ROOT DEVELOPMENT DOES NOT ANTAGONIZE REGENERATION OF DOUBLE NODE VEGETATIVELY PROPAGATED TEA (*Camellia sinensis*) CULTIVAR PROPAGATION

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Abstract

Gaps in tea farms and plantations resulting from poor planting material, storms, accidental mechanical operations and pests lead to monumental yield losses. Conventionally, single node cuttings have been used for infilling of such gaps in the past; however they take very long to regenerate and to eventually cover the gaps and are also expensive compared to normal seedlings. Therefore, double node cuttings can offer a faster and robust alternative for production of the infilling material. However, the fear has been that it poses greater demand for photosynthates for recovery and regeneration of shoots from more nodes and this may lead to competition between roots and shoots for assimilates and hence premature death instead. The current work was set to test the hypothesis that more nodes/ plus leaves lead to more demand of substrates from source to the nodes with little going to the roots, hence low chance of root initiation and thus not suitable for production of tea seedlings. A study to evaluate potential of different types of tea node cuttings (Single Node cutting SNC – and Two Node cutting TNC) as planting material was therefore conducted at Kangaita farm at the Kenya Tea Development Agency in Kirinyaga, Kenya. The experiment was laid as Complete Block Design with split plot arrangement in net shades. The regeneration of single node and double node cuttings from three popular commercial tea clones (TRFK 31/8, TRFK 6/8 and AHP S15/10) was assessed in relation to their root and shoot development in the nursery until transplanting at 8 months in screen house with polythene papers. Variables such as root length, root biomass, leaf size, shoot count, shoot length and survival were evaluated as a measure of root and shoot development. The results revealed that double node cuttings were more vigorous in generation of new shoots, which triggered early root development, with clear variation observable among the test clones. Clone TRFK 31/8 was more superior in root development while clone AHP S15/10 showed superior ability to produce two shoots. There results also revealed lack in difference in seedling survival from two types of regenerations. The study confirms that indeed there was no adverse competition between root and shoots for assimilates and demonstrates the potential of that double node tea cuttings to be used to generate robust planting material both for new fields and infilling. It is therefore recommended that this technique be adopted and more studies be done using cuttings with more nodes to optimize rooting and growth conditions during propagation.

Key words: *Nodal cutting, propagule survival, infilling, nutrient competition, root to shoot ratio*

INTRODUCTION

Tea (*Camellia sinesis* (L). O Kuntze) is a perennial plant belonging to the Theaceae family (Hara *et al*., 1995). The crop is managed as a low bush in a continuous phase of vegetative growth with three main varieties recognized commercially. These varieties are *Camellia sinesis* var. sinensis, a China type with small leaves, *C. sinensis* var. assamica, an Asian variety with broad leaves and *C. sinensis* var. combodiensis, a variety thought to be a hybrid between the first two and originating from Cambodia (Banerjee, 1992). These three tea varieties are extensively exploited commercially in the tea producing countries of the world (Banerjee, 1992). The main method of tea multiplication is through vegetative propagation (VP) using single node cutting as the most accepted and popular (Kamunya and Wachira, 2003). Tea production is a function of several factors which include; soil fertility management, proper plant density, and pruning, plucking cycle, pests and disease management. Death of tea plants occur periodically in tea fields due to unfavourable climatic conditions, lightening, pests, disease attack and drastic cultural operations such as pruning creating gaps in the tea stand (TRFK, 1986).

Most of the aging tea fields have many gaps, with these gaps being a source of weeds due to low ground cover and in addition, they increase the risk of soil erosion and fertility decline. The gaps also reduce the total area of light interception, hence a lower total rate of photosynthesis leading to a decline in biomass per unit area of land (Bazzaz, 1996). In north east India, about 10% open patches in mature tea plantations comprising a total area of 285,000 Ha which resulted in an estimated annual crop loss of 40 million Kgs made tea (Jain, 1980). Infilling of the gaps immediately after pruning is the recommended practice in most tea-growing regions like East Africa (TRFK, 2002), Central Africa (TRFCA, 1990), India UPASI (1987), and Sri Lanka (Sandanam,1986). However, infilled tea plants in Kenya show a poor establishment and do not perform as well as those planted in former forest sites implying a need to use a well-seasoned vegetatively propagated (VP) plants which should be at least 18 months old in the nursery and having been decentered at 6 inches to stimulate lateral aerial shoots and thus provide multi-stemmed vegetatively propagated tea plants (Bore, 1996). This is definitely a long time of waiting before the infilling takes place and there recovery poor.

