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Biomass Production and Nutrient Accumulation by Natural Rubber (*Hevea brasiliensis* Wild. Ex A. Juss.) Müell. Arg. Clones in a Humid Tropical Area in South India

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Abstract

Natural rubber (*Hevea brasiliensis* Wild. Ex A. Juss.) Müell. Arg. is an important commodity crop grown in world over for industrial raw material rubber latex for various products, mainly tyre manufacturing. *Hevea* propagation is through clones evolved by breeding as cultivars with desired characters. This article presented the biomass and nutrient accumulation of four important *Hevea* clones *viz*. RRII 105, RRII 118, RRII 203 and GT1 at 30 years age. Biomass and nutrient concentration of tree components *viz*. trunk, branches, leaf and root were assessed by uprooting the trees in the field and standing trees using allometric equation. Among the different clones, RRII 118 and GT1 recorded higher biomass compared to RRII 105 and RRII 203. Above-ground biomass (88-93 per cent) varied more than below-ground biomass (7-11 per cent). The high yielding clones had higher leaf and root biomass. Drought tolerant and timber clones *viz*. RRII 118 and RRII 203 recorded higher K and high yielding clone RRII 105 had higher Ca accumulation. Biomass removal of these clones may lead to deficiency of K and Ca in soil and hence needs the external supplements. The relation of high Ca content and leaf disease of fungal origin is promising for further studies. The higher accumulation of iron and manganese indicated the tolerance of *Hevea* to these elements and possibility of phytoremediation. The per cent contribution of nutrients to total biomass varied less between clones and was below 3 percent at the age of 30 years and this is evidence of adjustments in proportions of nutrients in *Hevea* irrespective of clonal variations.

Keywords

Biomass; Nutrient accumulation; Tree components; *Hevea* clones

Introduction

Natural rubber (NR) tree (*Hevea brasiliensis* Wild. Ex A. Juss.) Müell. Arg. is unique in the production of natural rubber, and it contributes almost 99 per cent of the requirements of the natural rubber in the world (Perron *et al*., 2021; Karunaichamy and Rajagopal, 2020). It is an important commercial source of natural rubber latex (Hytonen *et al*., 2019) and is a forest tree species native to Brazil found in the Amazon River basin (Rekha *et al*., 2016). It is included in family *Euphorbiacea* as a monocotyledon and growing in perennial nature with long duration of 30-32 years. The rubber tree is a quick growing sturdy tree with a tall trunk and thick canopy prevailing in tropical conditions. Rubber plants take 4 to 5 years for canopy closure and grow to full sized trees in 15 to 20 years (Karthikakuttyamma, 1997). The harvesting crop is the latex that flows from the bark of the tree by a systematic wounding called tapping. The productive economic life of rubber trees (Joseph and Jacob, 2020) is around 25-30 years. After 30-32 years the trees are cut down and replanted with new clones. Natural rubber (NR) is one of the major commodity crops in the economy of India because of its huge industrial application of which the important ones are the tyre manufacturing and export of value-added products (James *et al.*, 2018). In India, NR cultivation and establishment of large plantations were initiated more than one hundred years ago, and rubber cultivation is mainly confined (85 per cent) to the state of Kerala (Pradeep *et al*., 2020). Development of a clone is done by breeding programme (Abraham and Mydin, 2020; Chandra *et al*., 2020) through the selection of the desired characters. The clones are the modified versions of plants to improve latex, the economic produce of the rubber tree, and other secondary characters like drought cold and disease tolerance. Propagation of the rubber tree is vegetative through budding of the scion portion into the stock plant of the earlier raised seedlings from sprouted seeds. The clones are used as the important planting material having different varieties for rubber cultivation. Tree crops are more important for higher biomass production and nutrient accumulation with long residence in the soil (Perron *et al*., 2021). The quantification of biomass, nutrient reserve and partitioning characteristics of trees accounts towards the site productivity, plant activity and nutrient pattern (Jing *et al*., 2020). Beside these, an understanding about the biomass production, partitioning and nutrient accumulation in various plant parts has an important role in nutrient budgeting for the development of crop growth models and crop response for evolving strategies to enhance productivity (Hytonen *et al*., 2019). However, the accumulation for each nutrient is different. Primarily, certain nutrients are rich in concentration in certain plant varieties in accordance with the plant activities. The clone-wise biomass production and nutrient accumulation of rubber are useful in nutrient budgeting and in understanding the nutrient requirements of different rubber clones, role of nutrients to improve crop production, tolerance to biotic and abiotic stress, resistance to diseases, and wood properties of the trees. Biomass and nutrient accumulation data can be helpful in selecting the clones to use soil reserve judiciously. Biomass data is also very important in estimating carbon stock and carbon sequestration capacity and, thereby, in ascertaining the carbon crediting. Biomass and nutrient budgeting in clone RRII 105 at 20 years age in the traditional region of Kerala was reported by Karthikakuttyamma *et al*. (2004). The information on biomass and nutrient accumulation in different rubber clones deserves more attention because the data on this domain is scanty. The clones selected for the present study perform differently in terms of yield potential, stress tolerance, disease resistance and wood properties.

In view of above, the present study was aimed at studying the biomass characteristics, nutrient partitioning and nutrient accumulation in the four important clones of *Hevea* to know the variation between clones for exploring further the possibility of selection of suitable clones. The hypothesis of the study is that the clones selected have variability in biomass production, nutrient characteristics, nutrient partitioning, nutrient accumulation and related plant properties.

Materials and Methods

Site characteristics

The location of the study was the Central Experimental Station (CES) of The Rubber Research Institute of India, Rubber Board located at Chethackal in Pathanamthitta district, the south-eastern district of Kerala (9º22′ N and 76º50′E and 100 msl), India. The region received average annual rainfall of 3500 mm generally, with mean minimum and maximum air temperature of 22.4º C and 30.8º C, respectively, under humid tropical climate. The soil comes under the classification of clayey-skeletal, kaolinite, isothermic and Ustic Kanhaplohumult is the international classification name of type of a soil with a depth of 100 cm (NBSS-LUP, 1999). The general soil nutrient status was high in organic carbon (2.52 per cent), medium in available P (14 mg kg^{-1} soil) and medium in available K (92.5 mg kg^{-1}). The soil pH was 4.95, which is strongly the acidic.

