

Mini-seedling budding of *Hevea brasiliensis*: forty years of efforts in China

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Abstract— Good varieties are the basis for long-term high, stable and efficient production of rubber trees. Mini-seedling buddings have the characteristics of short nursery period, low labor intensity, large number of seedlings per unit area, easy transportation and planting, well-developed taproot and intact root system, high survival rate after planting, fast growth, strong tolerance to drought, wind and cold, and early tapping. The application of rubber mini-seedling buddings and its related research were summarized. Finally, the outlook for ongoing research on mini-seedling buddings was prospected.

Keywords— *Hevea brasiliensis*, mini-seedling budding, propagation, application.

I. INTRODUCTION

Mini-seedling budding of *Hevea brasiliensis* is a new propagation technique of elite clone on the basis of traditional budding method, which was developed by Rubber Research Institute, Chinese Academy of Tropical Agricultural Sciences [1-3], and was listed by the Agriculture Ministry of China as the first batch of main push varieties and technology of south subtropical crops during the 11th Five Year Plan period. Mini-seedling budding technique use 2-week-old seedlings, which are pulled out of the germination bed with seeds (nutrient provider for budding and further growing), with about 20cm plant height and leaf phenology between elongating stage to leaf-unfolding stage[4-5], as rootstock and green bud- patches as scion[6-14]. The budding operation process is done at indoor, which reduce labor intensity comparison with traditional budding in the field. After budding the budded seedlings are directly transferred into nursery bags or pre-planted on sand bed or nursery trays with some shade. After budding successfully, two compound leaflets are kept on the seedling stocks when topping to avoid dieback [15-16], rootstock bud are picked[17-18], scion bud sprouting [19-23], and the buddings are raised up to 2-3 leaf whorls during 5-8 months with above 0.4cm shoot diameter[24-27]. Raising polybag-budding by mini-seedling budding technique are 3-12 months less than those by traditional propagation, which reduce propagation costs and labor intensity and increase nursery productivity per unit area (Table 1). Mini-seedling budding shorten rootstock growth time by using small rootstocks for budding. The weight of polybag

buddings raised by mini-seedling budding is twice lighter than those raised by compared with the traditional method for raising budded stumps, resulting in lower cost and less labor intensity as well as higher productivity of the nursery per unit area. Mini-seedling buddings with good taproot and intact roots is easy to transport and transplant. In addition, mini-seedling buddings are characterized by high survival rate of field transplanting, fast growth after transplanting, wind tolerance [28], cold resistance [29], and drought resistance [30], early tapping (nearly one year shorter than traditional buddings). Taken together, in contrast to traditional buddings, mini-seedling budding can save cost and increase income by 0.5-1.0 yuan per plant [3, 31]. The cost of rubber plantation raised by mini-seedling buddings at nursery period and immature period is 6450 yuan per hectare less than that raised by traditional buddings, showing good economic benefits [32].

Table.1: Comparison on nursery time of buddings at 1-3 leaf whorls by different propagation

Nursery types	rootstock diameter(cm) at budding	nursery time(month)
polybag budded stumps	1.50-3.00	≤20
Green budding	0.60-1.40	≤12
Young budding	0.40-0.50	≤10
Mini-seedling budding	0.30-0.40	≤8

Note: nursery time is from sowing seeds to transfer budded plants out of the nursery.

II. RESEARCH AND DEVELOPMENT HISTORY

Mini-seedling budding of *Hevea brasiliensis* was begun research in 1980 and begun to take shape in 1983-1984. Reform of scientific research institutions in China was begun in 1986, which meant researchers had to fund their own research. In 1987, Huang Shoufeng group applied for a research loan (3000 yuan) from Rubber Research Institute to multiply mini-seedling buddings, sold them and repaid the loan. Meanwhile, mini-seedling buddings were transplanted in the field. After that, the research of mini-seedling budding stagnated without funding. Mini-seedling buddings were raised again in 1996 and prepared for the assessment of scientific and technological achievement in 1998. After appraisal, the research of mini-seedling budding stalled again without funding. Popularization of large-scale trial planting mini-seedling buddings was supported by the earmarked fund for China Agriculture Research System (CARS-34-YZ4) since 2005-2006.

III. POPULARIZATION OF LARGE-SCALE TRIAL PLANTING

Mini-seedling budding was introduced and outreached in Hainan Province [33], Yunnan Province [34-36] and Guangdong Province [37-42], respectively. Since its popularization in Yunnan Province in 2003, it has been rapidly recognized by the majority of seedling raising farms and planting farmers. In 2019, the number of rubber mini-seedling buddings promoted by the rubber tree seedling breeding base of Yunnan Institute of Tropical Crop Science had exceeded 1.2 million, covering an area of over 2666.67 hm². In 2005, Jinghong Farm of Yunnan Agricultural Reclamation introduced mini-seedling budding and conducted three training sessions on mini-seedling budding training, with a cumulative total of 310 training persons. From 2005 to 2011, 304,848 plants were transferred out of the nursery, with a survival rate of 46% and a nursery rate of 95.8% [36]. Jinghong branch of Yunnan Natural Rubber Industry Co., Ltd. could produce 50,000 mini-seedling buddings in 2007. In 2008, Xishuangbanna successfully introduced mini-seedling budding, and extended to production, and 22,200 mini-seedling buddings were raised in private breeding farms and planted in 50 hm² to rubber farmers [34]. The industrialization and large-scale production of mini-seedling budding has been realized in state farms of Guangdong Agricultural Reclamation Tropical Crop Research Institute. The annual production capacity of rubber mini-seedling budding has exceeded 1 million plants [37]. The problem is that the technical team of mini-seedling budding is unstable, which affects the

