Drought tolerance of Garcinia kola and Garcinia afzelii at the seedlings stage

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ABSTRACT

Garcinia kola and Garcinia afzelii are very useful forest tree species for chew stick and medicinal purposes in West Africa. They are currently receiving attention for cultivation due to the dwindling stocks of the species in the natural forest. Soil moisture is one of the most critical environmental factors that affect seedling growth in the field. The study was therefore conducted to test the response of the two species to variability in water availability. The seedlings were subjected to simulated no drought, 15-day drought, 30-day drought and 84-day drought. Seedling response to watering after 60-day simulated drought by withholding of watering was also monitored. Relative growth rate (RGR) in diameter decreased with the prolongation of the simulated drought period, and the difference was significant among all the treatments. For G. afzelii, simulated 84-day drought exhibited negative RGR in diameter, whereas for G. kola both the simulated 30-day and 84-day drought exhibited negative RGR in diameter. The RGR in height and leaf production was similar for the simulated no drought and the 15-day drought but significantly different from the 30-day and the 84-day drought period for G. afzelii. However for G. kola, it was the 15-day and 30-day simulated drought that were similar. Leaf production was highly correlated with height growth than with diameter growth. Release of drought-stress by the resumption of watering triggered leaf shedding up to a minimum value and thereafter increased. The relative rate of leaf production after re-watering increased, however the rate declined with the passage of time for G. afzelii but continued to increase for G. kola. Diameter growth was more affected than height by the dry conditions. The growth performance of G. afzelii was generally better than that of G. kola and both species have mechanisms for overcoming dry periods without experiencing mortality. Garcinia afzelii can be established in any of the forest zones, whereas Garcinia kola should preferably be established in Wet/Moist Forest and moist semi deciduous forest zones.

Keywords: Garcinia species, simulated drought, diameter growth, height growth, leaf production

INTRODUCTION

There are over 600 species of the genus *Garcinia*, belonging to the family Guttiferae, throughout the tropics. Most are upper–storey trees and shrubs, with some being large enough to be classified as timber (Steentoft, 1988). Sixteen species of the

Genus in West Africa have been classified as Non-Timber Forests Products (NTFPs) (Falconer, 1992). Among these are *Garcinia kola* and *Garcinia afzelii* Engl. The two species are widely distributed from Sierra Leone, in West Africa, to Cameroon, in Central Africa, and from Gabon to Congo Brazzaville, all in Central Africa. *Garcinia kola* grows mostly in moist forests whilst *G*. afzelii is mostly found in the dry forests of West Africa (Irvine, 1961). In Ghana, G. afzelii occurs in mainly the dry semi-deciduous forest zone with quite a good representation of all sizes in un-burnt rather than burnt forests (Hall and Swaine, 1981; Swaine et. al., 1997; Abbiw, 1990). This is an indication that the species is susceptible to fire. The two species are among the few commercial NTFPs that have several uses, the most important being chew stick and medicinal (Mshana et. al., 2000; Ayuk, et. al., 1999; Sosef et. al. 1998; Irvine, 1961). Split stems and twigs are used as chewing sticks in many parts of Africa, and have been commercialized for many years in the major cities, offering natural dental care, cash revenues and employment to hundreds of people. About 90% of the people in Ghana are reported to be using the chew sticks either often or occasionally for dental care (Adu-Tutu et al., 1979: Falconer, 1994). The Kumasi markets in Ghana alone provide the equivalent of between 2000 and 6500 harvested trees of the species every month (Falconer, 1994). It has been estimated that about two-thirds of residents of cities and almost all village folks in Nigeria use these species frequently for dental purposes (Mbakwe, 1983). The seeds of G. kola, together with other parts of the plant, are used in medicinal preparations. For instance, some of the preparations are used for the treatment of cirrhosis of the liver and have antiinflammatory, anti-diabetic, bronchial-dilator. anti-hepatoxic and antiviral compounds (Mbakwe, 1983). Particularly exciting is the observation that G. kola extract can prevent the Ebola virus from replicating itself (Anon. 1999). The bark of the stem is used in the tanning and dyeing industry. Thousands of tons of the bark were therefore exported from Ghana during the Second World War, between 1939 and 1945 (Irvine, 1961).

