage of stem diameter for rubber seedlings. Leaf color-related metabolic pathways involve flavonoid biosynthesis and anthocyanin biosynthesis [14-15].

3.3 EFFECT OF LEAF PHENOLOGY ON THE CHLOROPHYLL PARAMETERS

As shown in Figure2C, SPAD value, N content and leaf surface humidity of seedlings new leaf whorl showed a same and upward trend with leaf phenology. SPAD value of seedlings new leaf whorl at coloring stage, light green leaf stage, stable leaf stage and mature leaf stage were more 24.29% ($P < 0.01$), 32.55% ($P < 0.01$), 44.90% ($P < 0.01$), and 60.82% ($P < 0.01$) than that at bronze stage II, respectively. SPAD value of seedlings new leaf whorl at stable leaf stage and mature leaf stage were more 16.58% ($P < 0.01$) and 29.39% ($P < 0.01$) than that at coloring stage, respectively. SPAD value of seedlings new leaf whorl at stable leaf stage and mature leaf stage were more 9.31% ($P < 0.05$) and 21.32% ($P < 0.01$) than that at light green leaf stage, respectively. SPAD value of seedlings new leaf whorl at mature leaf stage were more 10.99% ($P < 0.01$) than that at stable leaf stage. The performance of leaf color in different phenological stages was significantly different, and the corresponding SPAD value was also different [16].

As shown in Figure2D, N content of seedlings new leaf whorl at coloring stage, light green leaf stage, stable leaf stage and mature leaf stage were more 16.84% ($P < 0.01$), 22.60% ($P < 0.01$), 31.53% ($P < 0.01$), and 42.60% ($P < 0.01$) than that at bronze stage II, respectively. N content value of seedlings new leaf whorl at stable leaf stage and mature leaf stage were more 12.57% ($P < 0.01$) and 22.05% ($P < 0.01$) than that at coloring stage, respectively. N content of seedlings new leaf whorl at stable leaf stage and mature leaf stage were more 7.28% ($P < 0.05$) and 16.31% ($P < 0.01$) than that at light green leaf stage, respectively. N content of seedlings new leaf whorl at mature leaf stage were more 8.42% ($P < 0.01$) than that at stable leaf stage.

As shown in Figure2E, leaf surface humidity of seedlings new leaf whorl at coloring stage, light green leaf stage, stable leaf stage and mature leaf stage were more 16.84% ($P < 0.01$), 22.60% ($P < 0.01$), 31.53% ($P < 0.01$), and 42.60% ($P < 0.01$) than that at bronze stage II, respectively. Leaf surface humidity of seedlings new leaf whorl at stable leaf stage and mature leaf stage were more 12.57% ($P < 0.01$) and 22.05% ($P < 0.01$) than that at coloring stage, respectively. Leaf surface humidity of seedlings new leaf whorl at stable leaf stage and mature leaf stage were more 7.28% ($P < 0.05$) and 16.31% ($P < 0.01$) than that at light green leaf stage, respectively. Leaf surface humidity of seedlings new leaf whorl at mature leaf stage were more 8.42% ($P < 0.01$) than that at stable leaf stage.

As shown in Figure2F, leaf surface temperature of seedlings new leaf whorl at coloring stage, light green leaf stage, stable leaf stage and mature leaf stage were more 3.95% ($P < 0.01$), more 12.38% ($P < 0.01$), more 0.72% ($P < 0.01$), and less 3.03% ($P < 0.01$) than that at bronze stage II, respectively. Leaf surface temperature of seedlings new leaf whorl at light green leaf stage, stable leaf stage and mature leaf stage were more 8.12% ($P < 0.01$), less 3.10% ($P < 0.01$) and less 6.71% ($P < 0.01$) than that at coloring stage, respectively. Leaf surface temperature of seedlings new leaf whorl at stable leaf stage and mature leaf stage were less 10.38% ($P < 0.01$) and 13.71% ($P < 0.01$) than that at light green leaf stage, respectively. Leaf surface temperature of seedlings new leaf whorl at mature leaf stage were less 3.72% ($P < 0.01$) than that at stable leaf stage. Leaf surface temperature of seedlings new leaf whorl at light green leaf stage showed a rapidly increase in comparison with other leaf stages. These results indicate that light green leaf was the sensitive stage to environmental temperature for seedlings.