Experimental design

Four important clones of natural rubber (*Hevea brasiliensis*) Müell. Arg., *viz*. RRII 105, RRII 118, RRII 203 and GT1, were selected for the study. The first three clones were evolved through breeding by the Rubber Research Institute of India (RRII) and the fourth one was an Indonesian clone Gondang Tapen (GT) brought to India under clone exchange programme. The clone RRII 105 is the popular clone included as the category1(officially released for planting after small scale, large scale and multi-locational on-farm evaluations) of the approved clone recommendations of the Rubber Board. It occupies 85 per cent of the total area under cultivation in India. It is widely cultivated in the traditional belt (extending from Kanyakumari district of Tamil Nadu state in the south through Kerala to Coorg district of Karnataka state in the north) and non-traditional region in India (*viz*. North-Eastern, Konkan and Eastern region). Traditional regions are having congenial agro-climatic conditions for rubber cultivation. In the non-traditional region, the soil is suitable but the climatic constraints like severe drought, cold stress and wind events, are the limitations. The clones RRII 203 and GT 1 are included in category 11 (allowed for planting in 50 per cent of the total area along with another 50 per cent under category 1 clones). RRII 118 is in category 111 (superior clones with proven merits and limited for planting for the experimental purpose) as reported by Mydin *et al*. (2017). To evolve the clones for the experiment, the seeds collected from the approved seed garden were germinated and seedlings were raised. The bud patches of scion portion were grafted and multiplied to make plants of each clone for the purpose of planting in the main field. The plants were grown through the immature phase (1-7 years), mature phase (7th year onwards) and latex harvesting stage (7th or 8th year onwards) up to tree felling age at 30 years. The trees were planted at a spacing of 4.9 m × 4.9 m in randomised block design (RBD) with 5 replications during June-July 1985. All cultural operations including establishment of leguminous ground cover *Pueraria phaseoloides*, regular weeding and spraying for disease management were followed uniformly as per the recommendations of the Rubber Board (1980). Since this is a clone evaluation trial, the management practices were identical for all the clones. Rubber has specific manurial practices for the immature phase (1-7 years after planting) and a mature phase (from 5th year onwards). Accordingly, the plants were dosed with 10-10-4-105 NPKMg fertilizer mixture, *viz*. 225 g $plant^{-1}$, three months after planting during September-October, 450 g $plant^{-1}$ (in two equal splits during April-May and September-October during 2nd year and 4th year), and 550 g $plant^{-1}$during 3rd year. From 5th year onwards, uniform fertilizer dose of 30:30:30 NPK by urea (65 kg), rock phosphate (150 kg) and muriate of potash (150 kg) on per hectare basis for mature trees (recommended dose) were applied annually in two equal splits during April-May and September-October, covering all clones in the productive yielding phase up to 25 years. Thereafter, no fertilizer was given to all the clones when they reached to tree felling stage at 30 years age.

Tree sampling and analysis

Two trees of four different clones at 30 years age in the same location of an experimental field of clone trial were selected for the study. Trees were uprooted and total height and girth at 150 cm from the bud union were taken as the basal parameters. Trees were divided into four morphological units as tree components, *viz*. trunk, branches, leaf (small twigs and petiole) and root in each clone. This was used for the biomass estimation and nutrient accumulation of clones. To assess the biomass, fresh weight of each component was recorded immediately after felling by using appropriate weighing balance in the field itself to avoid moisture loss. Representative sub-samples were taken from each component that were oven-dried at 65^0C for 72 hours and the dry weights were recorded. Using this, the total dry biomass of trunk, branches, leaves, and root of each clone was estimated. A portion of trunk, branches, leaves and roots were taken for chemical analysis to know the variation in nutrient concentration of these components. The per cent content of major- and micronutrients of all these components was estimated using a known quantity of the ground samples dried at 105ºC for constant weight by applying standard procedures, *viz*., nitrogen (N) estimation by micro-kjeldhal method using acid digestion and distillation, phosphorus (P) estimation and potassium estimation by stannous chloride method using spectrophotometer and direct reading flame photometry respectively. The calcium (Ca), magnesium (Mg), and micronutrients, *viz*. zinc (Zn), copper (Cu), manganese (Mn) and iron (Fe), were estimated by direct reading atomic absorption spectrophotometer.

To determine the biomass and nutrient accumulation in more trees, 10 replicates of standing trees of each clone. Thus, total 40 trees were selected at the same location. Girth (trunk) at 150 cm from the basal bud patch of the trees was recorded. Using the girth values, the aboveground biomass of these standing trees was determined by the Shorrock's equation (Shorrock *et al*., 1965). Total above ground dry biomass (kg) was 0.002604(G) ^ 2.7826), where 'G' is the girth (trunk) at 150 cm, which was validated (Ambily *et al*., 2012) for the rubber clones in India. Similar method of the estimation of biomass using allometric equation was reported for the coniferous and broadleaved mixed forest in north-eastern China (He *et al*., 2018) and among *Poplar* SRC clones (Dinko *et al*., 2017). The allometric equation for biomass estimation was also reported in *Olea europaea*, L. Subsp. *cuspidate* in Mana Angetu forest (Kebede *et al*., 2018) and mountain moist evergreen forest in Mozambique (Lisboa *et al*., 2018). Using the per cent contribution of biomass to the components (trunk, branches and leaf) of the uprooted trees, the corresponding biomass of the tree components in standing trees were estimated. Root biomass was around 10 per cent irrespective of the clones in the uprooted trees. Hence, to estimate the root biomass of standing trees, the corresponding root dry biomass per cent of uprooted trees of each clone were used. From this, the total biomass (above-ground + root) of standing trees of clones were calculated. A portion of sub-samples were collected from trunk, branches, leaves and roots of the standing trees (10 numbers each) of every clone to determine the nutrient concentration as per the method used for the uprooted trees. Nutrient accumulation was worked out by multiplying the nutrient concentration with dry biomass derived for the standing trees. Contributions of nutrients to the total dry biomass of the tree in each clone were also calculated.

Statistical analysis

Data were statistically analysed by one way analysis of variance (ANOVA) to compare the growth parameters, biomass, nutrient accumulation and distribution in plant components. Total nutrient accumulation in whole tree basis and contribution of nutrients to total biomass were also compared between clones using one-way ANOVA. When the data were significant at the 5 per cent significant ($p<0.05$) level, a multiple comparison by Duncan multiple range test (DMRT) were performed to describe the significant level of the clones for all parameters. All values shown are mean values for each clone. Means with different letters are statistically different ($p<0.05$). All analyses were conducted by OP stat (Sheorm, 1998).

Results

Growth

Growth characteristics (Table 1) were significantly different ($p<0.05$) among *Hevea* clones. Height recorded were 10.9 m for RRII 105 and 14.7, 14.8 and 15.3, respectively, for RRII 118, RRII 203 and GT 1. The

RRII (p=0.0001) height was recorded of the clone RR 105, and the height of other three clones was on par. Girth was higher (p=0001) in RRII 118 (146.8 cm) and GT 1 (138.7 cm) than in RRII 203 (111.8) cm and RRII 105 (103.8 cm). Moreover, it was observed that the same ratio of girth and height, which comes to 1:10, was observed for each of the clones except RRII 203 (of which the ratio was recorded slightly higher, i.e., 1:13 (p=0.0001)).

