SCREENING TEN GENOTYPES OF NATURAL RUBBER SEEDLINGS (*Hevea Brasiliensis* Mull. Arg) FOR DROUGHT TOLERANCE IN UMUDIKE, SOUTH EASTERN NIGERIA

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ABSTRACT
A field study was conducted in 2012 and 2013 cropping season at the Research Farm of National Root Crops Research Institute, (NRCRI) Umudike, in Abia State, to screen 10 rubber genotypes collected from Rubber Research Institute of Nigeria for tolerant to drought conditions. The aim was to select tolerant genotypes for development of new clones of natural rubber and to study the association (r) between the 9 traits used as indices for picking drought tolerant genotypes. The experiments were laid out in a Randomized Complete Block Design (RCBD) using three replications. Thirty rubber seedlings per plot were transplanted from pre-nursery to the main nursery at the spacing of 100 x 30 cm on a well prepared bed. Results obtained in the field over the two years indicated that variations existed in the level of drought tolerance among the genotypes. For agronomic traits, all the traits showed significant differences ($P<0.05$) among the genotypes. GT1, RRIM 600, PR107 and Nig 804 showed more tolerance to drought conditions from 0 to 40days than the other genotypes and PB5/51, RRIM 628 and Nig 800 were found to be more susceptible to drought when compared with the other genotypes in the field. Life saving irrigation may become an essential farm practice for the survival of these genotypes in the nursery field during the dry seasons or periods of drought.

INTRODUCTION
Natural rubber (*Hevea braziliensis* Mull. Arg) is an important agricultural commodity used in the manufacture of a wide range of products. Latex production from the tree *Hevea braziliensis* plays major role in generating income for many developing countries. The plant is quick growing, erect, with a straight trunk and smooth surfaces. It is the tallest species of the genus, *Hevea*. In the wild, trees may grow to over forty meters (40m) tall and live for over a hundred years. In the plantations they rarely exceed twenty- five metres (25m) because growth is reduced by tapping (Webster and Baulkwill, 1989).

As global demand for natural rubber increases, a major challenge facing the cultivation of rubber plants is inability to withstand unfavourable environmental conditions particularly lack of water (Nair *et al.,* 2010). Drought remains one of the most limiting factors among all environmental constraints. Drought stress can occur at any of the growing stages and cause complete loss of crops or reduce yield. In Nigeria, most rubber plantations were planted from a single popular clone RRIN 800 series. This clone is high yielding but with the demerit that it does not tolerate drought.

Intrinsic Water Use Efficiency in *Hevea* clones
Limited water availability is one of the major factors that affect productivity in crop plants. Generally, plants manage to survive water deficit to a certain extent either by morphological or physiological modifications that enable them to escape desiccation stress. In Nigeria, 85% of the natural rubber cultivation areas are in the traditional rubber growing regions where rainfall and other agro- climatic conditions are better suited for rubber cultivation. The rest 15% of rubber cultivation is in the western and eastern parts of Nigeria with varied climatic constraints like moisture stress, high and low temperatures, drought and cold stresses which are major factors that

limit growth and productivity of rubber in these regions. Among the various growth /productivity limiting factors, the negative impact of drought on yield has been well established in different *Hevea* clones (Jayasree *et al*., 2010). The intrinsic water use efficiency of plants is known to be related to the efficiency of plants to grow with limited availability of water (Majkenn *et al;* 2005).The drought tolerant capacity of the modern *Hevea* clones is largely unknown. The present study was aimed at selecting *Hevea* clones that are tolerant to drought stress. Natural rubber (*Hevea braziliensis)* is one of Nigerian's prominent industrial and export crops which apart from providing raw materials for local industries, also contributes to the export earnings of the nation. If properly developed, natural rubber and other crops can contribute a substantial part of Nigeria's foreign exchange earnings, thereby making the country less dependent on petroleum.

The traditional uses of natural rubber is well known in the manufacture of the following: tyres, tubes, car bumpers, bridge bearing, transmission belts, hoses, domestic and industrial gloves, balloons, adhesives, pillows and mattresses, building for earthquake resistance etc. Some other recent uses of interest which RRIN Scientists have developed are from rubber seed oil is the manufacture of alkyd resins, an important component in paint manufacture, glazy putty of which a pilot plant has been developed by RRIN with financial support of Raw Materials Research and Development Council (RMRDC), liquid soap, leather tanning, printing ink, biodiesel etc.

