WFL Publisher
Science and Technology

Journal of Food, Agriculture & Environment Vol.3 (1) : 153-156. 2005 *www.world-food.net*

Genetic variation in *Enantia chlorantha* (Oliv.) - a medicinal plant

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Received 9 August 2004, accepted 22 November 2004.

Abstract

Genetic variations in *Enantia chlorantha* from four sources were studied. Mature fruits of the species were collected from Lafe near Ore (lat. 6° 44^{1}N and longitude 4° 52^{1}E), Omo Forest Reserve – 1 & 2 (lat. 6° 35^{1}N – 7° 05^{1}N and longitude 4° 05^{1} - 4° 40^{1}E) and Okitipupa (latitude 6° N and longitude 5° E). Ten bunches were randomly selected from each source and the number of fruits per bunch were counted. The fruits were depulped and thirty seeds were randomly selected from each seed source and their weights, diameters and lengths were measured. Another one hundred seeds from each source were selected and sown in germination trays filled with washed and sterilized river sand and watered daily. Germination counts were taken daily. At the two-leaf stage, thirty uniformly growing seedlings from each source were transplanted into polythene bags filled with topsoil. Height, collar diameter and number of leaves per seedling were measured fortnightly for five months. Biomass assessment of the seedlings was carried out at the end of the 5 months period. The effect of seed sources was highly significant ($P \leq 0.01$) on the number of fruits per bunch in *E. chlorantha.* Bunches from Lafe had the highest mean number of fruit (37.5±1.96) followed by Okitipupa (28.4±2.55) while the lowest value (13.5±0.9) was recorded in Omo-1. Seed sources significantly ($P \leq 0.01$) affected all the metrical traits considered in this study. Seeds from Omo-1 had the greatest mean length, diameter and weight of 2.0±0.02 cm, 1.1±0.11 cm and 1.0±0.02 g respectively. Seeds from Lafe were the smallest with mean length of 1.1±0.02 cm, diameter of 0.7±0.08 cm and weight of 0.4±0.01 g. Commencement of germination and percentage germination varied among the seed sources. Germination was first observed at 25 days after sowing (DAS) among the seedlots from Omo-1 and Omo-2 while seedlots from Okitipupa and Lafe commenced germination at 26 and 27 DAS respectively. Seeds from Lafe had the highest percentage germination of 69 followed by Omo-1 with 61, Okitipupa (52%) and Omo-2 (38%). There were significant differences ($p \leq 0.05$) in germination among the sources. Seedlings from Omo-1 had the highest mean value in height (13.2 cm), collar diameter (2.6 cm) and number of leaves (9.4) while seedlings from Omo-2 gave the lowest values (height 8.8 cm, collar diameter 2.1 cm and number of leaves 5.8). Seedlings from Lafe had the highest mean total dry weight of 0.42 g, followed by Omo-1 with 0.4 g while Omo-2 gave the lowest value of 0.16 g.

Key words: Genetic variation, seed size, seedling growth, *Enantia chlorantha.*

Introduction

The amount of diversity available in thousands of useful tree species on earth constitutes inter-generational resources of vast social, economic and environmental importance [1]. However, these resources are continuously eroded through deforestation and subsequent changes in land use pattern, over exploitation and destructive collection of forest resources as well as inappropriate and illegal timber and wood harvesting practices. Variation in populations of species allows for conservation of unique genetic units and also provides a backup for the survival of these organisms. The extent of diversity in a population is determined by gene environment interaction (gei), but the gene component exacts a more definite control on the expression of traits by organisms from generations to generations. Often, there are wide latitude in growth rate, morphology and even constituents of the active ingredients in plants of the same species growing in the same or different environments. Zobel and Talbert [8] stressed that individual trees often vary a great deal from one another even when growing in the same stand. Genetic variation can play a vital role in the use of forest trees. Our ignorance of intra-specific variation of medicinal species is the causes of the criticism that – due to environmental and genetic variation – dosage of active ingredients in herbal medicine are difficult or impossible to accurately determine [2].

