J. Rubb. Res., **11(1)**, 43–51

Relationship between Rate of Laticifer Differentiation, Number of Laticifer Rows and Rubber Yield among 1981 IRRDB Germplasm Collections of Hevea brasiliensis

JUN-HONG YU*, XIA ZENG*, SHU-GUANG YANG*,
HUA-SUN HUANG* AND WEI-MIN TIAN*#

Early selection based on parameters related to yield is important in the Hevea breeding program. In an attempt to characterise such parameters, we investigated the variation in TNLD (time needed for laticifer differentiation) of the 1981 IRRDB germplasm collections by means of an experimental system for the induction of secondary laticifer differentiation in relation to RLCL (ratio of laticifer rows to the cell layers of the functional phloem) and rubber yield of the corresponding mature germplasm collections. The results demonstrated that TNLD varied among the 63 germplasm collections examined and was correlated to RLCL (r=0.5795, highly significant) and rubber yield (r=0.4558, significant level) of the mature plants. TNLD is expected to be a parameter candidate for early selection, which is better than the leaflet latex method in that it is related to the secondary laticifer differentiation and also better than test-tapping yield method in that early selection can be carried out with one-year-old epicormic shoot in the nursery. Large scale experiment is necessary to confirm this conclusion.

Key words: conventional breeding; early selection; *Hevea brasiliensis* Muell.Arg.; laticifer differentiation; mechanical wounding; 1981 IRRDB germplasm collections

Rubber tree is a kind of perennial and strongly outcrossing crops[1]. Conventional breeding of *Hevea* for the purpose of improving rubber yield is labour intensive and time consuming[2]. Early selection based on parameters related to yield is important in the *Hevea* breeding program. Both the amount of latex from the cut made on the petiolule or vein of leaflet (referred to as the leaflet latex method) and that from the bark of trunk by means of test-tapping (referred to as the test-tapping yield method) are considered to be related to rubber yield and used as parameters for early selection in *Hevea* breeding practice[1,3]. The leaflet latex method is better than the test-tapping yield method in that early selection can be carried out with one-year-old epicormic shoots in the nursery rather than with 3–4 year-old young trees in the field. However, the latex from primary laticifers are different from the secondary laticifers in the bark of trunk. Moreover, the leaflet latex method was

*Key Laboratory for Tropical Crops Cultivation and Physiology, Rubber Research Institute, Chinese Academy of Tropical Agricultural Sciences, Danzhou, Hainan 571737, P.R.China
Corresponding author (e-mail: wmtian1965@hotmail.com)

proved to be unreliable for the early selection of 1981 IRRDB germplasm collections of rubber tree[4]. On the other hand, test-tapping yield method is better than leaflet latex method in that latex from the secondary laticifers are essentially the same as those involved in commercial latex exploitation in the trunk of mature rubber trees[5]. The purpose of this study is to examine the occurrence of some parameters which are related to secondary laticifers and can be used for early selection with one-year-old epicormic shoots in the nursery.

Epicormic shoot developing from the latent buds on the pruned stem flushes five to six times a year. Such a shoot therefore consists of a series of foliage clusters, separated by lengths of leafless stem (*Figure 1*). Each of these morphologically distinct growth increments represents a growth flush, and is referred to as an extension unit (EU)[6]. Although the primary laticifers are present in the leaves and bark of every extension unit, there are no secondary laticifers in the bark of stem of the first and second extension units under natural conditions[5]. The secondary laticifer differentiation in these parts can be locally induced by mechanical wounding[7,8], which is essentially the same as the laticifer row formation in the trunk of mature rubber trees in tapping. So, the rate of laticifer differentiation induced by mechanical wounding may be related to the ability of the corresponding mature trees to differentiate laticifers and produce rubber. In an attempt to characterise some parameters for early selection of potential high-yield germplasm lines from the 1981 IRRDB germplasm collections, we investigated the relationship between the rate of secondary laticifer differentiation of the germplasm materials by means of an experimental system for the induction of the secondary laticifer differentiation and the number of laticifer rows and rubber yield of the corresponding mature trees.