Present day rubber cultivation faces various climatic constraints even in major growing areas and these constraints are expected to become more serious in future. Soil, atmospheric drought, high temperature and diseases susceptibilty are major environmental factors limiting growth and yield in *Hevea* (Mercy *et al*., 2010). Therefore, there is need to screen *Hevea* genotypes for tolerance to drought conditions for development of new clones of natural rubber and to study the association between indices used to identify drought tolerant of natural rubber seedlings.

MATERIALS AND METHODS

Experimental Site

The study was carried out at the research farm of the National Root Crops Research Institute (NRCRI), Umudike Abia State Nigeria (latitude 05^0, 29'N; longitude 07, 33'E) and is 122m above sea level. Umudike is in the tropics and has a total rainfall of about 2177 mm per annum with an average temperature of about 26^{0}C. The predominant vegetation type is rain forest (NEST, 1991), while the soil has been classified as sandy loam ultisol (Agboola, 1979). The rainfall pattern is bimodal. A long wet season from April to July is interrupted by a short August break followed by another short rainy season from September to October. Dry season stretches from early November to March.

Planting Materials

Genotypes: Ten (10) genotypes of rubber seeds (*Hevea brasiliensis* Mull. Arg), obtained from Rubber Research Institute of Nigeria, Iyanomo, Benin City, Edo State, were used. The genotypes used included:

RRIM 600, NIG 800, NIG 801, NIG 802, NIG 803, NIG 804, PR 107, RRIM 628, GT I, and PB 5/51. These genotypes were subjected to drought treatments.

Land Preparations

The experimental sites were cleared, ploughed, harrowed and a raised bed of 3m wide was made.

Pre- germination Nursery or Pre- nursery

The rubber seeds were spread in the pre- nursery bed where seeds were germinated before raising in the poly bag or ground nursery. The pre- nursery was small in size and was prepared by making a raised bed of length 3m x 2m. The four sides of the bed were bordered by bamboo sticks to keep the raised soil intact. The seeds were sown in the field at Umudike on September, 10 each year then at one month old when the seeds must have sprouted very well the seedlings were transplanted onto the main nursery. Planting in the pre-nursery and transplanting to the main nursery were carried out between September and October each year.

Ground Nursery or Main Nursery

Sprouted seedlings were transplanted to the main nursery at one month old on October, 10, 2012 and October, 10, 2013 in a randomized complete block design (RCBD) with three replications. Each plot size was $6m^2$ (3 x 2m) with 1m apart and contained 30 plants spaced 30cm within and 1m between the rows to give a seedling population of 33,333 seedlings per hectare. The seedlings were watered twice daily (morning and evening) during the dry spell, about three to four manual weeding were done to keep the field weed free. Recommended phosphate and urea (ratio 1:1) fertilizer at the rate of 150kg /ha (i.e 450g per plot) was broadcast after preparing the field.

Data Collection

After establishment of the 2012 experiment, there was no dry spell to enable me assess for drought tolerance. Therefore, the experiment was extended to the next year 2013. The rubber genotypes were exposed to drought stress 441 days after planting and scores were taken after subjection to 26, 33 and 40 days of drought stress making it 467,474 and 481days after planting. Data were collected on the following traits (plant vigor, leaf area and percentage plant survival) at various drought stress periods. Percentage plant survival was calculated by counting the number of seedlings that survived after transplanting to the main nursery field according to Samarappuli *et al.,* (1996) after establishment, at 26, 33 and 40 days of drought stress. Plant vigor was scored visually using scale ranges from 1-5 according to Onunka *et al.,*(2004) after establishment before the on-set of drought stress. Where: 1= very high vigor, 2= high vigor, 3= low vigor, 4 = poor vigor and 5= very poor vigor. Data on leaf area (cm^2) after establishment and after 26, 33 and 40 days of drought was measured and calculated. Leaf Area (cm^2) = L x W x K, where L is leaf length, W is maximum width of the leaf and K is a correction factor of 0.654.