Table 1: Growth characteristics (Height, Girth, Girth: height ratio, Shoot: root ratio and Root: shoot ratio of clones *viz*. RRII 105, RRII 118, RRII 203 and GT1. All values showed are mean values. Means with different letters are significantly different (p<0.05).

Clone	*Height (m)*	*Girth (cm)*	*Girth: height ratio*	*Shoot: root ratio*	*Root: shoot ratio*
RRII 105	10.9^{b}	103.8^{d}	$1:10^{b}$	8.01^{c}	0.12^{a}
RRII 118	14.7^{a}	146.8^{a}	$1: 10^{b}$	10.62^{a}	0.09^{a}
RRII 203	14.8^{a}	111.8^{c}	$1:13^{a}$	12.06^{d}	0.08^{a}
GT1	15.3^{a}	138.7^{b}	$1:11^{b}$	14.39^{b}	0.07^{a}

Biomass and partitioning

The variation in biomass was observed in the clones. The biomass production and the yield potential, as per the approved classification (Saraswathyamma *et al.*, 2000), were found different in four clones under study. The growth characteristics of RRII 105 was observed with tall trunk having good branches along with strong union. On the other hand, RRII 118 was a vigorous clone with short trunk having prominent branches like trunk along with secondary branches. In RRII 203, the trunk was long and straight with well distributed and balanced canopy; but in GT1, the trunk was upright and slightly kinked with main branch long and acute angled along with light secondary branches. The yield potentials of the clones *viz*. RRII 105, RRII 118, RRII 203 and GT1 reported by Saraswathyamma *et al.* (2000) were 2400, 1164, 1818, and 1400 $kg^{-1}ha^{-1}$ per year. It was observed that the high biomass accumulating clones was not good in yield.

Significant biomass difference (Figure 1) and biomass partitioning per cent to the total biomass (Figure 2) in plant components were observed between clones. The total dry biomass was 1214.43, 2489.09, 1102.29 and 2055.58 kg/tree for the clone RRII 105, RRII 118, RRII 203 and GT 1, respectively. Among the clones, RRII 118 and GT1 recorded higher biomass (p=0.0001) compared to RRII 105 and RRII 203.

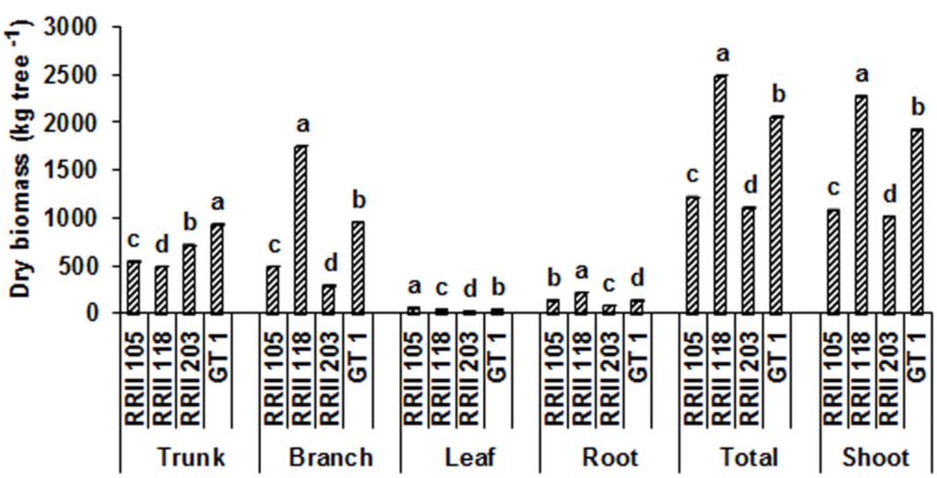

Figure 1: Dry Biomass accumulation (kg $tree^{-1}$) (Total, Shoot and in plant components (Trunk, Branches, Leaf and Root) of clones (RRII 105, RRII 118, RRII 203 and GT1). All values showed are mean values. Means with different letters are significantly different (p<0.05).

The clone RRII 203 recorded higher (*p*=0.0001) biomass (709.76 kg tree^{-1}) and per cent contribution (64.13 %) in trunk and recorded less biomass (289.31 kg tree^{-1}) and per cent contribution (26.14 %) in branches, compared to the clone RRII 118. RRII 118 recorded less biomass (489.67 kg tree^{-1}) and per cent contribution (19.67 %) in trunk and higher (*p*=0001) biomass (1752 kg tree^{-1}) and per cent contribution (70.39 %) in branches. But GT 1 and RRII 105 recorded an equal distribution: around 40 per cent in trunk and branches. Higher (*p*=0.0001) leaf dry matter was recorded in RRII 105 (54.17 kg tree^{-1}) and lowest in RRII 203 (18.81 kg tree^{-1}). Higher per cent leaf dry matter was observed in RRII 105 (4.46%), whereas other clones recorded leaf dry matter of less than 2 per cent. Root biomass was higher (*p*=0.0001) in RRII 118 (214 kg tree^{-1}) and lower in RRII 203 (84.41 kg tree^{-1}). However, the per cent contribution was higher in RRII 105 (11.1), while other clones recorded less than 10 per cent contribution of root biomass. When comparing the clones, 88-93 per cent shoot biomass and 7-11 per cent root biomass was observed at the age of 30 years. Shoot to root ratio in RRII 203 (12.1), GT 1 (11.1), and RRII 118 (10.6) is higher (*p*=0.0001) than RRII 105 (8.1).