Due to optimization of vegetative propagation procedures, tea has been propagated from single node cuttings. However, single node cuttings usually develop a single shoot and only develop lateral branching after decentering thus taking more time to reach the recommended VP tea plants for infilling (Bore, 1996). There is therefore a need to shorten the period of VP plants for infilling as well as having robust plants for establishing new tea fields. The concern has been pegged on the fact that there could more risk of resource competition between two nodes (more leaves and shoot) hence low rate of survival. According to Orians *et al*. (2011), this phenomenon, termed induced resource sequestration, refers to rapid injury induced changes in resource allocation patterns that result in an increase in export of existing or newly acquired resources from injured (TNC cutting) tissues (and/or systemic tissues with vascular connections) into storage organs. These resources are thus temporarily sequestered (unavailable) for growth, defense or storage in the tissues from which they were exported Babst *et al*. (2008). The current experiment tested if the two nodal system would fail due to competition between more leaves and root development.

MATERIALS AND METHODS

The study was conducted in Kenya Tea Development Agency (KTDA). Kangaita Tea Farm located in Kirinyaga County in Kenya. The sites was chosen on the basis of its cool climatic conditions. Kangaita tea farm is on the slopes of Mt. Kenya at 2,050 meters above sea level with average annual rainfall of 1,500 mm. The temperature ranges from 12^{o}C during the cold season (July) and 25^{o}C during hot months (January and September; (TRFK, 2012). The experiment was a Randomized Complete Block Design (RCBD) design with split plot arrangements where the main plots being tea clones (TRFK 31/8, TRFK 6/8 and AHP S15/10) while the subplot was the method of generating the vegetative propagation (i.e. Single node cutting SNC- and TNC- two node cutting). TRFK 31/8 a popular commercial clone was the standard check for adaptability, TRFK 6/8 check for high quality and AHP S15/10 for high yielding. Each treatment had a set of 200 V.P cuttings and were replicated thrice under enclosed transparent material that prevented loss of water and shade nets also provided moderate ambient temperatures. The standard TRFK rooting media and tea nursery management practices were adopted for all the treatments.

One month after planting (during development), ten plants per plot were randomly sampled and roots and shoot length and root biomass were measured. The root length and biomass was evaluated through measuring the length of the longest roots and the total weight of all roots after dissecting and washing the plant gently in a basin of water to avoid detaching any root. A 30 cm ruler to measure the length and a micro weighing scale were used respectively.

Data analysis

The data collected were subjected to analysis of variance (ANOVA) using the SAS statistical package version 9.00 TS Level 00M0 XP-PRO platform. Where significant differences existed, the means were appropriately separated by Fisher Least Significant Difference (LSD) test set at a significance level of 5%. Correlation analysis was also done using Pearson's correlation matrix.

RESULTS

Effects of Types of Cutting and Clones on Root Development

Clones varied in regards to increase in root biomass with significant differences ($p = 0.021$, sixth week; $p = 0.024$, twelfth week; $p = 0.047$, sixteenth week) being observed in some stages of growth, particularly in 6, 12 and 16 weeks under two node cuttings ((Table 1, TNC). The morphological features of the clones were not found to have significant influence on the root development since all the clones competed favorably well as shown in (Table 1, TNC). For example, clone TRFK 31/8 established from TNC was significantly superior in root biomass accumulation apart from week 16 where it was comparable with TRFK 6/8. The differences in root biomass accumulation as a result of method of vegetative propagation was however not observable as was expected (based on hypothesis that more shoot material, TNC would drain root reserves).