However, natural regeneration of the two species is poor; the seedlings are slow growing and can hardly be found in the forest floor (Taylor, 1960; Abbiw, 1990; Hawthorne, 1997; Gyimah, 2000). High demand for the species coupled with poor control over their exploitation have led to depletion of the species from the natural forest leading to near economic extinction in Ghana (Hawthorne, 1997; Wong 1997, IUCN 2004). The species are thus vulnerable and would be facing high risk of extinction in the wild in the mediumterm future (IUCN 2004). The two species are priority species immediate listed as for conservation actions in the Sub-Saharan Forest Genetic Resources (SAFOGEN) programme (Ouédraogo and Boffa, 1999).

To offset this imminent threat of extinction and to meet demand, cultivation appears as the only viable option. The species are among the focus species being investigated through the recently established African Tree Seed Research Network (Sacande et al., 2004). However, in order to cultivate them at any meaningful scale it is necessary to establish the optimum range of environmental factors that influence their growth. The species are shade tolerant and therefore grow under closed canopy forests where weather conditions and soil moisture levels are relatively stable (Hall and Swaine 1981). Their cultivation in open agricultural fields where the physical and the environmental factors fluctuate considerably appears challenging and needs to be properly guided by scientific information. Recently, there have been fluctuations in the climatic conditions in Ghana. The rainfall pattern has changed and has become less frequent with reduced intensity. Soil moisture however appears to be more critical in the survival of seedlings and growth after transplanting. Although drought is widely known to affect plant growth and survival, different species respond to drought stress differently and it is therefore important to define the specific tolerant levels for the species.

The questions that need to be answered are: How would the plant cope with varying degrees of drought stress? Can the plants recuperate when the drought stress is released? This study is therefore aimed at determining (i) the effect of varying degrees of draughtiness on seedling growth and (ii) seedling recovery ability from drought stress.

MATERIALS AND METHODS Study Site and Plant Materials

The study was conducted in a greenhouse at the Forestry Research Institute of Ghana at Fumesua near Kumasi at 6° 44' N. 1° 30' W of the Moist Semi-Deciduous Forest Zone (Hall and Swaine, 1981). From the climatic data recorded at the vicinity of the study site, from 2003 to 2007, the mean annual minimum and maximum temperatures, mean annual rainfall and mean annual relative humidity in the morning hours of 8:00 to 9:00 GMT were given as 22.2 $^{\circ}C \pm 1.66$ (SD), 31.1 °C ± 2.45, 1420 mm ± 235.6 and 87.1% \pm 10.5, respectively. The experiment was conducted in the greenhouse in order to control the environmental factors. The greenhouse had 50% ambient irradiance. It had a uniform relative humidity, RH, (%) that varied with temperature, θ (°C), the relationship obtained was,

 $RH = 341.2 \exp^{(-0.0554\theta)} (R^2 = 0.9904)$ (1)

Seedlings for the experiment were raised from seeds in poly-pots of dimension of 28 cm diameter and a length of 30 cm, resulting in a pot volume of 18.75 litres. They were raised for a period of one year in the greenhouse. A total of one hundred and fifty seedlings per species, with an average height of 25 cm, were selected for the application of the various treatments or watering regimes.

Experimental Procedure

Ten seedlings were selected per replicate, for the application of the various treatments or watering regimes. There were three replicates with the following treatments or watering regimes:

Treatment 1: Watering at three days interval

(TMT1). (This served as the control).

Treatment 2: Watering at fifteen days intervals (TMT2)

Treatment 3: Watering at thirty days intervals (TMT3).

Treatment 4: No watering for the whole period of 84 days (TMT4).

The volume of water applied for the watering of each seedling at a time was 1.6 litres. This volume of water applied at three days intervals within a month, was estimated to be equivalent to mean monthly rainfall of 125 mm. This was assumed to correspond to normal situation of no drought. Watering at fifteen days intervals corresponds to two weeks drought, thirty days intervals to one month drought and no watering to extreme drought.

Initial growth parameter assessment was carried out, and subsequently repeated at three weeks intervals. During the assessment, measurements were made of plant height, stem diameter above root collar and number of leaves present. At the beginning and end of the experimental period, ten seedlings were selected from each of the treatments and destructively sampled for the determination of root-shoot ratio. The roots and shoots were separately dried to a constant mass for 72 hours at 80 °C in an oven and then dry mass recorded after desiccation.