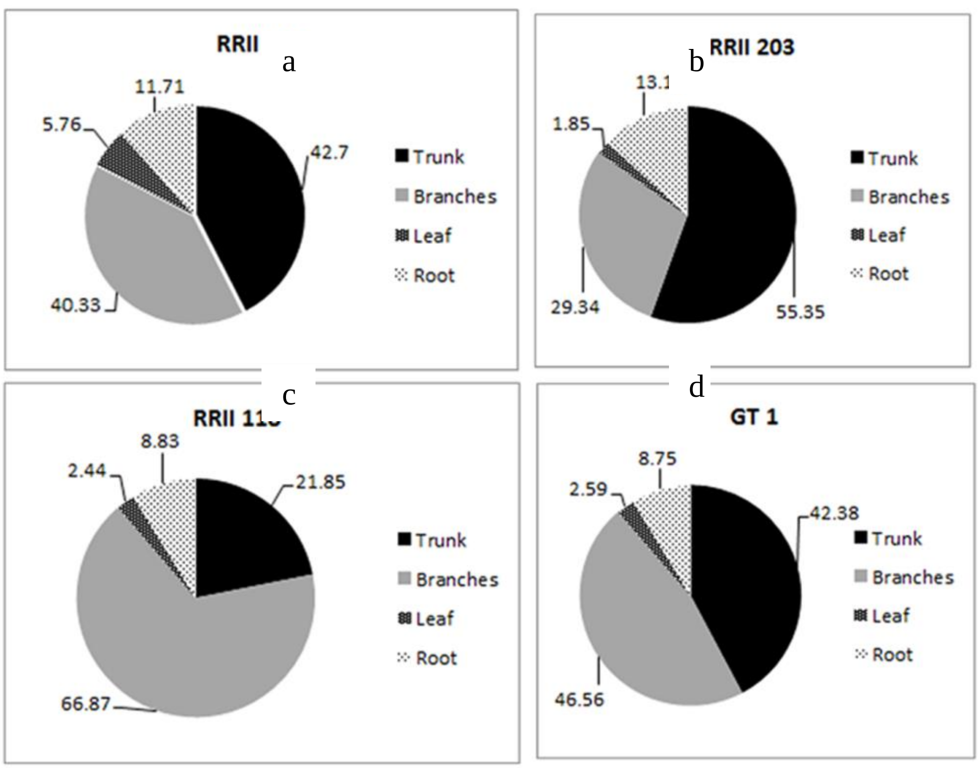

Figure 2: Biomass partitioning (%) of tree components in clone, RRI 105 (a), RRII 118 (b), RRII 203 (c) and GT 1(d). The data label denotes the per cent values of plant components in each clone.

Nutrient concentration

Nutrient concentration in tree components (Figure 3a-i) varied among different clones. Significant variation (*p*=0.0001) in nutrient concentration in tree components and between clones except N concentration in branches and Fe concentration in root were observed. Some indications such as high K (*p*=0.0001) in trunk, branches and leaf of RRII 118 and trunk of RRII 203 and high Ca (*p*=0.0001) in trunk and branches of RRII 105 is to be investigated further. Because RRII 118 and RRII 203 were known drought tolerant clones and RRII 105 is the popular high yielding clone, the difference in K and Ca content observed in these clones may be related to drought tolerance and yield, respectively. Therefore, this is to be considered for detailed studies to investigate whether there is any relation of these nutrients with the drought tolerance or yield. For the micronutrients also, there was significantly higher (*p*=0.0001) variation in Mn and Fe concentration in leaf of different clones. The clone RRII 118 recorded the highest (p=0.0001) Fe and Mn content in leaf. The clone RRII 105 also recorded higher (p=0.0001) Fe content in leaf. This is an indication of tolerance of these nutrients in rubber. The detailed study of the role of nutrients in rubber will enlighten further.

Table 2: Nutrient accumulation (N, P, K, Ca, Mg, Zn, Cu, Fe and Mn) of clones *viz*. RRII 105, RRII 118, RRII 203 and GT1 on per tree basis in kilogram per tree (kg/tree) All values showed are mean values. Means with different letters are significantly different (p<0.05). Nutrient accumulation in plant components (trunk, branches, leaf and root) in g/kg.

Clone & Tissues)	*Nutrient accumulation (kg/tree)*								
	N	*P*	*K*	*Ca*	*Mg*	*Zn*	*Cu*	*Fe*	*Mn*
RRII105	7.91[b]	0.59[d]	5.88[d]	14.79[a]	1.45[c]	0.03[c]	0.01[c]	0.04[c]	0.08[b]
(Trunk)	(4.29)	(0.36)	(4.24)	(12.74)	(1.34)	(0.02)	(0.01)	(0.29)	(0.05)
(Branch)	(4.11)	(0.33)	(3.87)	(15.14)	(0.81)	(0.02)	(0.01)	(0.18)	(0.07)
(Leaf)	(36.19)	(2.68)	(10.56)	(5.63)	(2.46)	(0.15)	(0.02)	(0.83)	(0.45)
(Root)	(6.06)	(0.09)	(8.45)	(1.84)	(1.49)	(0.03)	(0.01)	(0.48)	(0.03)
RRII118	9.79[a]	1.12[a]	26.29[a]	7.11[c]	3.8[b]	0.05[a]	0.03[a]	0.47[b]	0.21[a]
(Trunk)	(3.84)	(0.35)	(6.83)	(4.37)	(1.89)	(0.02)	(0.05)	(0.33)	(0.04)
(Branch)	(3.21)	(0.40)	(7.23)	(2.31)	(1.39)	(0.01)	(0.01)	(0.12)	(0.11)
(Leaf)	(38.58)	(2.79)	(18.62)	(7.51)	(2.51)	(0.18)	(0.12)	(0.91)	(0.78)
(Root)	(4.71)	(0.16)	(12.41)	(2.66)	(1.68)	(0.01)	(0.02)	(0.32)	(0.03)
RRII203	5.01[c]	0.84[c]	10.41[b]	3.24[d]	1.58[c]	0.02[d]	0.01[c]	0.04[c]	0.04[c]
(Trunk)	(4.11)	(0.71)	(11.7)	(3.19)	(1.67)	(0.15)	(0.01)	(0.48)	(0.03)
(Branch)	(3.52)	(0.52)	(3.17)	(1.81)	(0.54)	(0.01)	(0.02)	(0.14)	(0.03)
(Leaf)	(34.04)	(2.41)	(14.74)	(9.59)	(5.01)	(0.11)	(0.08)	(0.63)	(0.51)
(Root)	(5.09)	(0.14)	(13.15)	(3.21)	(1.66)	(0.02)	(0.02)	(0.42)	(0.03)
GT1	9.65[a]	1.02[b]	7.55[c]	9.87[b]	4.07[a]	0.04[b]	0.02[b]	0.63[a]	0.03[d]
(Trunk)	(4.41)	(0.55)	(4.89)	(5.94)	(3.06)	(0.16)	(0.01)	(0.42)	(0.41)
(Branch)	(3.81)	(0.24)	(1.38)	(3.71)	(1.11)	(0.01)	(0.01)	(0.19)	(0.06)
(Leaf)	(34.95)	(2.57)	(11.93)	(11.29)	(3.32)	(0.19)	(0.04)	(0.36)	(0.19)
(Root)	(3.83)	(0.!7)	(7.61)	(1.86)	(1.23)	(0.01)	(0.02)	(0.24)	(0.03)

(Values in parenthesis represents the nutrient content of tissues (g/kg)