The clonal variation in root length was noticeable during the growth, with TRFK 31/8 showing superiority in root elongation from week 10 to week 16 and this was irrespective of the type of nodal cutting (SNC or TNC; Table 2). A similar trend was observed on root biomass accumulation (Tables 1 and 2). This may be attributed to the fact that different clones have inherently varying leaf sizes and shoot lengths, with TRFK 31/8 having the longest shoot amongst the test clones (Fig.1).

However, there were no statistically significant differences (p > 0.05) in root elongation.

Table 1: Interactions between tea clones and type of nodal cuttings (single or two) on root biomass (g).

	Root weight [g]							
Cutting type	W2	W4	W6	W8	W10	W12	W14	W16
SNC								
Clones TRFK6/8	0[a]	0[a]	0.003[a]	0.019[b]	0.247[a]	0.682[a]	1.343[a]	1.804[a]
TRFK31/8	0[a]	0[a]	0.016[a]	0.132[a]	0.523[a]	1.001[a]	1.475[a]	1.854[a]
AHP S15/10	0[a]	0[a]	0.007[a]	0.066[b]	0.374[a]	0.741[a]	1.081[a]	2.578[a]
P-Value	.	.	0.316	0.009	0.340	0.675	0.422	0.519
TNC								
Clones TRFK6/8	0[a]	0[a]	0.005[b]	0.056[a]	0.409[a]	0.571[b]	2.086[a]	3.187[a]
TRFK31/8	0[a]	0[a]	0.025[a]	0.180[a]	0.742[a]	2.409[a]	2.633[a]	2.957[a]
AHP S15/10	0[a]	0[a]	0.006[b]	0.127[a]	0.449[a]	0.676[b]	1.713[a]	1.884[b]
P-Value	.	.	0.021	0.145	0.246	0.024	0.296	0.047

[*]Means followed by the same letter in a column are not significantly different at P≤0.05: SNC and TNC represent Single node cutting and two node cuttings respectively while W2,-W16 represent stand for weeks after planting from week 2 to week 16

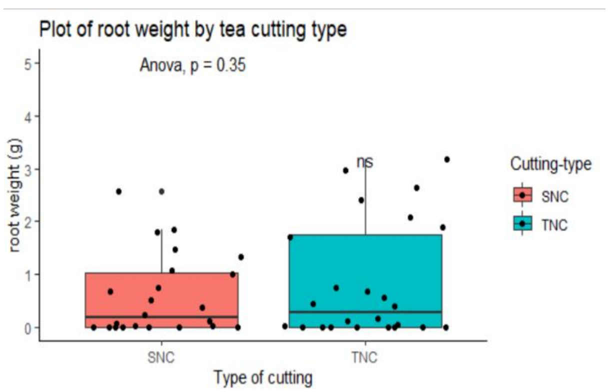

Figure 1. Mean root weight of stem cuttings as a function of stem cutting type, using data from the three genotypes that were rooted for 16weeks. Boxplots show rooting weight data as SNC (single node cuttings) and TNC (two node cuttings). Black dots show distribution of root weight data points collected every fortnight until 16th week from 3 varieties. Solid line in boxplot is the median root weight (g). No statistically significant differences (P=0.35) are noted between two types of tea cuttings was observed irrespective of variety.

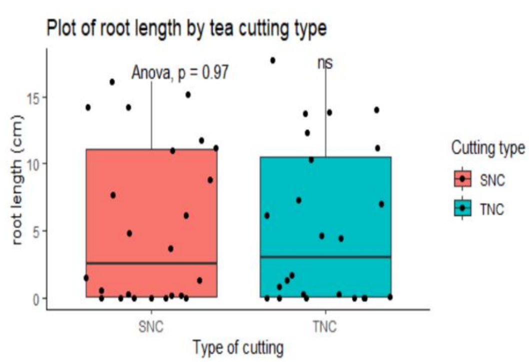

Figure 2. Mean root length of stem cuttings as a function of stem cutting type, using data from the three genotypes that were rooted for 16weeks. Boxplots show rooting length data as SNC (single node cuttings) and TNC (two node cuttings). Black dots show distribution of root length data points collected every fortnight until 16th week from 3 varieties. Solid line in boxplot is the median root length (cm). No statistically significant differences (P=0.97) are noted between two types of tea cuttings was observed irrespective of variety.