In another experiment, seedlings of both species were selected to monitor the recovery pattern of the species from drought stress. Thirty seedlings were selected from each of the two species and subjected to extreme drought stress by withholding watering for a period of 60 days. After this period, watering was resumed, with a volume of 1.6 litres, at three days intervals. Leaf abscission and flushing were monitored by counting the number of leaves on each seedling at weekly interval for six weeks.

RESULTS

Growth Trends

The root-shoot ratio was not affected by the various treatments in any of the species. The initial root-shoot ratio as well as that of the final was the same under the various treatments for both species, which was 1:4 and 1:2 for *G. afzelii* and *G. kola*, respectively. No mortality was recorded in any of the treatments.

Diameter

The pattern of growth in diameter among the various treatments was similar for both *Garcinia afzelii* and *Garcinia kola*. The initial diameter was similar for all the treatments. For *G. afzelii*, the differences in diameter growth among the various treatments became apparent even in the third week (Figure 1), with the Control (TMT1; 3-days interval watering) exhibiting the highest growth rate followed in a decreasing order by TMT2 (15-days interval watering), TMT3 (30-days interval

watering) and TMT4 (no watering). The differences continued to widen with the passage of time. With regard to TMT3, diameter growth apparently ceased after the third week and the diameter remained approximately the same for the entire period. For TMT4, the seedlings exhibited continuous negative growth rate from the third week onwards. This is an indication of dehydration. However, the diameter growth in TMT1 and TMT2 continued to increase but at different rates, with that of TMT1 being higher.

For *G. kola*, with the exception of TMT1, which had a higher diameter in the third week, that of the other treatments had similar diameter (Figure 1). The differences in diameter growth became more evident from the sixth week onwards, exhibiting a pattern similar to that of *G. afzelii*. For TMT3, growth apparently ceased from the sixth week onwards. However for TMT4, there was an exhibition of negative growth rate even after the third week.



Figure 1: Variation in diameter growth as influenced by the various treatments. TMT1, Watering at 3 days intervals (Control); TMT2, Watering at 15 days intervals; TMT3, Watering at 30 days interval; TMT4, No watering for 84 days.

Height

In general, there was a gradual increase in height with the passage of time, the rate of which depended on the type of treatment. For G. afzelii (Figure 2), the rate of height growth was similar with all the treatments for the first three weeks. The differences became prominent towards the sixth week and the gap among the treatments increasingly widened with the passage of time. The rate was similar for TMT1 and TMT2 after the third week and this trend continued, however there was a slight tendency for difference to occur after the twelfth week (if the experiment had continued). For TMT3, there was a gradual increase in height growth up to the ninth week and thereafter the rate tended to slow down. With regard to TMT4, height growth seemed to have

ceased after the third week.

The pattern of the height growth for *G. kola* (Figure 2) under the various treatments was quite different from that of *G. afzelii*. The height growth continued with approximately the same rate for the seedlings of TMT1 throughout the period. However with the other three treatments, height growth occurred almost at the same rate up to the sixth week and thereafter the growth ceased completely.



Figure 2: Variation in height growth as influenced by the various treatments. The legend is the same as in Figure 1.

Leaves

The pattern of changes in the number of leaves or leaf production with the passage of time under the different treatments was different for the two species. For *G. afzelii*, the rate of change was similar among the treatments up to the third week and thereafter separation occurred (Figure 3). As expected, TMT1 had higher rate of change than the other treatments. For TMT2 and TMT3, the rate of change remained similar up to the sixth week, and from there onwards TMT2 exhibited a higher rate of change than TMT3. For TMT4, there was a positive rate of change up to the third week and thereafter showed the tendency for negative rate of change.

For *G. kola*, only the TMT1 exhibited positive and continuous rate of change throughout the period (Figure 3). However with the other treatments, there was apparent cessation of leaf production after the third week, since their leaf number remained approximately the same throughout.



Figure 3: Leaf production as influenced by the various treatments. The legend is the same as in Figure 1.