Nutrient accumulation

The major and micronutrient accumulation, except Fe in root, in tree components (Figure 4a-i) varied among different clones. Of the major nutrients, *viz*. N, P and Mg accumulation in trunk was higher (p=0.0001) in GT 1. Similarly, higher (p=0.0001) K and Ca accumulation in trunk was recorded in RRII 203 and RRII 105, respectively. In branches, the higher (p=0.0001) accumulation of all nutrients compared to other clones was observed in RRII 118. Since the accumulation of nutrients is also a function of the biomass of components, the large biomass of branches in RRII 118 contributes to the higher accumulation of corresponding nutrients. In leaf, N, P and Mg were higher (p=0.0001) in RRII 105; whereas lower K and Ca were observed in RRII 203. In root, RRII 118 recorded higher (p=0.0001) N, K, Ca and Mg. Among the clones, micronutrients *viz*. Zn, Fe and Mn accumulation in trunk was higher in GT1. The Cu was lowest in RRII 105. In branches, all micronutrient accumulation was higher (p=0.0001) in RRII 118. In leaf, Zn and Fe were higher (p=0.0001) in RRII 105 with higher (p=0.0001) Cu in RRII 118. In root, Zn, Cu and Mn were higher (p=0.0001) in RRII118 compared to other clones. The nutrient accumulation is related to the biomass characteristics and nutrient concentration of the plant components, and both contribute to the observed differences among the clones. The role of nutrients to plant activities is to be further studied.

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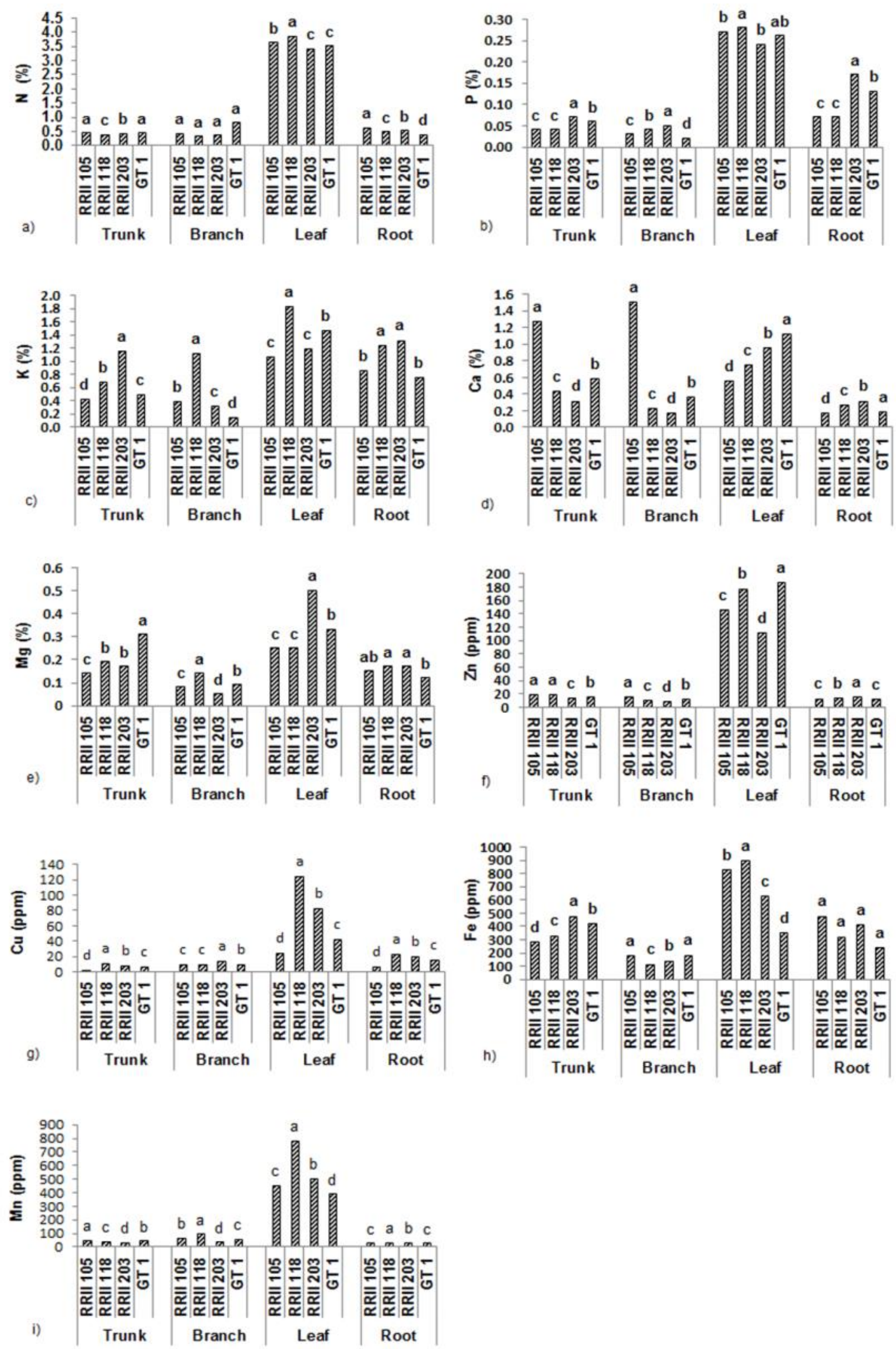

Figure 3: Nutrient concentration (N, P, K, Ca, Mg, Zn, Cu, Fe and Mn) in plant components (Trunk, branches, leaf and root) of clones *viz*. RRII 105, RRII 118, RRII 203 and GT1.All values showed are mean values. Means with different letters are significantly different (p<0.05).