Table 2: Interactions between tea clones and type of nodal cuttings (single or two) on root length (cm).

Type of nodal Cutting	WEEKS							
	W2	W4	W6	W8	W10	W12	W14	W16
SNC								
Clone TRFK6/8	0[a*]	0[a]	0.17[a]	0.50[b]	3.63[a]	7.64[a]	11.70[a]	15.20[a]
TRFK31/8	0[a]	0[a]	0.22[a]	1.50[a]	6.11[a]	11.01[a]	14.19[a]	16.13[a]
AHP S15/10	0[a]	0[a]	0.13[a]	1.33[a]	4.83[a]	8.82[a]	11.17[a]	14.17[a]
P-Value	.	.	0.8100	0.0088	0.0998	0.3423	0.2052	0.7523
TNC								
Clone TRFK6/8	0[a]	0[a]	0.22[a]	0.78[a]	4.64[a]	7.29[a]	11.18[a]	14.01[a]
TRFK31/8	0[a]	0[a]	0.26[a]	1.63[a]	6.13[a]	10.28[a]	13.86[a]	17.68[a]
AHP S15/10	0[a]	0[a]	0.10[a]	1.27[a]	4.43[a]	7.01[a]	12.29[a]	13.75[a]
P-Value	.	.	0.1580	0.4584	0.3179	0.0717	0.0680	0.0630

[*]Means followed by the same letter in a column are not significantly different at $P \le 0.05$: SNC and TNC represent Single node cutting and two node cuttings respectively while W2,-W16 represent stand for weeks after planting from week 2 to week 16

Effect of types of Cutting and Clone on Shoot Length and Propagule Survival

Significant differences in shoot length were observed up to week 8 followed with a slowdown in weeks 10, 12 and 14 in Kangaita, with seedlings established from TNC showing superiority (Table 3). The increase in shoot length was observed to peak again to significant levels in week 16. At week 16, it was clear that TNC had higher shoot length as well as more leaves, with TRFK 31/8 being more superior (just like in case of root length and root biomass –Tables 1 and 2). The TNC was always higher than SNC as regards shoot elongation in general (Fig.1). Slight differences in the effects of type of cutting and clone on survival rates were observed in the entire study in Kangaita, however these variations were not significant (Table 4). Despite this area experiencing moderately higher temperatures this did not translate to observable differences in survival of the tea clones grown either as SNC or TNC (Table 4). Implying that SNC is not more effective than TNC yet the latter will take shorter time before transplanting. To find out the relationships between root length, root biomass, shoot length, number of new leaves, leaf size and survival rate, correlation analysis was done whereby it was observed that all parameters apart from the ability to produce two shoots, showed strong positive correlation in Kangaita (Table 5). This was unexpected, particularly between roots and shoots or roots and leaves which seemed to be contrary to the theory that shoots drain/leaves drain substrates from roots and hence expected to be negatively correlated.

Table 3: Interactions between tea clones and type of nodal cuttings (single or two) on shoot length (cm).