RESULTS AND DISCUSSION

Percentage Plant Survival and Plant Vigor

The effects of drought stress on percentage plant survival and plant vigor of ten rubber genotypes at seedling stage in 2012 and 2013 is shown on Tables 1, 2, 3 and 4 respectively.

Table 1: Effects of drought stress and genotypes on percentage plant survival (%) of 10 rubber genotypes evaluated in the year 2012.

		Drought stress periods				
Genotypes	**initial No. of seedlings transplanted**	**0 (days)**	**26 (days)**	**33 (days)**	**40 (days)**	**Genotype mean**
GTI	90	84.444	71.111	71.111	71.111	74.444
Nig 800	90	82.222	62.222	62.222	62.222	67.222
Nig 801	90	70.000	50.000	50.000	50.000	55.000
Nig 802	90	80.000	50.000	50.000	50.000	57.500
Nig 803	90	67.778	48.889	48.889	48.889	53.611
Nig 804	90	81.111	58.889	58.889	58.889	64.444
PB 5/51	90	74.444	40.000	40.000	40.000	48.611
PR107	90	78.889	60.000	60.000	60.000	64.722
RRIM600	90	87.778	72.222	72.222	72.222	76.111
RRIM628	90	62.222	30.000	30.000	30.000	38.058
Drought Means	90	76.889	54.333	54.333	54.333	

F-LSD $_{(0.05)}$

Genotype means = 5.2698***

Drought stress means = 3.3329***

Genotypes * drought stress means = 10.5395*

Percentage plant survival per genotype varied from 62.22% in RRIM 628 to 87.78% in RRIM 600 before the on-set of drought stress. At 26 days of subjection to drought stress, percentage plant survival ranged from 30% in RRIM 628 to 72.22% in RRIM 600. Also at 33 and 40 days of drought exposure, percentage plant survival remained constant in RRIM 628 to 72.22% in RRIM 600. There were no statistical differences (P>0.05) among the genotypes across the drought stress periods. GT 1, RRIM 600 and Nig 804 exhibited the highest percentage plant survival of 84.44%, 87.78% and 81.11%) respectively at 441 days before the imposition of 26, 33 and 40 days of drought stress (Table 1). The long number of days was because of constant rainfall that disturbed drought assessments of the rubber seedlings after establishment. Then at 26, 33 and 40 days of drought stress imposition, percentage plant survival of the genotypes remained the same throughout the drought stress periods. This was because the rubber seedlings had over grown the drought sensitive stage as a result of constant rainfall that disturbed drought assessment in the field in the year 2012.

Even though there was statistical differences (P<0.05 among the genotypes over the drought stress periods in 2013, percentage plant survival ranged from 61.1% in RRIM 628 to 90% in RRIM 600 before the imposition of drought stress. At 26 days of subjection to drought stress, percentage plant survival ranged from 42.2% in RRIM 628 to 82.22% in GT1. Also at 33 days of drought stress exposure, percentage plant survival varied from 32.22% in RRIM 628 to 80% in GT1 whereas at 40 days of drought stress, percentage plant survival ranged from 22.22% in RRIM 628 to 67.7% in GT1. GT1 exhibited the highest percentage plant survival rate of 67.7% at 40 days of drought stress, followed by RRIM 600 with 63.33%. There was obvious negative influence of drought stress on the genotypes as their values decreased from establishment to 40 days of drought stress but the effects of drought stress was more pronounced in PB5/51 with percentage plant survival of between 55.5% and 26.6% from establishment to 40 days of drought stress followed by RRIM 628 with between 61.1% and 22.2% from establishment to 40 days of drought stress (Table 2). The survivability of the rubber seedlings decreased due to effect of drought on them. Genotypes GT1, Nig 804 and RRIM 600 had above 50% plant survivability rate across the drought stress. This implies that the genotypes are tolerant to drought conditions. RRIM 628 and PB5/51 recorded the least percentage plant survival rate of 22.22 and 26.67% at 40 days of drought which means that they are susceptible to drought conditions. Ravichandran *et al.*, (2010) reported that RRIM 600, GT1 and Nig 804 were found to be more tolerant in the field. He also suggested that life saving irrigation may be an essential farm practice for the survival of the other clones in the nursery.