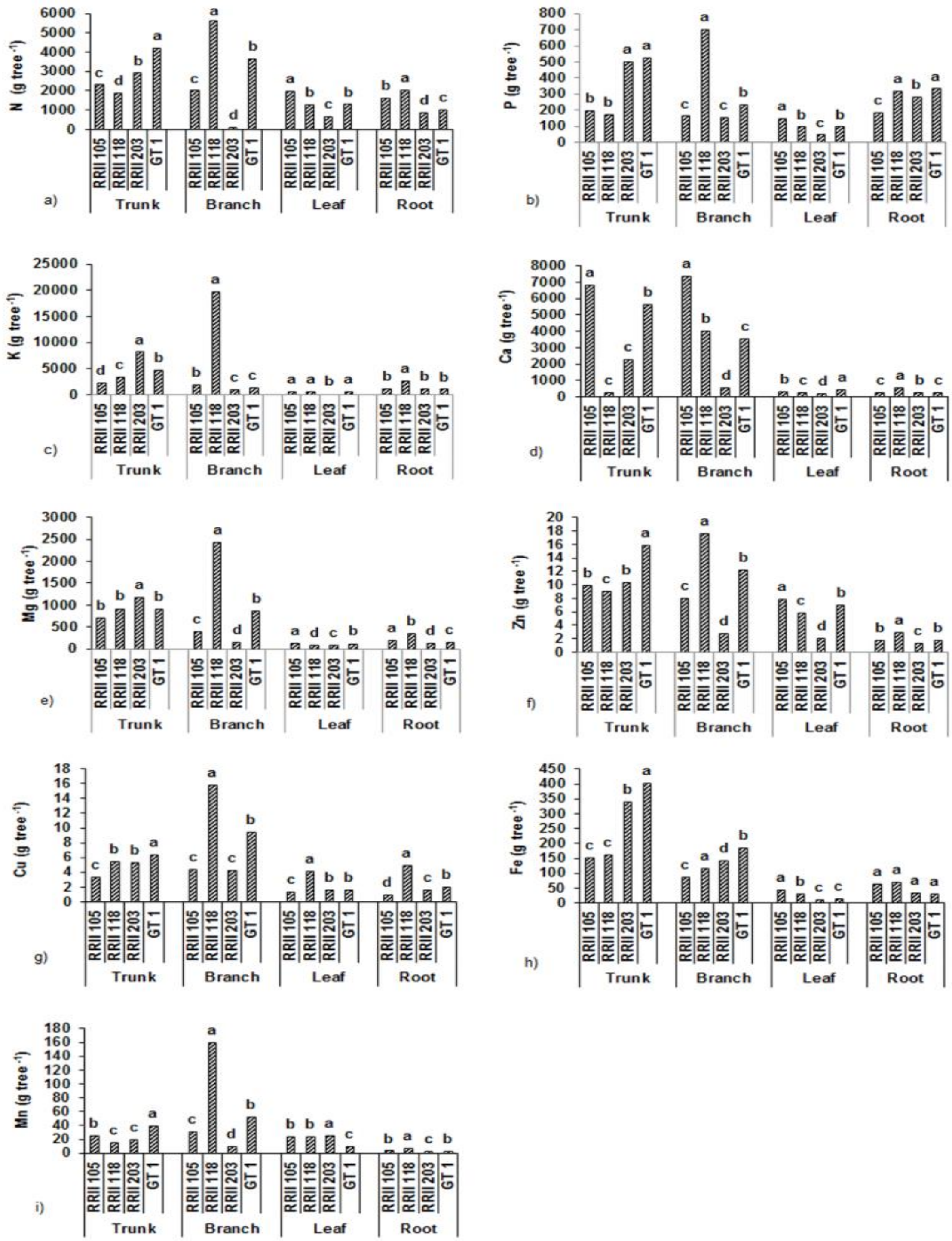

Figure 4: Nutrient accumulation (N, P, K, Ca, Mg, Zn, Cu, Fe and Mn) in plant components (Trunk, branches, leaf and root) of clones *viz*. RRII 105, RRII 118, RRII 203 and GT1.All values showed are mean values. Means with different letters are significantly different (p<0.05).

Nutrient accumulation in the whole tree (Table 2) showed variation between clones. The values of major and micronutrient accumulation in the whole tree were N (5.01-9.79), P (0.59-1.129), K (5.88-26.29), Ca (3.24-14.79), Mg (1.45-4.07), Zn (0.02-0.05), Cu (0.01-0.03), Fe (0.04-0.63) and Mn (0.03-0.21) kg tree^{-1}. Higher (p=0.0001) Ca accumulation was found in RRII 105. But RRII 118 and RRII 203 accumulated higher (p=0.0001) K than Ca. The nutrient order found in the studied clones was Ca>N>K>Mg>P (except in RRII 118 and RRII 203). Micronutrients were in the order of Fe>Mn>Zn>Cu in *Hevea* clones. Higher (p=0.0001) Zn, Cu, Fe and Mn accumulation was observed in clone RRII 118. The highest (p=0.0001) Fe accumulation was recorded in GT 1. Furthermore, detailed studies are required to know the role of these elements or any toxicity due to these elements in rubber. Is it a genetic character contributing to low yield in different cultivars?

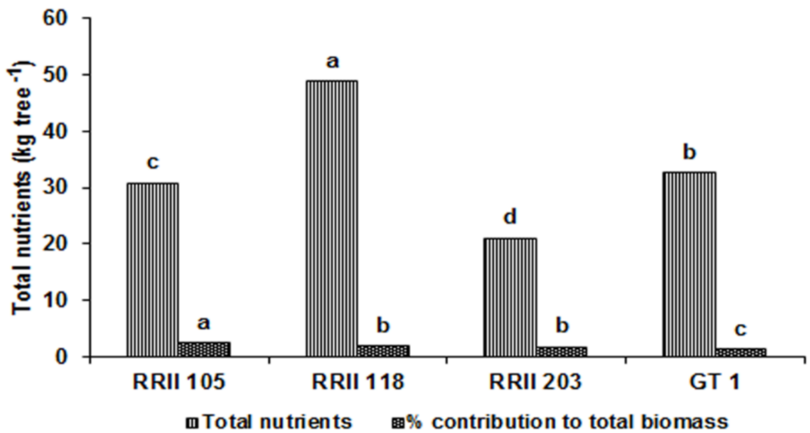

Figure 5: Total nutrients and per cent contribution of nutrients to total dry biomass in different clones. The bar in grey colour with vertical lines denotes the total nutrients and bar in black colour with dots denotes per cent contribution of nutrients to total dry biomass. All values showed are mean values. Means with different letters are significantly different (p<0.05).

Total of all nutrients in the clones and per cent contribution of nutrients to total dry biomass are presented in figure 5. Total of all nutrients in RRII 105, RRII 118, RRII 203 and GT1 were, respectively, 30.96, 48.86, 21.19 and 32.85 kg tree^{-1}. Total nutrients varied much as these were related to the biomass of the clones. But per cent contribution of nutrients was 2.55 for RRII 105, 1.96 for RRII 118, 1.92 for RRII 203 and 1.61 for GT 1. Among the clones under study, RRII 105 had higher (p=0.0001) per cent contribution of nutrients, whereas the nutrient concentration was lower (p=0.0001) in GT 1. RRII 118 and RRII 203 were on par. The yield potential of these two clones was also very much different. Even though the total nutrients showed much variation between clones, the per cent contribution was not varied correspondingly and was observed as below 3 per cent at the age of 30 years.

