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# Relationship between concentration of ATP in latex and yield potential in seedlings of *Hevea brasiliensis* and its implications in breeding

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In *Hevea brasiliensis*, the Para rubber tree, as in many other perennial trees, the breeding programme is a long-term strategy. It takes almost 23 years before any superior clone is released for commercial planting (Simmonds, 1989; Mydin, 2014). Utility of several physiological, molecular and biochemical parameters influencing rubber yield as early screening tools were investigated, and concentration of adenosine triphosphate (ATP) in latex was found as a potential marker for high yield considering its central regulatory role in energy metabolism-related to rubber biosynthesis (Sreelatha *et al*., 2004). Tapping or extraction of latex through the wounding of bark involves intense metabolic processes. These dynamic metabolic processes include rubber biosynthesis and reconstitution of sub-cellular components during latex removal by tapping, which involves a constant supply of energy in the form of ATP. The supply of sucrose and the availability of ATP plays a significant role in the latex regeneration processes. ATP is a source of energy for the mobilization of different solutes across lutoid membrane (Marin *et al*., 1981). Thus, high latex [ATP] could be an indicator for high rubber yield in *Hevea* (Sreelatha *et al*., 2004).

Previous studies using clones of *Hevea* have already demonstrated that high yielding clones possessed high latex [ATP]. Using clones with variable rubber yields, it was demonstrated that high-yielding clones RRII 105 and RRIM 600 also possessed high [ATP] (Sreelatha *et al*., 2004). The above study also showed that low yielding clones had very low [ATP]. In another study using immature plants and trees of ten clones with variable rubber yield, it was shown that high yielding clones like RRII 105, RRIM 600, PB 217 and PB 235 displayed consistently high [ATP]. In the same study, low yielding clones like RRII 33 and RRII 38 also had low [ATP], thus strengthening the view that high latex [ATP] could be an indicator of high rubber yield in *Hevea*. In a recent study, latex [ATP] was shown to have a significant positive correlation with crop efficiency of the polyclonal population (r=0.76) and selected genotypes (r=0.61) (Dey *et al*., 2018). However, so far, no studies have been carried out to assess the relationship between latex [ATP] and corresponding juvenile yield potential using seedlings.

In the *Hevea* breeding programme, 'test-tap' or juvenile yield has been used as an estimator of yield in young plants of *H. brasiliensis* (Varghese *et al*., 1989). Test tapping is carried out in two to three-year-old plants in a nursery, and the selections are evaluated in mature clonal trials after vegetative multiplication through budding (Mydin and Saraswathyamma, 2005). Previous studies have used mature trees of clonal and polyclonal origin for studying the relationship between yield based on normal tapping and latex [ATP] (Sreelatha *et al*., 2014; Dey *et al*., 2018). In the present study, we investigated the relationship between test-tap yield

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potential and corresponding latex [ATP] using a seedling population in a nursery evaluation trial.

The experimental population comprised of seedling progenies (full-sibs, half-sibs and open-pollinated polycross progenies), which were evaluated in a nursery trial in the farm of Rubber Research Institute of India (Kottayam, Kerala state, India). Details of the progenies and their pedigree are given in Table 1. Standard procedures were followed for assessing the juvenile yield through test-tapping (Mydin and Saraswathyamma, 2005). The progenies were planted at a spacing of 60 cm x 60 cm, and test-tapping was carried out at a height of 20 cm from ground level. After discarding latex from the first five test-tappings, latex from the subsequent 15 test tappings were collected, air-dried and weighed to compute mean test-tap yield as grams per tree per tap (g $tree^{-1}$ $tap^{-1}$). Test tapping was initiated from the third year of planting, and the yield recording was continued for two more years. ATP concentration in latex was determined in the third year of test-tapping (Amalou *et al*., 1992; Sreelatha *et al*., 2014). Data on test-tap yield and latex [ATP] of progenies were regressed to find the correlation.