Table 2: The mean effects of drought stress and genotypes on percentage plant survival % of 10 rubber genotypes evaluated in the year 2013.

		Drought stress periods				
Genotypes	**initial no. of seedlings transplanted**	**0 (days)**	**26 (days)**	**33 (days)**	**40 (days)**	**Genotype mean**
GTI	90	87.778	82.222	80.000	67.778	79.444
Nig 800	90	75.556	56.666	45.556	34.444	53.056
Nig 801	90	71.111	63.333	57.778	44.444	59.167
Nig 802	90	70.000	56.666	52.222	40.000	54.722
Nig 803	90	65.556	51.111	47.778	41.111	51.389
Nig 804	90	81.111	68.889	58.889	55.556	66.111
PB 5/51	90	55.556	44.444	37.778	26.667	41.111
PR 107	90	85.556	62.222	58.889	47.778	63.611
RRIM 600	90	90.000	77.778	77.778	63.333	77.222
RRIM 628	90	61.111	42.222	32.222	22.222	39.444
Drought Means		974.33	60.556	54.889	44.333	

F-LSD $_{(0.05)}$
Genotypes means = 17.198***
Drought stress means = 04.5524***
Genotype *drought stress means = 14.396021*

Plant Vigor: In the year 2012, plant vigor varied from 1.0 in RRIM 600 to 2.6 in Nig 803 before the commencement of the drought. At 26 days after exposure to drought stress, plant vigor ranged from 2.0 in GT1and Nig 804 to 4.0 in PB5/51 while at 33 days of exposure to drought, plant vigor varied from 2.3(high vigor) in GT1 and RRIM 600 to 4.6 (low vigor) each in Nig 800 and PB5/5.1 However at 40 days after imposing drought stress, plant vigor varied from 2.6 in GT1 to 4.6 (very poor vigor) in Nig 800, 801,803, PB5/51 and RRIM 628. After a total of 40 days drought, from the result analyzed the highest plant vigor was obtained from GT1. The effects of drought stress were more severe on Nig 800 and PB5/51with plant vigor of 3.0, 3.6 and 4.0 and RRIM 628 with 2.6, 4.0 and 4.0 at 26, 33 and 40 days of drought stress. Drought stress had no effect on GT1 and RRIM 600 at 33 and 40 days of drought stress (Table 3). In 2013, plant vigor varied from 1.0 in GT1and RRIM 600 to 1.6 in Nig 803 after establishment (0drought), at 26 days of subjection to drought stress, plant vigor ranged from 1.0 in GT1 and RRIM 600 to 3.33 in Nig 800. At 33 days of exposure to drought, plant vigor ranged from 2.0 (high vigor) in GT1 and RRIM 600 to 4.0 (poor vigor) in PB5/5 and Nig 800. However at 40 days after imposing drought stress, plant vigor ranged from 2.33 in GT1 to 5.0 (very poor vigor) in PB5/51. After a total of 40 days drought, from the result analyzed the highest plant vigor was obtained from GT1 with 2.33 (Table 4). Generally the effects of drought stress were more severe on all the genotypes except GTI with plant vigor of 2.33 after 40 days of drought stress and drought stress had no effect on GT1 and RRIM 600 after 33 days of drought stress in both years.

Table 3: Effects of drought stress and genotypes on plant vigor of 10 seedlings of rubber genotypes at nursery stage in the year 2012.

	Drought stress periods				
Genotypes	**0**	**26 (days)**	**33 (days)**	**40 (days)**	**Genotype mean**
GTI	1.333	2.000	2.333	2.667	1.667
Nig 800	1.333	3.000	4.667	4.667	3.000
Nig 801	1.667	3.333	3.667	4.667	2.333
Nig 802	2.000	3.000	3.667	4.000	2.500
Nig 803	2.667	3.333	3.667	4.667	2.333
Nig 804	1.333	2.000	3.000	3.000	2.333
PB 5/51	2.333	4.000	4.333	4.667	3.250
PR107	2.000	2.333	3.333	4.667	2.500
RRIM600	1.000	2.333	2.333	3.000	1.583
RRIM628	2.333	2.667	4.000	4.667	3.25
Drought means	2.700	3.200	3.000	4.000	

F-LSD $_{(0.05)}$
Genotype means = 0.3419***
Drought stress mean = 0.2162***
Genotypes * drought stress means = 0.6837**

Table 4: Mean effects of drought stress and genotypes on plant vigor of 10 seedlings of rubber genotypes at nursery stage in the year 2013.