Enantia chlorantha (Oliv.) is a member of the family Annonaceae and it is known locally as "Awopa or Yanni" in Yoruba. The extract from its bark is used in the treatment of malaria and other diseases. Keay [7] gave a detailed description of this species. *Enantia chlorantha* is a slow growing medicinal tree species with limited geographical range in the lowland rainforest of Nigeria and Cameroon. Due to its efficacy for the treatment of malaria fever, mass felling of the natural stand with attendant genetic erosion is a usual occurrence in Nigeria. The survival of this species has been left to the vagaries of the nature since the variations in its population have not been studied and documented to enhance selection and domestication of superior traits. Therefore, this study was carried out to assess the genetic variation in the morphological characters of seeds and seedlings of *Enantia chlorantha* from four sources in Nigeria.

Materials and Methods

Fruit collection and measurement: Mature fruits of *Enantia chlorantha* were collected in bunch from each of the four sources listed in Table 1. Ten bunches were randomly selected from each source and the number of fruits per bunch were counted. The mean number of fruits per bunch was computed and recorded. Three hundred fruits of the species from each source were depulped by gently squeezing the fleshy pulp to extract the seeds. Thereafter, thirty seeds were randomly selected from each seed source and their weight, diameter and length were measured using electronic balance, a digital caliper and metre ruler respectively.

***Seed germination**:* From each seed lot, one hundred good seeds (without pin holes or visible disease attack) were selected and sown in germination trays filled with washed and sterilized river sand and labeled according to seed source. The trays were kept under a high humidity propagator at the greenhouse of the Department of Forest Resources Management, University of Ibadan. Watering was done daily in the morning using a fine- meshed watering can. Germination counts were taken daily and germination was considered to have occurred, when the plumule has emerged and can be seen above the soil in the germination tray. Germination recordings were discontinued and considered to have been completed when no additional germination took place in a week.

***Seedling growth and biomass assessment**:* At the two-leaf stage, thirty uniformly growing seedlings from each provenance were transplanted into medium-sized polythene bags measuring 16 cm x 14 cm x 12 cm filled with topsoil. Additional five seedlings were transplanted from each provenance to serve as buffer for the experimental seedlings in the open nursery. The transplanted seedlings were initially left under shade for two weeks to wean. After the two weeks, the seedlings were transferred into the open nursery and laid out in a completely randomized design in three replicates. The following growth parameters: height, diameter at the collar and number of leaves per seedling were measured fortnightly for five months. Seedling height and collar diameter were measured with metre ruler and caliper respectively. Biomass assessment of the seedlings was carried out at the end of experimental period (5 months); five seedlings were randomly selected from each source and carefully uprooted. The uprooted seedlings were then divided into root and shoot components. The total leaf area of each seedling was determined using the grid method. Afterwards, root and shoot components of each seedling were oven-dried for 24 hours at 80°C. The samples were allowed to cool to a constant temperature before the dry weights were measured using an electronic weighing balance. The data collected were subjected to statistical analysis using the SAS package.

Results

***Number of fruits per bunch in E. chlorantha**:* Bunches from Lafe had the highest mean number of fruits (37.5) followed by Okitipupa (28.4) while the lowest value (13.5) was recorded in Omo-1. The fruit bunches from Omo-2 were not intact on collection hence they were not included in the assessment. The coefficient of variability was 21.1% (L.S.D = 5.2) (Table 2). The effect of fruit sources was highly significant (p≤0.01) on the number of fruits per bunch. However, there were no significant differences in the number of fruits per bunch within source (Table 3).

***Morphological traits of seeds**:* The summary of the mean values of metrical characters of seeds of *Enantia chlorantha* from four sources is shown in Table 4. Analysis of variance showed that source had a very high significant effect (P≤0.01) on length, diameter and weight of seeds (Table 5). Seeds from Omo-1 had the highest mean values for length (1.96 cm), diameter (1.14 cm) and weight (1.01 g). On the other hand, seeds from Lafe had the lowest mean values (length 1.14 cm, diameter 0.7 cm and weight 0.63 g). The coefficient of variability (%) was highest for seed weight (19.5%) and lowest (5.3%) for seed diameter.