industrialization and large-scale production of mini-seedling budding. Therefore, it is necessary to train a group of skilled mini-seedling budding workers before the beginning of budding production every year, the new and old mini-seedling budding workers should be trained to be as skilled workers as possible before taking up the job[37]. Furthermore, there are 4 standards related to the study of seedling bud grafting, including one national industry standard [43], one national standard [44], and two local standards [45-46].

According to the above-mentioned production and promotion of mini-seedling budding, the survival rate of bud grafting needs to be further improved, and the increased demand for skilled workers for bud grafting during the season of bud grafting needs to be solved.

IV. THE OUTLOOK FOR ONGOING RESEARCH AND DEVELOPMENT

Breakthrough on technology and mechanism of rubber seed storage Rubber seeds are moderately recalcitrant seeds that can tolerate moderate dehydration and slightly higher temperatures[47], with a critical moisture content of 13% [48], and germination decreased by about 40% when seeds are stored indoors for half a month after falling from the tree. However, rubber seed has a short viability period when exposed to direct light (Table 2).

Table.2: Germination percentage of rubber seed exposed to sunlight

Time of exposure to sunlight(day)	Germination percentage(%)
1	95
2	68
3	9
4	1
5	0

Source: Prang Besar Research Station (PBRS)

Therefore, seeds must be collected daily or alternate daily and set for germination immediately. If rubber seeds are not sown in 10-15 days, they lose viability on storage as a result of the production of hydrocyanic acid (HCN), but over 90% of the cyanogenic material is consumed to form non-cyanogenic compounds during seedling development, and the cyanogenic glucosides are believed to be transported and metabolized in the young growing tissues[49].

Taken together, the initial seed quality, seed moisture content and preservation environment are the three major factors affecting the longevity of seeds. Due to the wide variation in maturity and development stages of rubber seeds, the initial quality of the seeds during storage is not consistent, which affects the stability and repeatability of different storage methods (Table 3). The seed storage technology of *Hevea brasiliensis* can not only be used in seedling raising, but also can be used for rootstock screening. The breakthrough of this technology and mechanism will greatly improve the technical level of rubber tree seedling nursery.

Table.3: Different storage methods of rubber seeds

Core of storage	Storage time
-7-7°C [50]	About a year
Germination and storage in cold storage at 12-13 °C [51]	more than three months
River sand and compound for seed [52]	short and medium term
Paraffin embedding [53]	About a year
polyethylene glycol 1500[49]	Six months
7°C-10°C [54]	three months
Sawdust and 10°C/22°C/27°C[55]	three months
Pick seeds from the rubber tree [55]	92-100 days
Water storage [56]	one month

Mechanism on budwood quality control of mini-seedling budding Budwood quality mainly include diameter and rejuvenation of bud patches. The older the bud stick, the lower budding survival rate and bud breakout rate, the more sensitive to disease of leaf and root, resulting in rubber clone degradation and the decline of growth and yield. The increase rate of rejuvenated rubber buddings in yield was more than that in stem growth [57]. Therefore, rejuvenation of bud stick plays a very important role in improving quality of rubber buddings. Bud sticks rejuvenation is required in rubber tree seedlings (NY_1686-2009), which is regenerated by the method of repeated budding with healthy and strong rootstock and renewed every 5 years. In the technical regulation of rubber tree seedling propagation (NY_1686-2018), rejuvenation of bud stick is required once every 4-5 years by cutting stock and re-budding or re-planting and bud stick is from bud wood resource nursery. The diameter of rootstock used for seed seedling bud grafting is small

(about 0.4cm). The survival rate of s mini-seedling budding can be improved by selecting the bud strips with the same diameter as the rootstock. At present, the methods of bud proliferation used in mini-seedling budding are as follows: the green cluster buds in the outdoor by repeatedly cutting off and re-sprouting [44], and the young bud stick in the outdoor bud wood nursery (not lignified, 5-8cm in the length and 0.3-0.5cm in diameter) [58], the proliferation buddings with more than 3 whorls in green house[53], and the micropropagation of anther tissue culture [51]. As mentioned above, bud stick propagation mainly focused on the diameter, but there is little systematic study on the effect and mechanism of bud stick rejuvenation (such as rejuvenation times), on the quality of bud stick and budding. The juvenility characteristics of bud stick can be recovered by rejuvenation (repeated budding of the old bud stick on the new seedling stock or by tissue culture) , and the survival rate of mini-seedling budding can be significantly increased by juvenile bud stick, and the budding trees from juvenile buds showed fast growth and high yield in the field. The higher yield of juvenile clones of *Hevea brasiliensis* was demonstrated by the differential expression of some genes [58-63] , transcriptome level [64] , proteome level [65] and epigenetic level [66](Table 4) , and these results can provide inspirations for the quality control of min-seedling budding propagation, and further more consistent material for early yield screening [67].