	WEEKS							
CUTTINGS	W2	W4	W6	W8	W10	W12	W14	W16
SNC								
TRFK6/8	0.72[a*]	1.81[a]	3.08[a]	3.41[a]	6.12[b]	6.26[a]	6.26[a]	6.70[b]
TRFK31/8	1.25[a]	2.41[a]	3.90[a]	4.55[a]	9.18[a]	9.51[a]	9.51[a]	9.95[a]
AHP S15/10	1.37[a]	2.18[a]	3.79[a]	3.90[a]	6.65[b]	6.58[a]	6.58[a]	7.22[b]
P-Value	0.0951	0.4142	0.3804	0.1502	0.0071	0.3163	0.3163	0.0010
TNC								
TRFK6/8	2.48[a]	3.04[a]	4.87[a]	5.39[a]	4.86[a]	4.86[a]	7.78[a]	8.37[b]
TRFK31/8	1.75[a]	4.02[a]	5.11[a]	6.39[a]	12.54[a]	12.54[a]	13.48[a]	14.18[a]
AHP S15/10	2.89[a]	3.83[a]	5.92[a]	6.38[a]	8.57[ab]	8.57[ab]	6.75[a]	8.30[b]
P-Value	0.3419	0.3247	0.4345	0.5299	0.0456	0.0456	0.2510	0.0047

[*]Means followed by the same letter in a column are not significantly different at P≤0.05: SNC and TNC represent Single node cutting and two node cuttings respectively while W2,-W16 represent stand for weeks after planting from week 2 to week 16

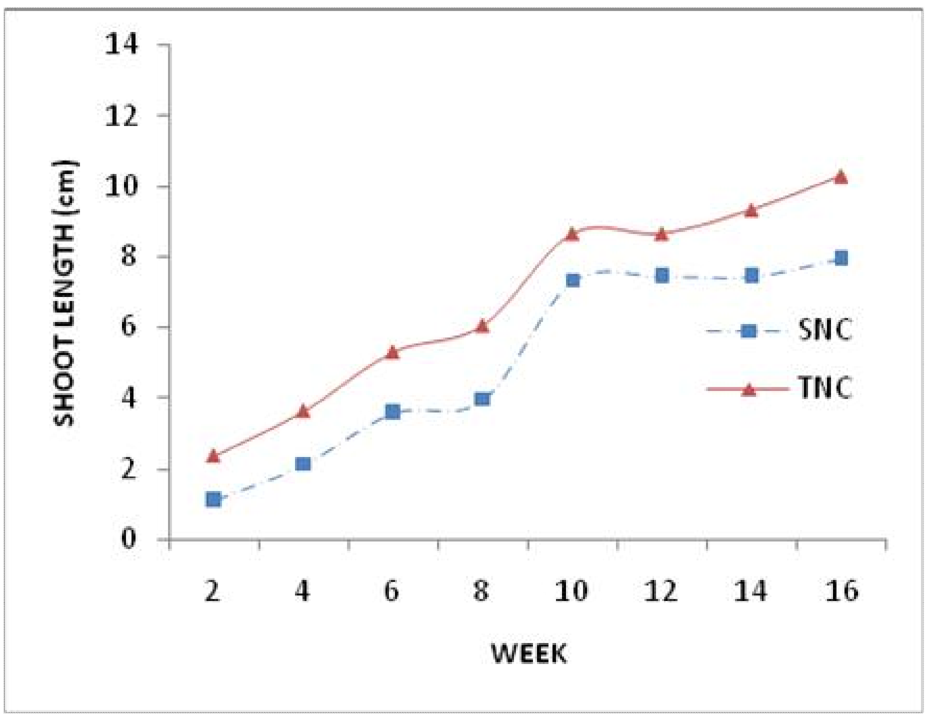

Fig 3: Effects of node cutting (Single Node Cutting and Two Node Cuttings; SNC and TNC respectively) on shoot length at Kangaita with time (weeks after transplanting)

Table 4: Effects of type of cutting and clones and on seedling survival (score out of 10 seedlings) at Kangaita.