***Seed germination**:* Commencement of germination (days after sowing - DAS) varied among the seed sources. Germination was first observed at 25 DAS among the seed lots from Omo-1 and Omo-2 while seed lots from Okitipupa and Lafe commenced germination at 26 and 27 DAS respectively. Percentage germination varied among the sources. Seeds from Lafe had the highest percentage germination (69) followed by Omo-1 (61), Okitipupa (52) and Omo-2 (38). Also, the rate of germination varied substantially among the sources (Fig. 1).

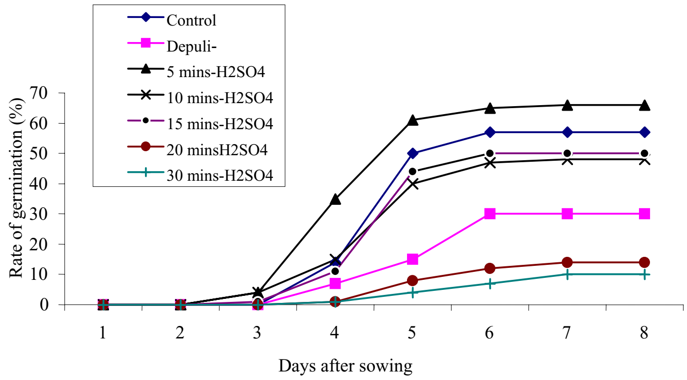

Figure 1. Rate of germination of *E. chlorantha* seeds under different pretreatments.

***Seedling growth height**:* Seedlings from Omo-1 had the highest mean height growth of 13.2 cm followed by seedlings from Lafe (12.5 cm) and Okitipupa (8.9 cm) while Omo-2 had the lowest mean height value of 8.8 cm. The coefficient of variability in seedling height across the sources was 8.72% (Table 6). The effects of sources, replication and age as well as interactions between source and age and source and replicate were highly significant (p≤0.01) on seedling height growth. The interaction between age and replicate did not have any significant effect on seedling height (Table 7).

***Collar diameter**:* Seedlings from Omo-1 had the highest mean diameter of 2.62 cm followed by seedlings from Lafe (2.6 cm) and Okitipupa (2.4 cm) while seedlings from Omo-2 had the lowest mean diameter value (2.1 cm). The coefficient of variability in diameter of seedlings from the sources was 5.39% (Table 6). Seed sources, age, replicate and the interactions between source x age and source x replicate all had highly significant effects (p≤0.01) on seedling diameter growth. However, the interaction between age and replicate had no significant effect on the diameter growth of seedlings (Table 7).

***Number of leaves**:* The highest mean number of leaves of 9.4 was recorded among Omo-1 seedlings followed by seedlings from Lafe (9.3) and Okitipupa (6.9), while the lowest value of 5.8 was obtained in seedlings from Omo-2. The highest coefficient of variability of 11.6% in all seedling traits was recorded in number of leaves per seedling from the different sources (Table 6). Number

Table 1. Sources of fruits and seeds for the study of genetic variation in *E. chlorantha.*

S/N	Fruit/Seed Source	State	Country	Latitude	Longitude
1	Lafe	Ondo	Nigeria	6° 44^{1}N	4° 52^{1}E
2	Omo Forest Reserve-1	Ogun	Nigeria	6° 35^1-7° 05^{1}N	4° 05^1- 4° 40^{1}E
3	Omo Forest Reserve-2	Ogun	Nigeria	6° 35^1-7° 05^{1}N	4° 05^1- 4° 40^{1}E
4	Okitipupa	Ondo	Nigeria	6°N	5°E

Table 2. Mean number of fruits per bunch of *Enantia chlorantha.*

Source	Number of fruits per bunch
Lafe	37.5*
Okitipupa	28.4
Omo – 1	13.5
C.V. (%)	21.1
L.S.D.	5.2

*Values are means of 10 bunches

Table 3. Analysis of variance for the number of fruits per bunch of *E. chlorantha* collected from four sources.