Relative Growth Rate

Growth performance of the seedlings was analysed in terms of relative growth rate (*RGR*). The *RGR* was calculated as;

$$RGR = \frac{\ln[x_2] - \ln[x_1]}{\tau_2 - \tau_1} \tag{2}$$

Where, X_1 and X_2 are the size of a seedling parameter (diameter, height or leaf production) at the beginning and end of period of interest, respectively, and T_1 and T_2 are the corresponding sampling period in days. The *RGR* in diameter, height and leaf production of the seedlings under the various treatments for *Garcinia afzelii* and *Garcinia kola* are given in Tables 1a and 1b, respectively. Analysis of variance was used to test the differences among the treatments, and *Tukey's w* test was used for mean separation.

Relative growth rate in diameter decreased with the prolongation of the simulated drought and it was found to be significantly different among all the treatments for both species. For *G. afzelii*, the *RGR* in diameter was 0.0302, 0.0178, 0.0038 and -0.0103, while for *G. kola*, the values were 0.0205, 0.0123, -0.0026 and -0.0181 week⁻¹ for TMT1, TMT2, TMT3 and TMT4, respectively. For *G. afzelii*, there was a negative *RGR* for TMT4, whereas for *G. kola*, both TMT3 and TMT4 exhibited negative *RGR*. The negative *RGR* values are indication that the final diameter was smaller than the initial diameter. The *RGR* in height for *G. afzelii* was 0.0562, 0.0573, 0.0245 and 0.0042, while that for *G. kola* was 0.0236, 0.0080, 0.0061 and 0.0041 week⁻¹, for TMT1, TMT2, TMT3 and TMT4, respectively. Analysis of variance indicated significant differences among the treatments. The difference between treatments tested with *Tukey's w* test showed that the *RGR* in height for TMT1 and TMT2 were not significant ($P \le 0.05$) for *G. afzelii*. However for *G. kola*, differences between TMT3 and TMT3 as well as between TMT3 and TMT4 were not significant ($P \le 0.05$). The growth performance of *G. afzelii* in terms of diameter and height was found to be better than that of *G. kola* (Tables 1a and 1b).

Table 1a: Relative growth rate in diameter, height and leaf production (week ⁻¹) of the	Garcinia
afzelii seedlings		

Treatment		Diameter	Height	Leaves
TMT1	Average	0.0302 (a)	0.0562 (e)	0.0391 (<i>h</i>)
	Minimum	0.0256	0.0503	0.0346
	Maximum	0.0338	0.0644	0.0417
	SD	0.0042	0.0073	0.0039
TMT2	Average	0.0178 (<i>b</i>)	0.0573 (e)	0.0379 (<i>h</i>)
	Minimum	0.0160	0.0505	0.0292
	Maximum	0.0203	0.0622	0.0427
	SD	0.0023	0.0060	0.0075
TMT3	Average	0.0038 (c)	0.0245 (f)	0.0241 (i)
	Minimum	0.0033	0.0223	0.0204
	Maximum	0.0041	0.0269	0.0276
	SD	0.0004	0.0023	0.0036
TMT4	Average	-0.0103 (d)	0.0042 (g)	0.0043 (j)
	Minimum	-0.0139	0.0036	0.0041
	Maximum	-0.0082	0.0045	0.0046
	SD	0.0031	0.0005	0.0002

SD; Standard Deviation; Similar letters in parenthesis indicate no significant difference TMT1, Watering at 3 days intervals (Control); TMT2, Watering at 15 days intervals; TMT3, Watering at 30 days interval; TMT4, No watering for 84 days.

Treatment		Diameter	Height	Leaves
TMT1	Average	0.0205 (a)	0.0236 (e)	0.0323 (<i>h</i>)
	Minimum	0.0201	0.0208	0.0294
	Maximum	0.0211	0.0274	0.0356
	SD	0.0005	0.0034	0.0032
TMT2	Average	0.0123 (b)	0.0080 (f)	0.0126 (i)
	Minimum	0.0115	0.0069	0.0116
	Maximum	0.0133	0.0098	0.0145
	SD	0.0009	0.0015	0.0017
TMT3	Average	-0.0026 (c)	0.0061 (fg)	0.0118 (i)
	Minimum	-0.0029	0.0051	0.0107
	Maximum	-0.0024	0.0066	0.0125
	SD	0.0003	0.0009	0.0010
TMT4	Average	-0.0181 (d)	0.0041 (g)	0.0079 (j)
	Minimum	-0.0183	0.0040	0.0071
	Maximum	-0.0180	0.0041	0.0090
	SD	0.0002	0.0001	0.0010