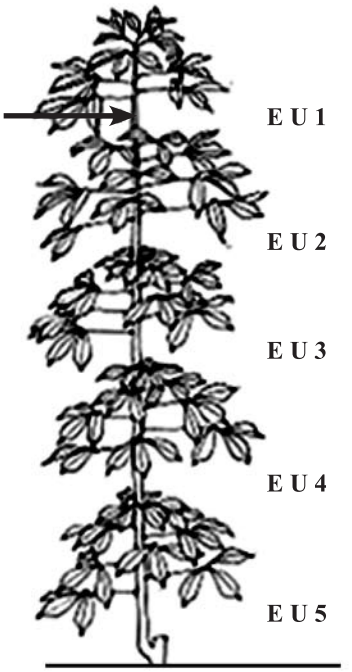

Figure 1. Diagram illustration of the epicormic shoot with five extension units. The site for wounding is as pointed by the arrow.

MATERIALS AND METHODS

Planting Materials

1981 IRRDB germplasm accessions were introduced from the Rubber Tree Genetic Resource Center in Malaysia during the 1983–1985 period. The introduced germplasm materials were conserved in the National Rubber Tree Germplasm Nursery of China on Hainan Island with a spacing of 1 m × 1 m between plants. Each germplasm accession consisted of two to four trees. To investigate the variation among the germplasm collections in the ability to differentiate laticifer, we selected 63 different germplasm collections of which epicormic shoots were at a phenophase suitable for laticifer differentiation induction[7,8].

One hundred and sixty different germplasm collections planted in the field during the 1986–1994 period (in 1986, 1987, 1988, 1994) were selected from the 1981 IRRDB germplasm collections by means of the leaflet latex method[8]. The germplasm plants were planted at a spacing of 3 m × 7 m between plants in a randomised complete block design with two replications except for those planted in 1994 which had no replications. Each replication consisted of six trees while each of those planted in 1994 consisted of four trees. Tapping commenced eight years after the germplasm plants were planted. The tapping system adopted was 1/2S d/3, together with an application of 1.5% ethephon every half a month except for the germplasm plants planted in 1994 (without ethephon application). Twenty-eight mature germplasm materials out of the 160 different mature germplasm materials as well as the corresponding germplasm collections (*i.e*., epicormic shoots in the nursery) were chosen on the basis of phenophase of the epicormic shoots which were at a suitable stage for inducing laticifer differentiation.

For mechanical wounding, the epidermis and outer parts of cortex of 0.2 cm × 1.0 cm area were scratched with a razor blade at two sites, 1 cm and 4 cm from the lowest foliage leaf of the first extension unit respectively, for each epicormic shoot (*Figure 1*). Bark samples (including parts of xylem) were collected five days and nine days after wounding from the wounded sites respectively. Bark samples of mature trees were punched from the trunk opposite to the tapping side 100 cm from the trunk base during the period of yield determination in 2004.

Light Microscopical Identification of the Induced Laticifers

To eliminate tannin-like substances which may be mistaken for the rubber inclusions in laticifers, samples were fixed in 80 % ethanol for 24 h at room temperature, then treated with iodine and bromine in glacial acetic acid[9], and embedded in paraffin after dehydration. Sections (thickness 20 μm) were cut with a microtome and stained with fast green. The laticifers in sections could be recognised since the rubber inclusions in the laticifers were brown due to iodine-bromine treatment[9].

Criteria for Laticifer Differentiation in the Stem of Epicormic Shoots

The criteria for laticifer differentiation was that more than five laticifer cells appeared continuously in the cross-section of the stem under light microscope.

Measurement of the Functional Phloem of Mature Trees

Measurement of the functional phloem was made following Hao and Wu[6]. The secondary laticifer rows in the functional secondary phloem were regular and easy to be distinguished. The number of secondary phloem cell layers was measured by counting the radial cell lines in the secondary phloem axial system, including phloem parenchyma cells, laticiferous cells and sieve elements, but not companion cells. Data were collected from five fields of vision that were chosen randomly in one section for each sample.