	Drought stress periods				
Genotypes	**0 (days)**	**26 (days)**	**33 (days)**	**40 (days)**	**Genotype mean**
GTI	1.000	1.000	2.000	2.333	1.500
Nig 800	1.333	3.333	4.000	4.667	2.333
Nig 801	1.333	3.000	3.333	4.000	2.000
Nig 802	1.333	3.000	3.333	4.667	2.000
Nig 803	1.667	2.000	3.333	3.667	2.1667
Nig 804	1.333	2.000	2.333	3.667	1.833
PB 5/51	1.333	3.000	4.000	5.000	2.5833
PR 107	1.333	2.333	3.333	4.000	1. 833
RRIM 600	1.000	1.000	2.000	3.000	2.583
RRIM 628	1.333	2.000	3.000	4.667	3.583
Drought Means	1.300	1.567	3.367	4.933	

F-LSD $_{(0.05)}$
Genotypes means = 0.344***
Drought period means = 0.2182***
Genotype * drought stress means = 0.689896**

Leaf area: Leaf area varied from 3056.2cm^2 in PB5/51 to 12319.1cm^2 in GT1 before the on-set of drought stress while at 26 days after exposure to drought stress, leaf area ranged from 2382.55cm^2 in PB5/51 to 15820.8cm^2 in GT1. At 33 days after imposing drought stress, leaf area ranged from 2313.26cm^2 in PB5/51 to 16504.5cm^2 in GT1 while at 40 days after subjection to drought stress, leaf area varied from 1833.08cm^2 in PB5/51 to 17277.9cm^2 in GT1. There were significant differences ($p<0.05$) in leaf area among the genotypes (RRIM 628, PB5/51 Nig 801, Nig 800 etc) at 26, 33 and 40 days of drought stress. However the highest leaf area was obtained from GT1 (17277.9cm^2) at 40 days of drought stress followed by RRIM 600 with 12088.45cm^2 and PR107 with leaf area of 11168.45cm^2 (Table 5). In 2013, leaf area varied from 164.49cm^2 in PB5/51 to 314.81cm^2 in GT1 after establishment (0drought) then at 26 days after imposing drought stress, leaf area ranged from 293.89cm^2 in PB5/51 to 503.73cm^2 in GT1.

Table 4.5: Effects of drought stress and genotypes on leaf area (cm^2) of 10 rubber genotypes evaluated in the year 2012.

	Drought stress periods				
Genotypes	**0 (days)**	**26 (days)**	**33 (days)**	**40 (days)**	**Genotype mean**
GTI	12319.1	15820.8	16504.5	17277.9	15480.56
Nig 800	6842.66	7979.71	7821.53	6874.11	7379.50
Nig 801	7875.00	7940.36	8539.35	3429.92	6946.16
Nig 802	7541.30	9132.81	10170.2	9692.35	9134.09
Nig 803	7543.08	9964.43	11375.9	11066.2	9986.80
Nig 804	10298.2	11407.6	11603.4	10789.6	11024.7
PB 5/51	3056.24	2382.55	2313.26	1833.08	2396.28
PR107	10941.2	10977.8	11541.2	11168.1	11157.1
RRIM600	10890.9	10928.07	12186.7	12088.5	11523.5
RRIM628	3405.63	2397.30	2601.91	1923.75	2582.15
Drought period Means	8071.33	8893.15	8761.79	8614.34	

F-LSD $_{(0.05)}$
Genotype means = 7.5053***
Drought stress mean = 4.7468***
Genotype * drought stress means =15.0106*