Mean test-tap yield and latex [ATP] of the progenies are given in Table 2. Test-tap yield ranged from 0.3 to 31.5 g $tree^{-1}$ $tap^{-1}$, indicating very high variability in the experimental population (Table 2). Among full-sibs, 14/C33 (RRII 414 x 90/21), followed by 14/A216 (RRII 414 x RO 230), recorded a high mean yield of 31.5 g $tree^{-1}$ $tap^{-1}$ and 19.1 g $tree^{-1}$ $tap^{-1}$, respectively. Among hybrids of RRII 105, one hybrid (14/A143) gave a mean yield of 4.8 g $tree^{-1}$ $tap^{-1}$. The remaining two hybrids, 14/A240 and 14/A236 recorded very low mean yields ranging from 0.3 to 1.0 g $tree^{-1}$ $tap^{-1}$.

Half-sibs of Fx 516 had a mean yield ranging from 9.5 in 14/HS/Fx516/D5 to 28.2 g $tree^{-1}$ $tap^{-1}$ in 14/HS/Fx516/D3. Similarly, there was a wide variation in yield among half-sibs of RRII 430 and RRII 414. The mean yield of half-sibs of RRII 414 ranged from 6.6 in 14/HS/RRII414/D205 to 13.6 g $tree^{-1}$ $tap^{-1}$ in 14/HS/RRII414/D97. Half-sibs of RRII 430 had a mean yield ranging from 5.9 in 14/HS/RRII430/D83 to 29.9 g $tree^{-1}$ $tap^{-1}$ in 14/HS/RRII430/C64. The half-sib of *H. spruceana* (14/HS/HSP/A154) gave the lowest yield of 0.4 g $tree^{-1}$ $tap^{-1}$.

Progenies exhibited very high variation for latex [ATP] (Table 2). Maximum ATP concentration

**Table 1. Details of progenies and their parentage**

| Progeny identity | Parentage |
|---|---|
| 14/HS/Fx516/D3 | HS of Fx 516 (F 4542 x AVROS 363) |
| 14/HS/Fx516/A248 | HS of Fx 516 |
| 14/HS/Fx516/A95 | HS of Fx 516 |
| 14/HS/Fx516/D5 | HS of Fx 516 |
| 14/HS/HSP/A154 | HS of *H. spruceana* |
| 14/HS/RRII414/C3 | HS of RRII 414 (RRII 105 x RRIC 100) |
| 14/HS/RRII414/D97 | HS of RRII 414 |
| 14/HS/RRII414/D205 | HS of RRII 414 |
| 14/HS/RRII414/D214 | HS of RRII 414 |
| 14/HS/RRII414/D209 | HS of RRII 414 |
| 14/HS/RRII414/C5 | HS of RRII 414 |
| 14/HS/RRII414/D215 | HS of RRII 414 |
| 14/HS/RRII430/C48 | HS of RRII 430 (RRII 105 x RRIC 100) |
| 14/HS/RRII430/D248 | HS of RRII 430 |
| 14/HS/RRII430/C64 | HS of RRII 430 |
| 14/HS/RRII430/D142 | HS of RRII 430 |
| 14/HS/RRII430/D231 | HS of RRII 430 |
| 14/HS/RRII430/D226 | HS of RRII 430 |
| 14/HS/RRII430/D19 | HS of RRII 430 |
| 14/HS/RRII430/D227 | HS of RRII 430 |
| 14/HS/RRII430/D26 | HS of RRII 430 |
| 14/HS/RRII430/D78 | HS of RRII 430 |
| 14/HS/RRII430/D225 | HS of RRII 430 |
| 14/HS/RRII430/D66 | HS of RRII 430 |
| 14/HS/RRII430/D83 | HS of RRII 430 |
| 14/HS/RRII430/D250 | HS of RRII 430 |
| 14/HS/RRII430/D71 | HS of RRII 430 |
| 14/HS/RRII430/D138 | HS of RRII 430 |
| 14/OP/OPCES/A197 | Polycross progeny |
| 14/A143 | RRII 105 (Tjir 1 x Gl 1) x Fx 516 |
| 14/A240 | RRII 105 x RO 230 |
| 14/A236 | RRII 105 x RO 230 |
| 14/C33 | RRII 414 x 90/21 (RRII 105 x RO 142) |
| 14/A216 | RRII 414 x RO 230 |