3.4 CORRELATION ANALYSIS BETWEEN THE GROWTH INDEX AND THE CHLOROPHYLL PARAMETERS AT LIGHT GREEN LEAF STAGE

As shown in Figure3A-D, there was a significant positive correlation between plant height and stem diameter at bronze stage II ($r=0.77$, $p<0.01$), coloring stage ($r=0.73$, $p<0.01$), light green leaf stage ($r=0.70$, $p<0.01$), stable leaf stage ($r=0.86$, $p<0.01$), respectively. There was a significant positive correlation ($r=1$, $p<0.01$) among SPAD value, N content, leaf surface humidity and leaf surface temperature at different leaf stages. Plant height had no significant correlation with chlorophyll parameters (SPAD value, N content, leaf surface humidity and leaf surface temperature). Stem diameter had no significant correlation with chlorophyll parameters (SPAD value, N content, leaf surface humidity and leaf surface temperature). These results indicate that there is no significant correlation between growth index (plant height and stem diameter) and chlorophyll parameters (SPAD value, N content, leaf surface humidity and leaf surface temperature) for rubber seedlings at different leaf stages.

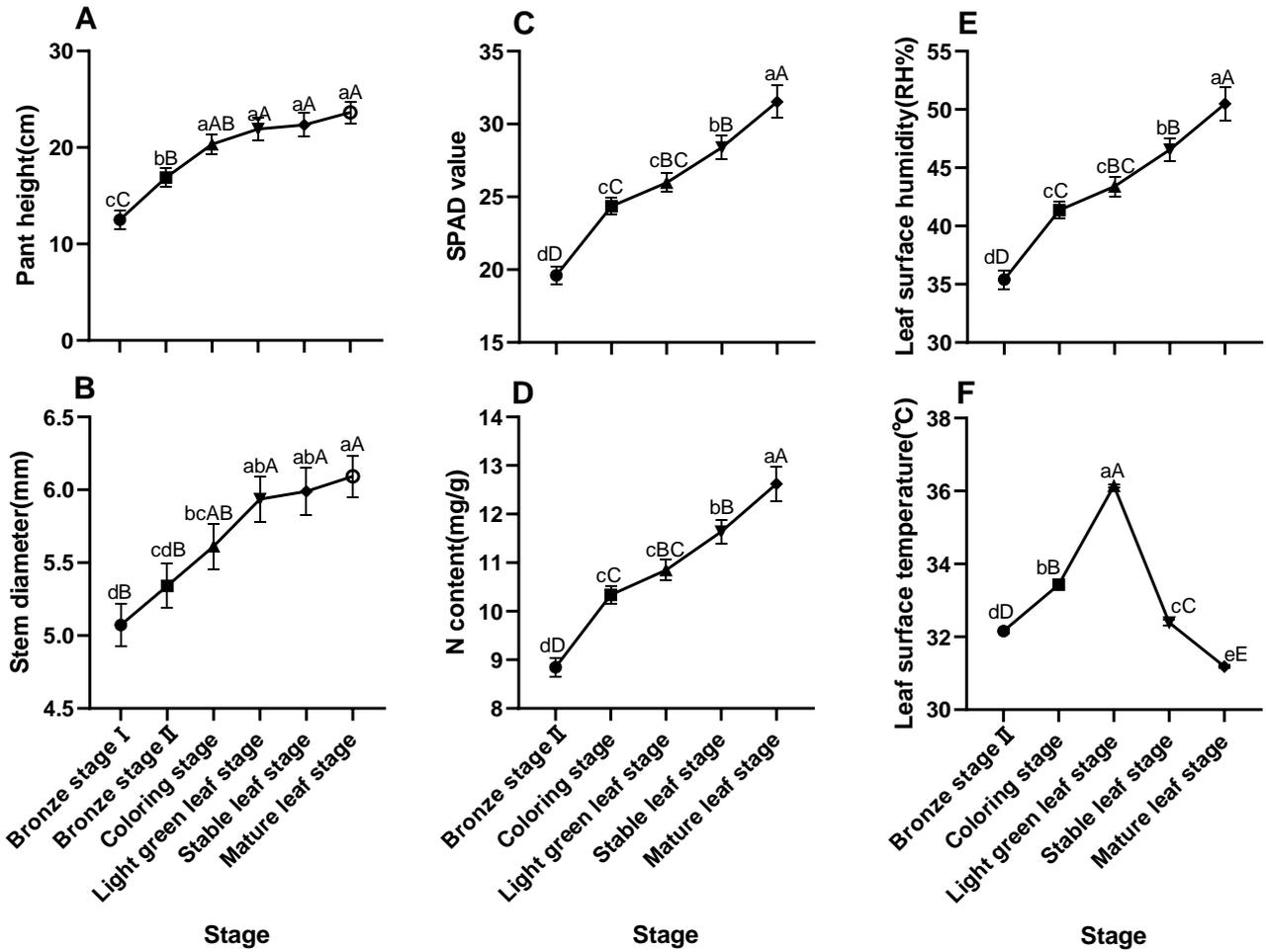


Fig. 2. Comparison on plant height (A), scion stem diameter (B), SPAD value (C), N content (D), leaf surface humidity (E) and leaf surface temperature (F) in rubber seedlings at different leaf phenology