At 33 days after exposure to drought stress, leaf area ranged from 316.55cm^2 in PB5/51 to 724.77cm^2 in GT1 while at 40 days after subjection to drought stress, leaf area varied from 200.47cm^2 in RRIM 628 to 982.80cm^2 in GT1 (Table 6). There were influences of drought stress on the leaf area of the rubber genotypes at 26, 33 and 40 days of drought stress but effects of drought was most severe on PB5/51 with leaf area of 2382.55cm^2, 2313.26cm^2 and 1833.08cm^2 after 26, 33 and 40 days drought stress respectively and RRIM 628 with leaf area of 2397.3cm^2, 2301.91cm^2 and 1923.75cm^2 after 26, 33 and 40 days of drought stress which showed that they were more susceptible to drought conditions than the other genotypes in 2012. Mean while in 2013, there were significant differences ($p<0.05$) in leaf area among the genotypes (RRIM 628, PB5/51 Nig 801, Nig 800 etc) at 26, 33 and 40 days of drought stress. However the highest leaf area was obtained from RRIM 600 (1015.3cm^2) at 40 days of drought stress followed by GT1 with leaf area of 982.8cm^2 and Nig 804 with 590.96cm^2. Effects of drought stress was most severe on PB5/51 with leaf area of 293.89cm^2, 293.89cm^2 and 218.19cm^2, RRIM 628 with leaf area of 306.26cm^2, 306.26cm^2 and 200.47cm^2 after 26, 33 and 40 days of drought stress respectively. Burning of lamina, drying and senescence were also observed causing considerable reduction in leaf area of these genotypes. Genotype RRIM 600 performed better in similar drought stress condition. Under acute drought stress in field the young nursery grown rubber seedlings of PB5/51 and RRIM 628 produced leaves with reduced leaf area and low chlorophyll content and this may cause considerable reduction in total functional leaf area of the plant possibly due to enhanced photo-oxidative damage leading to pigment bleaching.

Table 6: Mean effects of drought stress on leaf area (cm^2) of 10 rubber genotypes evaluated in the year 2013.

	Drought stress periods				
Genotypes	**0 (days)**	**26 (days)**	**33 (days)**	**40 (days)**	**Genotype mean**
GTI	314.81	503.73	724.77	982.80	631.53
Nig 800	172.41	390.07	458.69	262.29	320.86
Nig 801	195.14	370.79	443.46	435.42	361.20
Nig 802	177.74	429.33	499.97	470.49	394.38
Nig 803	197.79	350.06	482.33	501.91	383.02
Nig 804	199.85	402.22	502.37	590.96	423.85
PB 5/51	164.49	293.89	293.55	218.19	248.28
PR 107	243.46	448.06	515.50	451.32	414.59
RRIM 600	285.12	491.27	680.38	1015.3	618.01
RRIM 628	171.44	306.26	306.26	200.47	254.44
Drought period Means	212.26	398.57	435.76	512.92	

F-LSD $_{(0.05)}$
Genotypes means = 1.848***
Drought stress means = 1.169***
Genotype *drought stress mean = 3.697**

Reducing leaf area is a major phonological adaptation of deciduous trees to seasonal drought. Many deciduous trees drop all their leaves during seasonal drought and sprout new ones just before or after the first rain. The leaf area of GT1, RRIM 600 and Nig 804 increased consistently across the drought stress periods in both years (Tables 1 and 6). This is so because they are tolerant to drought conditions. These findings had already been reported by Samarappuli *et al.,* (1992b) who found that RRIM 600, RRIC 121 and GT1 grow better under drought stress as exhibited by introduced genotypes. In contrast PB5/51, RRIM 628 and Nig 800 were more susceptible to drought at 33 and

40 days. Abrams (1988) reported genetic variation in rubber leaf morphology due to drought effect on rubber trees.

CONCLUSION

Genetic variability for drought tolerance existed among the genotypes used. Genotypes GT1, Nig 804 and RRIM 600 were found to be drought tolerant while genotypes susceptible to drought were RRIM 628 and PB/5/51. Nig 800 was badly affected in 2013 (seedling evaluation) while in 2014 it was tolerant. Genotypes PR107, Nig 801, Nig 802 and Nig 803 were moderately tolerant to drought stress. Drought tolerant genotypes like RRIM 600, GT1, Nig 804 and PR107 should be selected for rubber improvement in Nigeria. Screening for drought tolerance in rubber plant should be done at seedling stage.

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