	WEEK							
CUTTINGS	W2	W4	W6	W8	W10	W12	W14	W16
SNC								
TRFK6/8	8.7[b*]	9.7[a]	10[a]	10[a]	10[a]	10[a]	10[a]	10[a]
TRFK31/8	8.7[b]	10[a]	9.3[a]	10[a]	10[a]	10[a]	10[a]	10[a]
AHP S15/10	10[a]	10[a]	10[a]	10[a]	10[a]	9.7[a]	9.7[a]	10[a]
TNC								
TRFK6/8	9.7[a]	10[a]	10[a]	10[a]	10[a]	10[a]	10[a]	10[a]
TRFK31/8	9.0[a]	10[a]	10[a]	10[a]	10[a]	10[a]	10[a]	10[a]
AHP S15/10	9.7[a]	10[a]	10[a]	10[a]	10[a]	10[a]	10[a]	10[a]

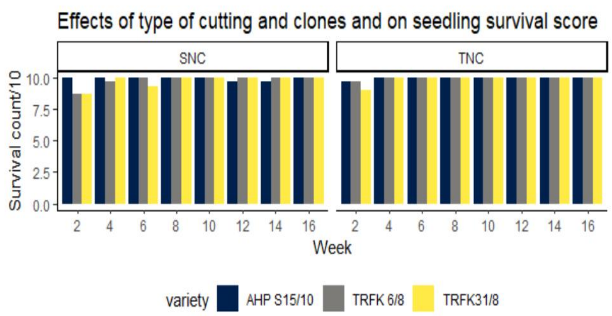

Fig 4. Survival count of rooted cuttings as a function of propagation duration from single node (SNC) and two node (TNC) stem cuttings from three commercial tea varieties AHP S-15/10, TRFK 6/8 and TRFK 31/8.

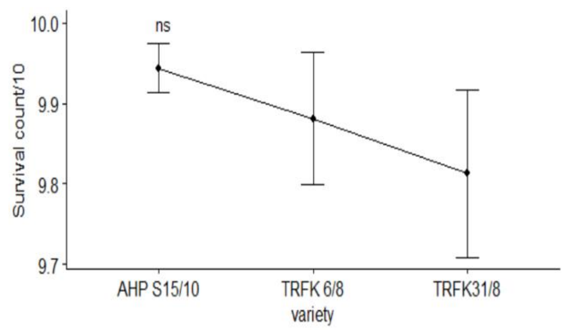

Fig 5. Survival count of rooted cuttings from the three varieties AHP S 15/10, TRFK 6/8 and TRFK 31/8. There is no significant (ns) differences ($p < 0.05$) in survival count of rooted cuttings of tea varieties after 16weeks. Vertical lines represent error bars on mean survival count for each variety.

Table 5: Correlation coefficients of seedling tea Parameters at Kangaita Site

	Root biomass	New leaves	Leaf size	Shoot length	Two shoots	Survival
Root length	0.85923 {<0.0001}	0.77371 {<.0001}	0.78350 {<0.0001}	0.66202 {<0.0001}	0.06266 {0.6010}	0.23247 {0.0050}
Root biomass		0.74648 {<.0001}	0.76967 {<.0001}	0.64652 {<.0001}	-0.03210 {0.7889}	0.19961 {0.0165}
New leaves			0.86933 {<0.0001}	0.83222 {<0.0001}	0.15844 {0.1838}	0.34680 {<0.0001}
Leaf size				0.87621 {<.0001}	0.05919 {0.6214}	0.26105 {0.0016}
Shoot length					0.04972 {0.6783}	0.32546 {<0.0001}
Two shoots						0.49453 {<0.0001}

{}-values in parentheses are p values

DISCUSSION

It was apparent that the root length did not differ significantly ($p<0.05$; Tables 1 and 2) between the node cutting during the study period. This could have been attributed to the fact that the adventitious root formation in tea is not dependent on the initial number of nodes but can be influenced by many factors among them the actively growing axillary buds, size of the stem (stalk) and maturation of the leaves (Wilson, 1993). The current work also agrees with those of Hartmann *et al*. (1997). According to Hartmann *et al*. (1997), the ability to form adventitious roots has also been associated with the characteristic of the cutting material and in many case use of inoculating with mycorrhizae strains. Indeed, root callusing has been shown to be enhanced by inoculating with mycorryzhae (Chelagat *et al*., 2021a and b) leading vigorous plant growth supported by leaf development. It therefore implies that the use of mycorrhizae inoculation can complement both single and two and node cuttings. Not surprising, it has previously observed that among the characteristics identified to influence the ability of the cutting to form adventitious roots are size and age of leaves as earlier documented (Kawase, 1972; Wilson 1993), presence of active buds proximity of nodes to the base of the cutting (Hansen, 1986) size of the stem and according to Wilson (1994), the position of cuttings on the stock plant. Nevertheless, plant growth is highly regulated and coordination of shoot and roots ensures that energy production in the leaves is fine tuned to the availability of water and nutrients (Paul and Foyer, 2001). The arguments by Paul and Foyer (2001) supports our finding, which proves that the clones were able to adjust their increase in root biomass based on resource availability regardless of their morphological features.