Table 1b: Relative growth rate in diameter, height and leaf production (week⁻¹) of the *Garcinia kola* seedlings

The legend is the same as in Table 1a

Like the relative growth rate in diameter and height, that of leaf production also decreased along the gradient of increasing drought (TMT1 to TMT4) for both species. However, the difference between that of TMT1 and TMT2 for *G. afzelii* (Table 1a) was not significantly different but they were different from that of TMT3 and TMT4. In the case of *G. kola*, it was the difference between TMT2 and TMT3 that was not significantly different (Table 1b).

Seedling height growth was positively related to leaf production as depicted in Figure 4. The relationship, which was exponential, obtained for *G. afzelii* was;

$$Ht = 8.811 \exp^{(0.037L)}$$
 (*R*² = 0.943). (3)

And for *G. kola* was;

$$Ht = 8.458 \exp^{(0.092L)}$$
 (*R*² = 0.884). (4)

Where, Ht and L represent seedling height and number of leaves, respectively. The coefficient relates to the initial seedling and the exponent is an index of intrinsic height growth rate in relation to number of leaves.



Figure 4: Height growth as a function of leaf production

Seedling Response to the Release of Drought Stress

Re-watering the drought-stressed seedlings triggered leaf shedding within one week (Figure 5). There was about 42% reduction in leaf number for *G. afzelii*, whilst in *G. kola* the reduction in leaf number was about 60% within the first one week. The leaf number later remained steady for another one week before the production of new leaves was resumed and this continued for the subsequent days

The relative growth rate in leaf production was very high in the third week after re-watering for *G. afzelii*, but the rate declined in subsequent weeks. However for *G. kola*, the *RGR* generally continued to increase with the passage of time. The *RGR* value was 0.2054, 0.1582, 0.0763 and 0.0709 week⁻¹ for *G. afzelii*, while for *G. kola* it was 0.2113, 0.2136, 0.1431 and 0.2624 week⁻¹, for week 3, 4, 5 and 6, respectively.



Figure 5: Leaf shedding and production of the plants in response to re-watering after simulated drought.

DISCUSSION

Different watering regimes had significant influence on seedling mean diameter growth. Drought period of eighty-four days resulted in negative relative growth rate in G. afzelii and G. kola seedlings, and for G. kola even withholding of watering for thirty days resulted in negative RGR in diameter. According to Kozlowski et. al. (1991), drought causes shrinkage in the lower part of the stem. Shrinkage in diameter results from loss of turgor; this inhibits the enlargement of the xylem initials (Kozlowski, 1982). Similarly, decreased supply of auxin to the differentiating xylem derivatives and decreased supply of metabolites to the cambial region could be a probable cause (Larson, 1964). Hence, these might probably have resulted in shrinkage of mean

diameter observed in those seedlings that were drought-stressed for thirty days and beyond.

Height growth involves the elongation of shoot tips of plants, and positive turgor is required by plants for this process (Nonami, 1998; Mohr and Schopfer, 1995; Hsiao and Bradford, 1983; Cosgrove, 1981 and Jones et. al., 1981). The trend in mean height growth under different drought regimes does not diverge from the general observation that drought limits plant growth. Relative growth rate in height was greatly influenced by the availability of water for both G. kola and G. afzelii and ceased when they were not watered throughout the eighty-four days. Similarly, drought-stressed Cedrela odorata and Schizolobium amazonicum were reported to have showed virtually no increase in height growth

(Poorter and Hayashida-Oliver, 2000).

Generally increasing watering led to corresponding better growth, although for G. afzelii watering at three days and fifteen days intervals produced similar relative growth rate. These observations are similar to those made by Burslem (1996); except that watering treatments have more pronounced effect on seedling growth in the present study. Retardation in height growth may be attributed to negative turgor resulting from reduced water uptake (McIntyre, 1987). Reduced water uptake can be linked to lack of water in the soil. Limitation to tree height growth could be attributed to reduction in water supply to the upper leaves and branches (Waring and Schlesinger, 1985). In a related study, Rundell and Stecker (1977) reported a negative relationship between reduced water supply and size and number of water conduction elements and this might explain what was observed with the two species. Retardation in height growth may also be due to lack of water, hindering plant nutrition. According to Baron (1986), dissolution of mineral elements, such as nitrogen, phosphorus, potassium, etc. and transport of the mineral solution to sites of synthesis require hydrated protoplasm.