Determination of Rubber Yield

Rubber yield was determined in the form of dry rubber weight. Latex was collected from four to six mature trees for each germplasm collection at early, middle and late May, July and October (three times each month).

Analysis of the Correlation among TNLD, RLCL and Rubber Yield

Data were analysed with the SAS 6.12 software and Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Variation in Laticifer Differentiation Induced by Mechanical Wounding among 1981 IRRDB Germplasm Collections

Secondary laticifers appeared in the stem of the first extension unit of epicormic shoot in response to mechanical wounding[7,8]. There was no difference in the time needed for laticifer differentiation (TNLD) for the same clone between different epicormic shoots either on the same stock or on the different stocks (data not shown) while variation in TNLD occurred among the 1981 IRRDB germplasm collections. The secondary laticifer differentiation could be induced by mechanical wounding within 5 days for 46.0% of the 63 germplasm collections tested (*Figure 2a*) whereas it took more than 5 days but less than 9 days for 41.3% of the germplasm collections. No laticifer differentiation occurred for 12.7% of the germplasm collections 9 days after mechanical wounding (*Figure 2c*).

Correlation between TNLD, RLCL and Rubber Yield

The epicormic shoots of germplasm collections that differentiated laticifers within 5 days were related to a high ratio of laticifer rows to cell layers in the fuctional phloem (RLCL) of the corresponding mature germplasm collections (*Figure 2a* and *b*) while the mature germplasm trees of the germplasm collections that did not differentiate secondary laticifers within 9 days had low RLCL (*Figure 2d* and *c*). The RLCL of the mature trees of which the epicormic shoots initiated laticifer differentiation within 5 days was significantly higher than that of the mature trees of which the epicormic shoots did not differentiate laticifers within either 5 days or 9 days (*Figure 3*). SAS correlation analysis showed that there was a significant correlation among TNLD (*Figure 4b*), RLCL (*Figure 4c*), and rubber yield (*Figure 4a*). The coefficient of correlation was -0.6447 (high significant level) for TNLD and RLCL, 0.5795 (high significant level) for RLCL and rubber yield, and -0.4558 (significant level) for TNLD and rubber yield.

TNLD may be a Parameter Candidate for Early Selection of Potential High-Yield Germplasms

Twenty-eight mature germplasm collections were divided into three groups, *i.e*., high-yield group, mid-low-yield group and low-yield group, based on the determination of rubber yield[4]. The high-yield group consisted of twelve germplasm accessions from 87-37 to 94-1 (*Figure 4a*) and its yield was significantly higher than that of the mid-low-yield group. The mid-low-yield group consisted of ten germplasm accessions from 94-22 to 87-53 and the low-yield germplasms included the remaining six accessions (*Figure 4a*). There was no significant difference between the rubber yields of mid-low-yield group and the low-yield group.

Gomez demonstrated that the number of laticifer rows was one of the most important factors that determined rubber yield[10]. Here, we provided evidence that in the functional secondary phloem, the ratio of laticifer rows to the cell layers (RLCL) was closely related to the ability of mature germplasm plants to produce rubber (*Figures 4a* and *c*). About 90 percent of the mature germplasm plants in the high-yield group have high RLCL while 87 percent of the mature germplasm plants in the mid-low-