Improvement of sprouting uniformity of mini-seedling budding After cutting back, mini-seedling buddings take different days to sprout, and lead to unsynchronized growth of leaf phenology, which increased intensity of grading buddings before taking out of nursery and production cost. Therefore, to reduce nursery intensity, sound preparation for budding like rejuvenation of bud patches, enough and strong bud patches, vigorous rootstock with strong stem and healthy taproot, pre-planting in trays after budding (Table 5) and cutting back at the same time [68] to sprout and grow evenly.

Table.5: Effect of pre-planting on dieback rate of mini-seedling budding after transplanting in 6 months

Pre-planting	dieback rate (%)
Trays with coir	9.31±4.79bB
Sand bed	13.95±4.01aA

Note: For trays, 63 repeats, each repeat contains 240 plants. For sand bed, 36 repeats, each repeat contains 231 plants.

Table. 4: Genetic, transcriptomic, proteomic, and epigenetic differences between juvenile and mature clones of rubber trees.

Clones	Tissue	Differential expression
Unknown	Leaf, flower, bud, latex, bark	<i>HbCMT1</i> transcripts accumulated in various examined tissues, with high expression levels in the leaf and low levels in the latex. <i>HbCMT1</i> transcript expressed at different levels with the lower in self-rooting juvenile clones than in their donor clones [58]. <i>Hb14-3-3a</i> and <i>Hb14-3-3b</i> were differentially expressed in flower, leaves, barks and latex of <i>Hevea brasiliensis</i> . <i>Hb14-3-3a</i> transcripts accumulated at relatively high levels in the barks, while <i>Hb14-3-3b</i> transcripts accumulated at relatively high levels in the leaves [59]. <i>HbHDT1</i> was differentially expressed in the flower, callus, embryos, leaf, bud and latex, and was not induced in the latex by jasmonate acid and ethylene [60]. A translationally controlled tumor protein (<i>TCTP</i>) was constitutively expressed in the latex, leaves and barks, and induced by ethylene [61].
Haiken 2[62]	latex	Suppression subtractive hybridization method showed that comparison with DCs, 95 genes were upregulated and 81 downregulated in self-rooting JCs, respectively. Systematic analyses of the differentially expressed genes between self-rooting JCs and DCs suggest that rubber biosynthesis, production, and scavenging of reactive oxygen species may have significant functions in high-yielding self-rooting JCs.
CATAS7-33-97, Haiken2[63]	Flower, somatic embryo, leaf, callus, latex	<i>HbTRX1</i> was constitutively expressed in all tested tissues. <i>HbTRX1</i> transcripts accumulated at relatively low levels in the flower, somatic embryo, and leaves, while <i>HbTRX1</i> transcripts accumulated at relatively high levels in the callus and latex. The <i>HbTRX1</i> transcript was expressed at different levels, with higher levels in self-rooting juvenile clones than in their donor clones.
CATAS 7-33-97, Haiken 2[64]	latex	Comparative transcript profiling indicated that 1716 genes were identified as differentially expressed between self-rooting JCs and DCs. Functional analysis showed that the genes related to the mass of categories were differentially enriched between the two clones. Several genes involved in carbohydrate metabolism, hormone metabolism and reactive oxygen species scavenging were up regulated in self-rooting JCs.
Haiken 2[65]	latex	The proteomic approach showed that comparison with donor clones, 13 proteins were upregulated, 11 proteins were downregulated in self-rooting juvenile clones. These proteins were classified as carbohydrate and energy metabolism, secondary metabolism, signal translocation, transcriptional regulation-related, protein synthesis and degradation, transport, nucleoside acid process, lipid metabolism.
CATAS 8-79[66]	leaf at bronze stage	The genomic DNA methylation showed that the juvenile clones was 33.2% and the mature clones was 22.9%, and different expressed fragments were related with metabolism and cell growth.

Container of mini-seedling budding and mechanized seedling nursing

Different container mini-seedling buddings can be grown depending on the environment in which they are planted, e.g., mini-seedling buddings raised in small root-tubes with 2-3 leaf whorls are suitable for remote mountainous areas, mini-seedling buddings raised in large container with 4-6 leaf whorls are suitable for flat and of high value for intercropping. Currently, sales of rubber buddings are also severely affected due to the ongoing downturn in rubber prices, and the exploration of mechanized seedling nursery in container of mini-seedling budding seedling is still lagging.

V. CONCLUSION

Mini-seedling buddings are widely raised in China rubber plantation for the characteristics of short nursery period, low labor intensity, large number of seedlings per unit area, easy transportation and planting, well-developed taproot and intact root system, high survival rate after planting, fast growth, strong tolerance to drought, wind and cold, and early tapping. However, there are several scientific problems such as seed storage, budwood quality, sprouting uniformity and mechanized container nursing to be solved in the future.

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