Discussion

In *Hevea*, the evolution of clones through breeding with improved qualities is ultimately for achieving enhanced productivity and environmental sustainability. There are different reports that clones were different in their performances (Mydin *et al*., 2017; Reju *et al*., 2020; Ambily *et al*., 2012; Meenakumari *et al*., 2013) including the girth, biomass especially above-ground biomass, yield, stress, disease tolerance and wood properties. Shorrock (1965) reported the historic initial time studies on girth and above-ground biomass of clones in Malaysia and found a variation between clones. Swamy *et al*., (2006) had observed around 1.5 times increase in girth among two clones of *Populus deltoids* in agrisilviculture plantation. The growth variation of *Eucalyptus* clones was observed by Saravanan (2019). In the present study, the clones studied were different in girth and above-ground biomass. The reason for a similar girth:height ratio observed for all clones except one clone being a timber clone with long straight trunk is found as a clone characteristic of *Hevea.* In the present study, the root biomass was only slightly different as compared to the above-ground biomass, and the root biomass was about 10 per cent irrespective of the clones. In earlier studies (Karthikakuttyamma, 1997), the shoot to root ratio was reported to be 5.92 for the clone RRII 105 at 20 years age. Jessy (2008) reported that the shoot to root ratio to be 4.81 for the clone PB 217 at 19 years age. The root to shoot ratios in these studies were 0.17 and 0.21, respectively, which are different from the clones (0.1-0.12) at 30 years age used in the present study. This indicated that there is age-wise difference existing in the shoot to root and root to shoot ratios in rubber clones. Biomass partitioning of rubber is a species character as reported in other species where the partitioning was in a different manner (Albaugh *et al*., 2006) having root to stem ratio of 0.7-0.5, 0.4 and 0.47 in *Pinus taeda* trees at three sites. Thus, shoot to root ratio is around 50 per cent in *Pinus taeda* trees. Ludovici *et al*. (2002) reported that the root: stem ratio of 0.43 in loblolly pine was 30 per cent root and 70 per cent shoot mass. In agrisilviculture plantations, the root to shoot ratio of *Populus deltoids* was different (0.2 to 0.35) between clones (Swamy *et al*. 2006). In rubber, a different pattern of partitioning was observed in the present study. In *Picea likiangensis*, 14.8 per cent root biomass was reported at 32 years age in Southern China (Davidson *et al*., 1999). The high biomass accumulated clones, *viz*. RRII 118 and GT 1, had lower yield potential as reported in approved cultivar classification (Saraswathyamma *et al*., 2000). Therefore, the inverse relation of biomass and yield was observed in clones of the present study.

The variation in biomass production, partitioning and per cent contribution to plant components of clones was reported by many workers (Dinko *et al*., 2017; Swamy *et al*., 2006; Saravanan, 2019). Similar observation of the higher branch biomass in the highest biomass accumulated clone was found in the hybrid aspen (*Populus tremula* × *P. tremuloides*) clone (Hytonen *et al*., 2020). Variations in biomass partitioning and per cent contribution to total biomass in clones was also found in *Eucalyptus* clones (Saravanan, 2019).

The clonal difference in nutrient concentration was reported in hybrid aspen (*Populus tremula* × *P. tremuloides*) in Finland (Hytonen *et al*., 2020) and natural rubber *Hevea* in Thailand (Hytonen *et al*. 2019; Hytonen *et al*., 2020) and was found different from the present study. This may be attributed as species and location-wise difference. General order of macronutrient content of rubber tree observed (Karthikakuttyamma, 1997; Jessy, 2008) was Ca>N>K>Mg>P and the difference in the nutrient of two clones of the present study can be attributed as a clonal character related to the different plant activities in these clones. The different pattern of nutrient distribution in the tree *Picea likiangensis* in Southern China was reported by Liu *et al.* (2004). Nagaraju *et al.* (1997) reported that the plant species differ in their nutrient elements in plant components. Kleiber *et al*. (2019) have reported that the per cent nutrient content of Lime tree and Horse chestnut tree differed in their health indicated the species specificity in nutrient pattern. In the present study, there was variation in nutrient concentration in different plant components. The higher Ca in the trunk and branches in the clone RRII 105, high K in all the plant components of clone RRII 118, high K in the trunk and root of RRII 203 and high Ca and K in the leaf of clone GT1 may relate to different plant activities like yield variation, drought tolerance, disease resistance and timber properties. Higher leaf K was reported as an index of adaptation to drought stress in *Hevea* (Ambily *et al*., 2020). The observation of higher K in the clones of the present study indicated the role of K in drought tolerant property in *Hevea* and can be further studied for breeding for drought tolerant clones. The clone RRII 105 is a high yielding clone and RRII 118 and RRII 203 are drought tolerant. This may be a clone specific difference due to that the Ca content of plants is, to a large extent, genetically controlled and little affected by the Ca supply in the root medium (Lungstrom and Stjernqust, 1993). Higher Ca may be attributed as the higher plants often contain Ca in appreciable amounts. Calcium is largely immobilized in cell walls and would be expected to accumulate with age (Lungstrom and Stjernqust, 1993). It was reported (Fromm, 2010) that Ca and K application was beneficial for the formation of wood in trees. The role of Ca and K is in cambial activity, xylem development and xylogenesis. The clones already identified as timber clones had a high K in the present study; it is also somewhat related to the role of K in wood formation in *Hevea*. The higher K content also relates to the drought tolerance and the observed high leaf K and root K in RRII 118 and RRII 203 may be due to drought tolerant property of these clones. The drought tolerant property of *Hevea* clones based on the physiological properties was reported by Neethu *et al*.

(2021). Antony *et al*. (2018) reported that RRII 203 showed high K in leaf and root in the present study, which had better performances in dry areas in Karnataka. This is also evidence of a relation of K and drought tolerant properties of this clone. However, the clone RRII 105 with low K content was found as susceptible to more leaf drying to drought stress also support the role of high K in drought tolerance of clones. As far as the leaf diseases are considered, abnormal leaf fall and powdery mildew caused by *Phytophthora palmivora* and *Oidium hevea steinm*, respectively, are the major crop loss resulting diseases in India (Mazlan *et al*., 2019). Among the clones studied, the clone RRII 105 and GT1 were reported as resistant clones to *phytophthora* leaf fall on prophylactic spraying (Edathil *et al*., 2000). In a recent report (Khompatara *et al*., 2019), *Sargassum polycystum*, a seaweed extract, was found effective to increase resistance to the *Phytophthora* mediated leaf fall disease in rubber seedlings. Bharat *et al*. (2018) reported that an alga, *Sargassum polycystum*, has the elemental concentration of sodium (85.3 mg L^{-1}), chlorine (75.02 mg L^{-1}) and calcium (69 mg L^{-1}) in higher quantity and among this Ca was in appreciable quantity. This pointed out the role of Ca in controlling *Phytophthora* leaf fall disease. The enhanced resistance may be due to high Ca content in these clones. Disease control using chemical fertilizers usually have an adverse effect on the environment, soil and a reason for toxicity of living beings associated (Khompatara *et al*., 2019). In consideration of these, the identification of inherently resistant clones is more beneficial and easier as a control measure. Therefore, the observation of high Ca in the clones can be a basis for the detailed study of the elemental role of Ca and further in the breeding and selection of resistant clones. The nutrient order of macronutrient content of rubber clone RRII 105 and PB 217 observed was Ca>N>K>Mg>P (Karthikakuttyamma, 1997; Jessy, 2008). But the nutrient order in the present study varied among clones even though clone RRII 105 and GT1 had similar order of macro nutrient concentration. Similar order of nutrient elements was reported in orange trees (Mattos *et al*., 2003). Species difference is evident as reported by Davidson *et al.* (1999) in the nutrient accumulation of the two species *viz*., *Inga dens flora* and *Pollalesta discolor*, in which nutrient order is N>K>Mg>P>Ca. This was different from *Hevea* clones studied. Similar concentration pattern of *Hevea* clones except RRII 118 was reported in Apple trees in Himachal Pradesh (Sharma and Bandari, 1995). Kumar *et al*. (2005) reported the nutrient concentration in bamboo (*Bambusa bambos*) tree in a different manner from observed in *Hevea*.