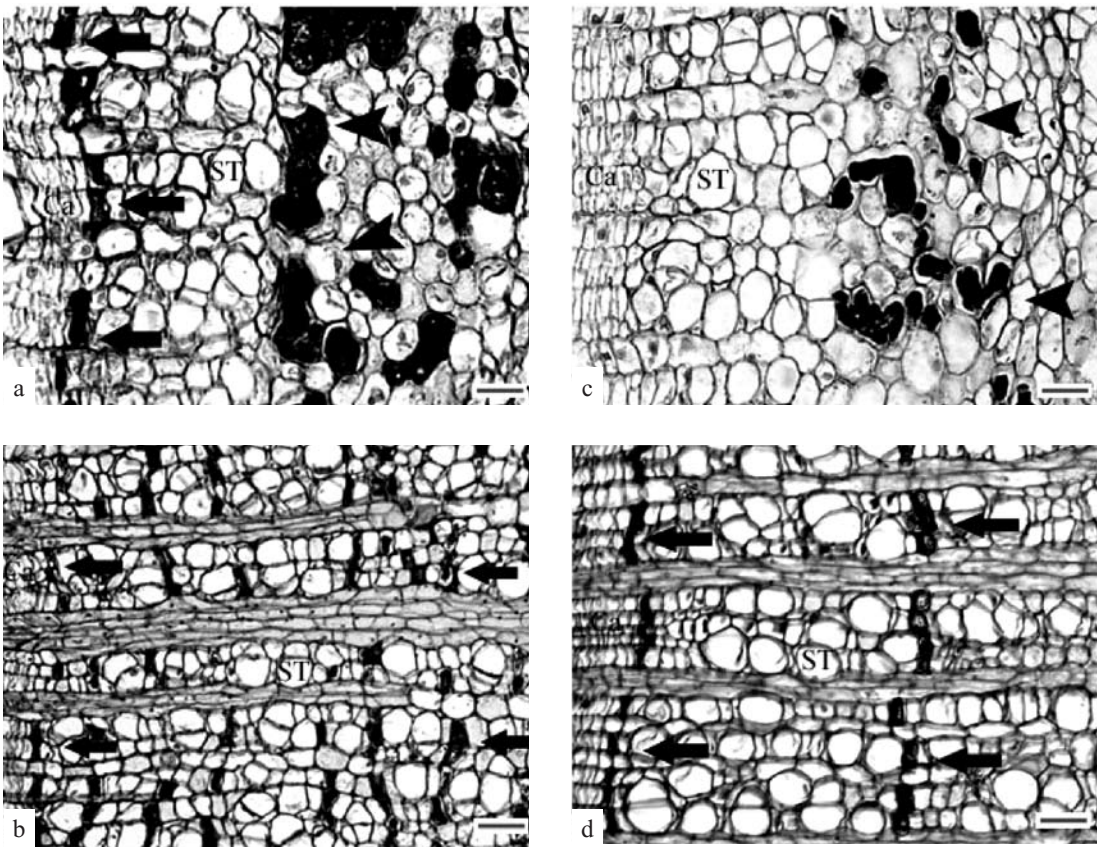

Figure 2. Cross-sections of bark, showing the secondary laticifer differentiation in different germplasm accessions. (a), a secondary laticifer row (black arrows) appeared in the stem of epicormic shoot 5 d after mechanical wounding; (b), more than five laticifer rows occurred in the functional secondary phloem (black arrows) from the mature tree of the same germplasm accession as that in (a); (c), no secondary laticifers appeared in stem of epicormic shoot 9 d after mechanical wounding; (d), less than three laticifer rows (black arrows) were present in the functional secondary phloem from the mature tree of the same germplasm accession as that in (c). Black arrowheads, the primary laticifers; Ca, cambia; ST, sieve tube. Bars=20 μm in (a) and (c), and 40 μm in (b) and (d).

yield and low-yield groups had low RLCL (*Figure 4c*). RLCL was much better than the number of laticifer rows in functional and non-functional secondary phloem since laticifer rows in non-functional secondary phloem was irregular and thus was difficult to be counted accurately. Nevertheless, RLCL reflected the ability of mature trees to differentiate laticifers and thus was not a suitable parameter for early selection.

TNLD reflected the variation in secondary laticifer differentiation in the stem of epicormic shoots caused by mechanical wounding. There was significant correlation between TNLD and RLCL, and between TNLD and rubber yield.