Among the characteristics identified in previous studies to influence the ability of cuttings to form adventitious roots are the size and age of the leaves (Wilson, 1993; Kawase 1972). Temperature is an important factor in cell elongation hence the reason why the roots were longer at Kagochi site which was warmer (data not shown) Previous studies done on root development at different temperatures revealed that root growth in pine increased almost exponentially as the temperature increased and likewise root growth were inhabited at low temperatures (Farrar, 1988; Teskey *et al*., 1984). Physiological changes caused by variation in soil temperatures such as decreased or increased leaf conductance to water vapour (Teskey *et al*., 1984). Though all the clones belong to the same species (*Cammelia sinensis*) the variation in their genetic composition could have contributed to the difference in allocation of assimilates to the roots hence the reason for longer roots in clone TRFK 31/8.

The number of leaves and leaf size affects the total leaf surface area which determines the number of stomata and light inception, CO_2 assimilation and amount of food reserves contained in the node cutting. TNC had seemingly higher shoot length than SNC (Fig.1) owing to the fact that it had two leaves and a longer stalk and therefore more food reserve to support the growth in the initial stages up to the point of reliability on root nutrient absorption from the soil. The argument is in tandem with our positive correlation between root length and shoot length, as well as root length/biomass and leaves (Table 5). This is contrary to the arguments by Paul and Foyer (2001), who think that partitioning of biomass into the root and shoot is a dynamic process, in the condition of ample water and nutrient supply an excess root system act as a strong sink and reduces the growth of the shoot due to the competition for carbohydrates.

Conversely, a fast growing root system might provide more water and nutrients to the shoot thereby enhancing shoot growth. This explains the reason why longer shoot in TNC was found to have greater root biomass (Fig 1, table 5). The root biomass and shoots had positive correlation, this is supported by the previous research data which demonstrate that an increased root system does not necessarily act as strong sink as postulated by Vercuyssen *et al*. (2011). It has been proven that optimizing root growth result in more production as it enhances water and nutrient absorption Vercuyssen *et al*. (2011). Mobilization of carbohydrate from the root during intense shoot growth has been described but considered as minor as argued by Alatou *et al*. (1989) and this contradicts the hypothesis expounded by Orians *et al*. (2011). Furthermore our results (table 5), where good positive correlation between shoot and root growth is supported by research by Willaume and Pages (2011). Incidentally, Alaoui-Sosse (1994), also reported that carbohydrates stored in basal segments were mobilized only during second-flush development. In the current experiment the carbohydrates were not determined but it could be a good reason for the results observed. In control seedlings, high sucrose concentrations on day 10 suggest an important flow of carbohydrates between aerial and root parts at the end of aerial growth (Willaume and Pages (2011), Alaoui-Sosse (1994). These authors demonstrated that mobilization of starch was partial and occurred relatively late which partially explains good root growth of TNC just as much as SNC. The strong aerial sinks mainly used carbohydrates exported from leaves of the first flush and starch reserves from the first-flush stem whereas mobilization in the roots was moderate, Alaoui-Sosse (1994).

Conclusion

The study confirms that indeed there was no adverse competition between root and shoots for assimilates and demonstrates that double node tea cuttings can be used to generate robust planting material both for new fields and infilling as this would reduce the waiting time and need for decentering the growing. It can therefore be recommended to farmers for use to refill the gaps caused by natural calamities in tea fields.

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