(375.5 µM) was detected in 14/C33, which also possessed the maximum rubber yield (31.5 g $tree^{-1}$ $tap^{-1}$). Very low [ATP] (46 µM) was recorded in 14/A236 (RRII 105 x RO 230), which also had a very low rubber yield of 1.0 g $tree^{-1}$ $tap^{-1}$. Very low yielding progenies 14/A240 (RRII 105 x RO 230; 0.3 g $tree^{-1}$ $tap^{-1}$) and 14/HS/HSP/A154 (half-sib of *H. spruceana*; 0.5 g $tree^{-1}$ $tap^{-1}$) also had very low [ATP] of 80 and 53 µM, respectively. Overall, high-

**Table 2. Juvenile test-tap yield and latex [ATP] of progenies.**

| Progeny | Test-tap yield (g tree$^{-1}$ tap$^{-1}$)*,# | Latex [ATP](μM) |
|---|---|---|
| 14/C33 | 31.5 | 375.7 |
| 14/HS/RRII430/C64 | 29.9 | 309.9 |
| 14/HS/Fx516/D3 | 28.2 | 315.3 |
| 14/HS/RRII430/C48 | 28.0 | 355.2 |
| 14/A216 | 19.1 | 268.1 |
| 14/HS/Fx516/A248 | 19.0 | 305.9 |
| 14/HS/RRII430/D227 | 18.1 | 206.1 |
| 14/HS/RRII430/D142 | 17.3 | 266.8 |
| 14/HS/RRII430/D19 | 16.6 | 206.3 |
| 14/HS/RRII430/D26 | 15.0 | 202.9 |
| 14/HS/RRII430/D78 | 14.9 | 193.0 |
| 14/HS/RRII430/D248 | 14.8 | 339.0 |
| 14/HS/RRII430/D66 | 14.1 | 171.0 |
| 14/HS/RRII414/D97 | 13.6 | 261.7 |
| 14/OP/OPCES/A197 | 13.0 | 241.3 |
| 14/HS/RRII414/C3 | 12.8 | 359.4 |
| 14/HS/RRII430/D231 | 12.4 | 240.2 |
| 14/HS/Fx516/A95 | 12.2 | 273.4 |
| 14/HS/RRII430/D71 | 11.6 | 141.7 |
| 14/HS/RRII430/D225 | 11.4 | 173.4 |
| 14/HS/RRII414/D209 | 11.2 | 145.4 |
| 14/HS/RRII430/D138 | 11.2 | 122.6 |
| 14/HS/RRII414/D215 | 10.8 | 122.3 |
| 14/HS/RRII430/D226 | 10.2 | 215.6 |
| 14/HS/Fx516/D5 | 9.5 | 134.0 |
| 14/HS/RRII414/C5 | 8.7 | 124.4 |
| 14/HS/RRII414/D214 | 7.0 | 171.8 |
| 14/HS/RRII430/D250 | 7.0 | 161.6 |
| 14/HS/RRII414/D205 | 6.6 | 182.3 |
| 14/HS/RRII430/D83 | 5.9 | 164.0 |
| 14/A143 | 4.8 | 124.3 |
| 14/A236 | 1.0 | 46.1 |
| 14/HS/HSP/A154 | 0.4 | 52.9 |
| 14/A240 | 0.3 | 79.9 |
| Mean | 13.2 | 207.5 |
| Range | 0.3-31.5 | 46.1-375.7 |
| S.E. | 1.3 | 15.1 |

*Mean over three years; #Progenies are listed in descending order, based on their yield performance.

yielding half-sib selections of RRII 414, RRII 430 and Fx 516 also had very high latex [ATP] of more than 300 μM.