Species difference was observed in the case of micronutrients also. The significant clonal difference in the concentration of Zn and Cu was reported by Hytonen *et al*. (2020). In two tree species *viz*., *Inga densiflora* and *Pollalesta discolor*, the micronutrient concentration was in the order of Mn>Fe>Zn>Cu (Davidson *et al*., 1999), which was different from that observed in rubber. This may be attributed to the differences in the uptake and metabolism, according to the requirement of the crops. Generally, Fe toxicity is happening when Fe concentration exceeds 1,000 ppm. But in rubber, Fe concentration in the leaf itself was 899 ppm in clone RRII 118 without toxicity symptoms. Mn is also very important in the sense that it is having biochemical functions. Mn exceeding 160 ppm causes toxicity (Alejandio *et al*., 2020). In acidic soils high in manganese availability, plants can take up considerable amounts of Mn so that levels in the order of 1,000 ppm Mn in the dry matter are not uncommon (Alejandio *et al*., 2020). But when it exceeds 2,000 ppm, toxicity is often observed. In the clone RRII 118, Mn concentration in the leaf was 780 ppm and in other three clones it ranged from 387 to 499 ppm. Higher level of Fe and Mn in leaf in *Hevea* clones indicates that *Hevea* is tolerant or accumulates these elements. Yan *et al*. (2020) reported the recent development of phytoremediation, an eco-friendly technique for the removal of metal pollutants by growing plants having ability to accumulate these elements. This indicated that the tolerance of higher Fe and Mn concentration in *Hevea* may have the possibility for phytoremediation. The Zn and Cu concentration is comparatively less in rubber. Usually, copper is taken up by plants in only a very small quantity. Pietrini *et al*. (2019) reported that, in most of the plants, Cu is important in physiological functions in a concentration range of 3-20 ppm. The nutrient requirement and role of nutrients is to be further explored in *Hevea*.

The total dry biomass (t/tree) in rubber tree in Thailand had 2.4, 0.2, 3.4, and 4.8 kg N, P, K, Ca, respectively, and 380–700, 36–64, 530–980 and 750–1,360 kg per hectare basis (Hytonen *et al*., 2019). This was different from the nutrient accumulation in plant components, total nutrients and per cent contribution of nutrients to total dry biomass observed in *Hevea*. Usually in plant composition, C, H and O comes to around 94-99.5

and nutrient composition is 0.5-6.0 per cent (Mills and Jones, 1996). In the present study, the nutrient contribution was below 3 per cent and within this limit. The total nutrients and per cent contribution of nutrients to total dry biomass in clones indicated that there were adjustments in the nutrient proportions as a species characteristic of *Hevea* and related to the clonal characteristics like variation in growth, yield, and disease and stress tolerance. This is to be further studied in detail to obtain confirmed results to relate the biomass and nutrient accumulation with clonal characteristics. In the present study, clones were different in biomass, nutrient concentration and nutrient accumulation. This is evidence of clonal variation in biomass and nutrient accumulation in *Hevea* clones. The biomass and nutrient budget of the clone RRII 105 was reported by Karthikakuttyamma *et al*. (2004), but the data for different clones is useful for the further detailed study of the role of nutrients in rubber tree and for the nutrient management to clone-wise recommendations judiciously for productivity enhancement and sustainability of rubber ecosystem.

Conclusion

The present study indicates that natural rubber (*Hevea brasiliensis*) clones differ in their biomass production and nutrient accumulation. Biomass partitioning and nutrient distribution pattern was also varying in different clones. Highest yielding clones (RRII 105 and RRII 203) recorded higher leaf and root biomass compared to low yielding (RRII 118 and GT1) clones. The inverse relation of biomass and yield potential was recorded in these clones. While above-ground biomass showed much variation, the below-ground biomass not varied much and, irrespective of clones, about 10 per cent root biomass was observed. This was found as a clone characteristic of *Hevea*. There were no characteristic variations in leaf concentration between clones. High K content in the drought tolerant clones RRII 118 and RRII 203 may be related to drought tolerance and timber properties. High Ca in high yielding clone RRII 105 is a relation of Ca to high yield and high Ca in RRII 105 and GT 1 may be due to a tolerance to *phytophthora* leaf disease in *Hevea*. The observed nutrient relation is pertinent in the relation of these nutrients in yield, wood properties, and drought and disease tolerance. During biomass removal of these clones, there is a possibility of deficiency of K and Ca in the soil. The per cent contribution of nutrients to total biomass varied less between clones and was below 3 per cent at the age of 30 years for all clones and this is evidence of adjustments in proportions of nutrients in *Hevea*. Higher accumulation of iron and manganese indicated that *Hevea* is tolerant to these elements and is a potential for phytoremediation. Detailed study may provide more insight into the relation of biomass and nutrient accumulation to various plant activities in rubber tree and different clones of *Hevea* so as to utilize the soil reserves more efficiently and for further breeding to improved varieties and selection of clones to increase the productivity of rubber.

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Authors' Declarations and Essential Ethical Compliances

Authors' Contributions (in accordance with ICMJE criteria for authorship)

Contribution	Author 1	Author 2
Conceived and designed the research or analysis	Yes	Yes
Collected the data	Yes	Yes
Contributed to data analysis & interpretation	Yes	Yes
Wrote the article/paper	Yes	No
Critical revision of the article/paper	Yes	Yes
Editing of the article/paper	Yes	No
Supervision	No	Yes
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Research involving human bodies (Helsinki Declaration)
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