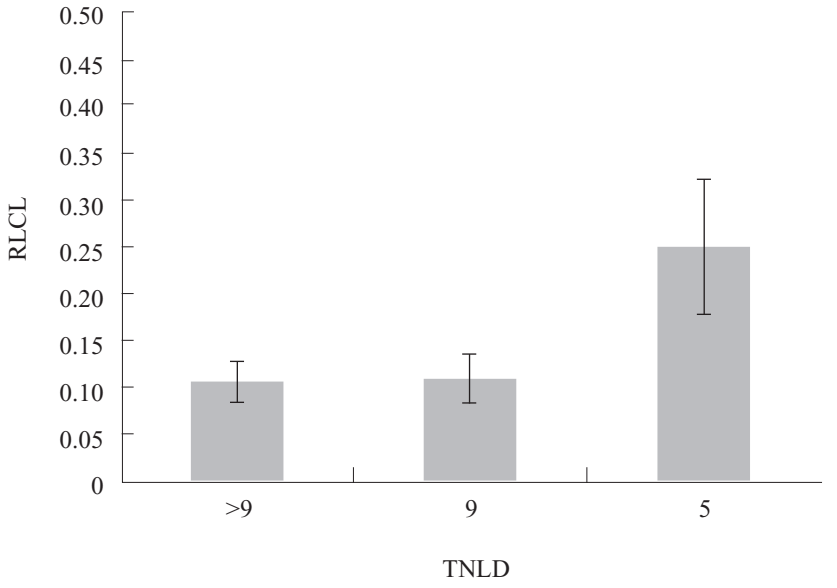

Figure 3. Variation in RLCL of the mature germplasm trees which were divided into three groups on the basis of TNLD of the corresponding germplasm collections. RLCL, ratio of laticifer rows to the cell layers of the functional secondary phloem; TNLD, time needed for laticifer differentiation; 5, TNLD was 5 days; 9, TNLD was longer than 5 days, but less than 9 days; >9, there was no laticifer differentiation within 9 days.

So, it was reasonable that TNLD was related to the ability of mature germplasm plants to differentiate secondary laticifer and produce rubber and thus may be a parameter for early selection. TNLD was five days for 90% of germplasm collections of which the mature germplasm plants in the high-yield group had high RLCL and more than five days for all the germplasm collections of which the mature plants in the low-yield group had low RLCL (*Figures 4a–c*). TNLD was five days for 92% of germplasm collections corresponding to the mature germplasm plants in the high-yield group and more than five days for all the germplasm collections of the mature plants in the low-yield group (*Figures 3a* and *b*). However, TNLD was also five days for 50% of the germplasm collections corresponding to the mature germplasm plants in the mid-low-yield group (*Figures 3a* and *b*), and there was no significant difference in the number of the induced laticifer cells between this kind of germplasm collections and those of the mature germplasm plants in the high-yield group (*Figure 4*). The accuracy of TNLD for early selection of the potential high-yield germplasm collections was 78.6%. Therefore, TNLD was expected to be a parameter candidate for early selection, which was better than the leaflet latex method in that it was related to the secondary laticifer differentiation and also better than the test-tapping method in that early selection can be carried out with one-year-old epicormic shoots in the nursery. A large scale experiment is necessary to confirm this conclusion.