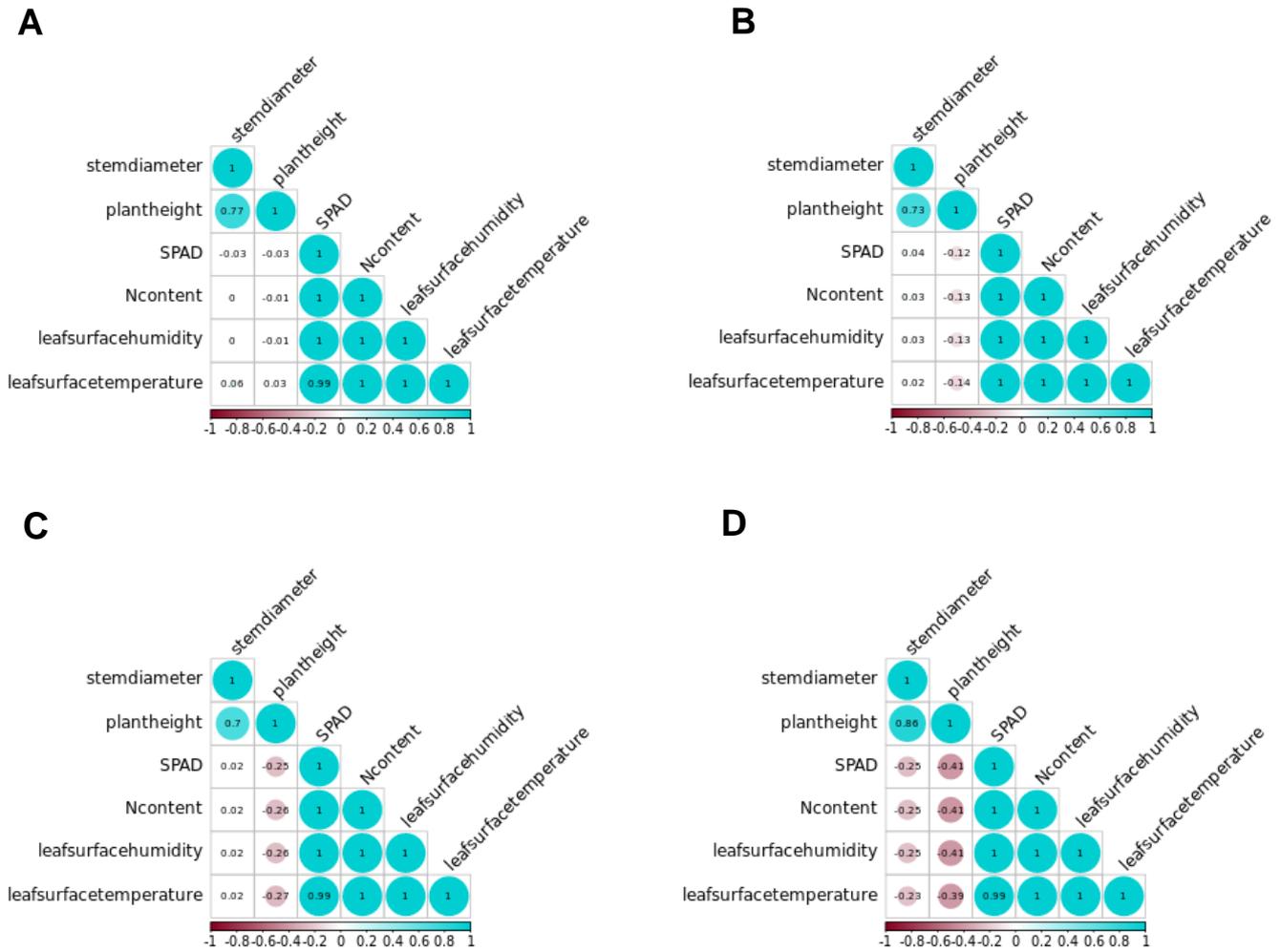


Fig. 3. Correlation analysis among stem diameter, plant height, SPAD value, N content, leaf surface humidity and leaf surface temperature at bronze stage II (A), coloring stage (B), light green leaf stage (C), and stable leaf stage (D)

4 CONCLUSION

For rubber seedlings propagation, bronze stage was the rapid growth and regulation stage of plant height, bronze stage I was the rapid growth and regulation stage of stem diameter, and light green leaf stage was the sensitive to environmental temperature.

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AUTHORS' CONTRIBUTION

X.H. Chen conceived the experiments, R. Wang and X.H. Chen conducted the experiments, R. Wang and X.H. Chen analyzed the data and drafted the manuscript, R. Wang, X.H. Chen, and J. Wang discussed the results and finalized the manuscript.

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