Watering the seedlings every three days resulted in a steady increase of leaf production in both species confirming the observation that water supply to seedlings leads to large number of leaf production (Wright, 1991). It was observed that watering intervals exceeding one month caused a gradual decline in leaf number in both species. It was also observed that the seedlings rarely exhibited shedding of leaves during droughtstressed periods. This was similar to an observation made with Brosimum lactescens, a shade tolerant species (Poorter and Hayashida-Oliver, 2000). This finding confirms that cell and leaf expansion are the processes in plants that are sensitive to water deficits (Fitter and Hay, 1987). Maintaining some amount of leaves may be one necessary strategy by seedlings to continue photosynthesizing. Notwithstanding this, they may require mechanism that will reduce injury to the meristematic tissue (Kramer, 1983). The plant may achieve this by increasing cuticular resistance to transpiration (Kozlowski *et. al.* 1991). The thickness of the leaves of the species coupled with their leaf rolling character during drought may probably offer this resistance.

Re-watering of seedlings that were subjected to sixty days of drought resulting in withering of leaves, caused substantial shedding of leaves of both species within seven days. However, continuous watering afterwards induced leaf flushing. Poorter and Hayashida-Oliver (2000) reported that some plants neither shed their leaves nor produce new ones during drought until they are re-hydrated. Perhaps re-hydration is necessary for resumption of metabolic activities. This seems to suggest that resumption of metabolic activity is necessary for the formation of the abscission layer so that leaf fall can start. Fast resumption of leaf growth seems to suggest that there was virtually no injury to the meristematic tissues. Such a strategy is beneficial for the survival of the seedlings (Kramer, 1983). Hence the two species do not lack dehydration tolerance according to the definition of McIntyre (1987).

It is known that among tree seedlings, a reduction in the availability of water leads to greater relative emphasis on roots growth (Bradford & Hsaio, 1982). However, both *G. kola* and *G. afzelii* seedlings showed no increase in root growth relative to shoot growth upon the application of different watering regimes. The root-shoot ratio at the end of the eighty-four days was very similar to that at the beginning of the experiment. Notwithstanding the fact that the roots are the last to be stressed in drying soils, the relative decline in growth in both shoots and roots was to a large extent the same. The absence of apparent increase in root dry mass relative to shoot dry mass may be attributed to the observation that the seedlings did not significantly shed their leaves. Probably, the roots were not so stressed to induce the release of established

abscisic acid (ABA), which is needed for leaf abscision (Zhang *et. al.*, 1987). Nonetheless this does not necessarily suggest that the trend would have been the same if drought had been extended over eighty-four days.

No mortality was recorded in any of the seedlings in any of the treatments thus suggesting that seedlings of *G. kola* and *G. afzelii* are capable of surviving drought for at least 84 days. Despite the fact that drought had profound influence on leaf production, diameter and height growth, seedling mortality may occur only at extreme conditions of drought. Water deficit has nonetheless been observed as a major cause of seedling death in forest gaps (Brown and Whitmore 1992; Turner 1990). Perhaps mortality may occur earlier in the field due to competition for water by other plants.

CONCLUSION

Diameter growth was more affected by dry conditions compared to height growth. Height growth was also found to be a function of leaf production. The growth performance of G. afzelii was generally better than that of G. kola. This is a reflection of their respective natural habitat or distribution. Garcinia afzelii is generally found in the dry forest where some dry periods are prevalent compared to G. Kola, which is naturally distributed in the moist forest. However, both species have mechanisms for overcoming dry periods without experiencing mortality. In establishing stands of the two species, care should be taken in the selection of sites. Garcinia afzelii can be established in any of the forest zones, ranging from dry semi-deciduous to wet evergreen forest, since it has been found to be more tolerant to drought than G. kola. Garcinia afzelii exhibited positive RGR in diameter at TMT3, with watering

at 30-days interval, whereas *G. kola* had negative *RGR*. *Garcinia kola* should preferably be established in wet/moist evergreen forest and moist semi-deciduous forest zones, due to its relatively low tolerance to drought.

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