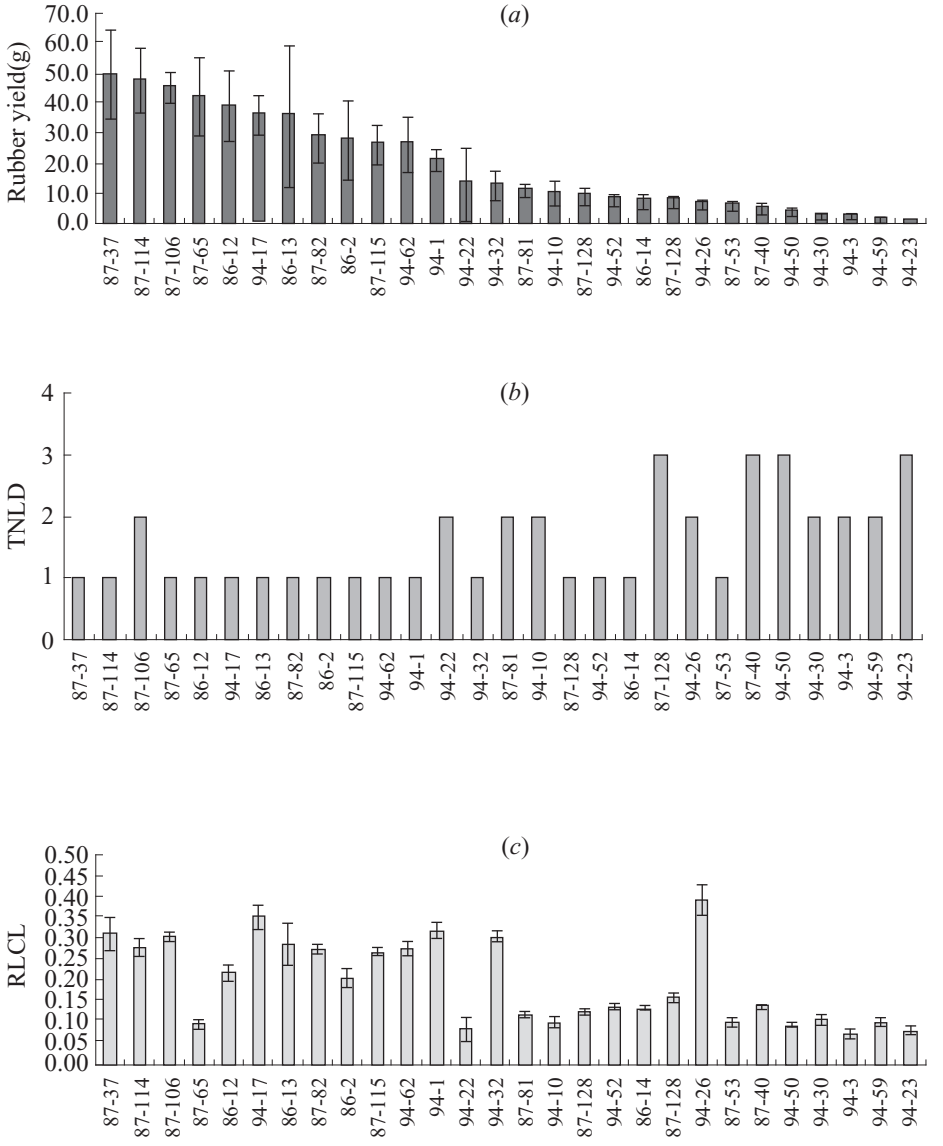

Figure 4. TNLD (b) in relation to RLCL (c) and rubber yield (a) of 28 different Hevea *germplasm collections. The germplasm materials were numbered by three to five Arabic numerals of which the first two numerals represented the year when they were planted and the third to fifth numerals the order number of the germplasm plants planted in the same year. RLCL, ratio of laticifer rows to the cell layers of the functional secondary phloem; TNLD, time needed for laticifer differentiation. 1, 2, 3 in (b) represented that TNLD was 5 days, 9 days and longer than 9 days, respectively. TNLD was considered to be longer than 9 days if there were no secondary laticifers in the stem of epicormic shoot 9 days after mechanical wounding.*