Regression analysis revealed a direct relationship ($R^2$=0.66) between latex [ATP] and test-tap yield of the progenies (Fig. 1), which conformed with the earlier findings from investigations with clones (Sreelatha *et al*., 2014; Dey *et al*., 2018). In general, high-yielding progenies had more latex [ATP] than low-yielders and *vice versa*. ATP is a direct source of energy for the conversion of sucrose to poly-isoprene molecules (rubber). It indirectly affects rubber yield, mediated through lutoid membrane ATPase activity (Sreelatha *et al*., 2014). Hence, higher levels of ATP in latex of high yielding progenies indicated that ATP could be involved in increasing the efficiency of rubber biosynthesis, as reported earlier (Sreelatha *et al*., 2014).

Presently, '*test tapping*' or juvenile tapping of seedlings at the age of two to three years in the nursery stage is the only recommended strategy for early screening of large populations for assessing their yield potential and shortlisting the selections before proceeding to further clonal evaluation (Ho, 1976; Tan, 1987). Only a very low percentage of selections (based on test-tap yield) from seedling nurseries turned into high yielders. Test-tapping is mandatory and cannot be dispensed with until more reliable methods for phenotype selection are evolved (Mydin, 2012). Nevertheless, the correlations between nursery yield (based on test-tapping) and mature yield though significant, are still not very strong enough (r = 0.22 to 0.26) to make juvenile yield evaluation by test-tapping alone a completely reliable method for early selection as only 25 per cent of selections from seedlings gave significantly higher yield at mature stages (Tan, 1998; Mydin, 2012). Thus, more detailed information on major yield components and stable sub-components, including biochemical components in the immature phase, could help evolve more reliable parameters for early prediction of yield with more precision (Licy *et al*., 1998; Prabhakaran Nair, 2010).

Although the high correlation between latex [ATP] and mature yield has already been established using clones of various ages and mature polycross population (Sreelatha *et al*., 2004, Sreelatha *et al*. 2014, Dey *et al*., 2018), so far, no such studies have been carried out using young seedlings. The present study conducted using nursery seedlings of a heterogeneous population consisting of half-sib, full-sib and polycross progenies similarly demonstrated

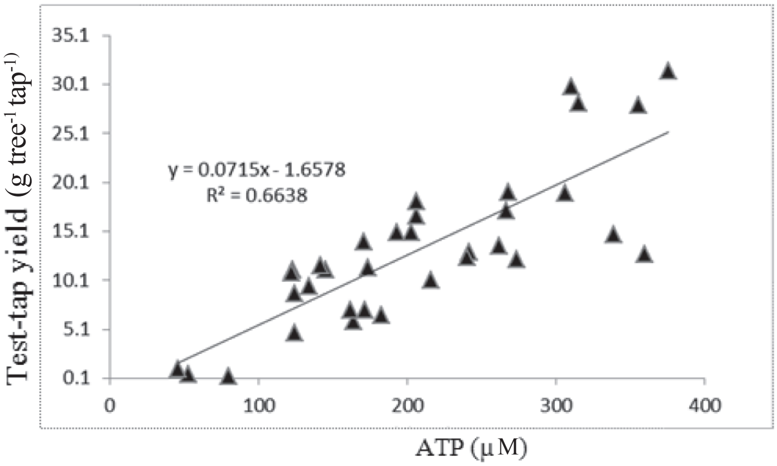


**Fig. 1. Relationship between test-tap yield and latex [ATP]**

a very strong relationship between juvenile test-tap yield and latex [ATP] irrespective of sibling composition. Thus, only seedlings with high test-tap yield and high latex [ATP] should be selected in order to ensure more precision in the recovery of high yielding genotypes to forward them to subsequent stages of clonal evaluation in larger field trials.

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