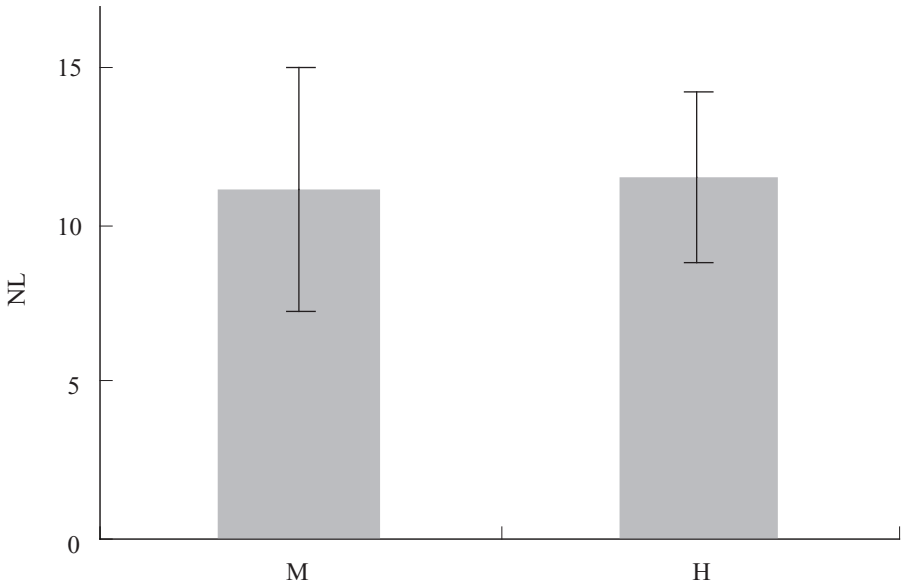

Figure 5. Number of laticifer cells (NL) induced by mechanical wounding within 5 days between the germplasm collections of which the mature trees in mid-low-yield group (M) and high-yield group (H).

ACKNOWLEDGEMENT

This work was supported by the National Natural Science Foundation of China (30160006), and 973 Pre-Project of China (2006CB708205).

Date of receipt: February 2007
Date of acceptance: November 2007

REFERENCES

1. ZHOU, Z.-Y., GUO, Q.-Y., ZHAN, S.-R., AND LIU, J.-X. (1994). Early Yield Predicting for *Hevea* Seedlings from Hand-pollination. *Chin. J. Trop. Crop*, **15**, 1–9.

2. VENKATACHALAM, P., PRIYA, P., AMMA, C.K.S., AND THULASEEDHARAN, A. (2004). Identification, Cloning and Sequence Analysis of a Dwarf Genome-specific RAPD marker in rubber tree (*Hevea brasiliensis*) Muell. Arg. *Plant Cell Rep*., **23**, 327–332.

3. GUO, X.-Y., ZHOU, Z.-Y. AND ZHAN, S.-R. (1995). The Second Validation Report of Early Yield Prediction in *Hevea brasiliensis* (1982–1994). *Chin. J. Trop. Agr*., **15**, 9–13.

4. ZENG, X., HU, Y.-S., HUANG, H.-S. AND FANG J.-L. (2006). The Evaluation Report of 1981'IRRDB Rubber Tree Germplasm—1987-Field-Planted Germplasm. *Chin. J. Trop. Crop*, **27**, 34–38.

5. HAO, B.-Z. AND WU J.-L. (2004). Biology of Laticifers in *Hevea* and Latex Production. *Chin. J. Trop. Crop*, **25**, 1–7.

6. HAO, B.-Z. AND WU, J.-L. (2000). Laticifer Differentiation in *Hevea brasiliensis*:

Induced by Exogenous Jasmonic Acid and Linolenic Acid. *Ann. Bot*., **85**, 37–43.

7. WU, J.-L., HAO B.-Z. AND TAN H.-Y. (2002). Wound-induced Laticifer Differentiation in *Hevea brasiliensis* Shoots Mediated by Jasmonic Acid. *J. Rubb. Res*., **5**, 53–63.

8. TIAN, W.-M. SHI, M.-J.,YU, F.-Y., WU, J.-L., HAO, B.-Z. AND CUI, K.-M. (2003). Localized Effects of Mechanical Wounding and Exogenous Jasmonic acid on the Induction of Secondary Laticifer Differentiation in Relation to the Distribution of Jasmonic acid in *Hevea brasiliensis*. *Acta Bot. Sin*., **45**, 1366–1372.

9. SHI Z.-Q. AND HU, Z.-H. (1965). A Histological Method for Rubber-bearing Tissues of Plants. *Acta Bot. Sin*., **13**, 179–182.

10. GOMEZ, J.B. (1982). Anatomy of *Hevea* and its Influence on Latex Production. Kuala Lumpur: Malaysia Rubber Research and Development Board.