Source of variation (SV)	DF	MS	F Value
Source	2	1468.03	47.08**
Within - source	9	49.13	1.58ns
Error	18	31.18	

** = Significant at 1% probability level; ns = not significant.

Table 4. Morphological characters of the seeds of *E. chlorantha* from four sources.

Source	Seed length (cm)	Seed diameter (cm)	Seed weight (g)
Omo – 1	1.96^a	1.14^a	1.01^a
Okitipupa	1.84^b	1.05^b	0.60^b
Omo – 2	1.53^c	0.9^c	0.50^c
Lafe	1.14^d	0.7^d	0.39^d
Mean	1.62	9.41	0.63
CV (%)	6.93	5.31	19.53
SE	± 0.11	± 0.50	± 0.12
L.S.D (5%)	0.06	0.26	0.06

Values in the same column with similar letters are not significantly different.

Table 5. Analysis of variance for morphological characters of *E. chlorantha* seeds from four sources.

		Length		Diameter		Weight	
SV	DF	MS	F Value	MS	F Value	MS	F Value
Source	3	4.045	321.8**	120.29	481.7**	2.211	148.4**
Replicate	29	0.016	1.34ns	0.28	1.14ns	0.01	0.68ns
Error	87	0.013		0.25		0.015	

** = Significant at 1% probability level; ns = not significant

Table 6. Means of metrical characters of seedlings of *E. chlorantha* from four sources.

Source	Seed height (cm)	Seedling diameter (cm)	Number of leaves
Omo – 1	13.18^a	2.62^a	9.42^a
Lafe	12.51^b	2.61^a	9.26^a
Okitipupa	8.94^c	2.38^b	6.88^b
Omo – 2	8.78^c	2.07^c	5.84^c
Mean	10.85	2.42	7.85
CV (%)	8.72	5.39	11.49
S.E.	± 0.95	± 0.13	± 0.90
L.S.D (5%)	0.29	0.04	0.27

Values in the same column with similar letters are not significantly different.

Table 7. Analysis of variance of metrical characters of seedlings of *E. chlorantha from* four sources.

		Seedling height		Seedling diameter		Number of leaves	
SV	DF	MS	F value	MS	F value	MS	F value
Source(R)	3	1611.5	1798.22*	20.03	1173.79*	944.05	1160.6*
Age (A)	9	1074.8	1199.30*	24.62	1442.64*	1633.45	2008.1*
Replicate(R)	29	8.51	9.50*	0.20	11.4*	7.77	9.6*
S x A	27	62.10	69.30*	0.90	53.03*	49.60	60.7*
A x R	261	0.75	0.83ns	0.02	0.87ns	0.57	0.7ns
S x R	87	11.75	13.11*	0.29	16.78*	12.69	15.6*
Error	783	0.90		0.02		0.81	

*= Significant at 5%; ns = not significant at 5% probability level

Table 8. Analysis of variance of biomass production in seedlings of *E. chlorantha* from four sources.

		Root dry weight		Stem dry weight		Leaf dry weight		Leaf area		Total dry weight	
SV	DF	MS	F value	MS	F value	MS	F value	MS	F Value	MS	F value
Source	3	0.003	9.90*	0.015	0.78ns	0.0021	2.19ns	466124.75	5.56*	0.046	2.23ns
Replicate	2	0.0003	1.25ns	0.021	1.15ns	0.0012	1.19ns	21720.25	0.26ns	0.025	1.2ns
Error	6	0.0003		0.019		0.0010		83883.58		0.020	

* = Significant at 5%; ns = not significant at 5% level of probability

Table 9. Mean biomass of seedlings of *E. chlorantha* from four sources.

Source	Root dry weight [g]	Stem dry weight [g]	Leaf dry weight [g]	Leaf area (cm^2)	Total dry weight [g]
Omo-1	0.11^a	0.19^a	0.10^a	1366^a	0.40^a
Lafe	0.10^{ab}	0.23^a	0.09^a	1286^{ab}	0.42^a
Okitipupa	0.07^{bc}	0.15^a	0.05^a	732^{bc}	0.27^a
Omo-2	0.04^c	0.07^a	0.04^a	577^c	0.15^a
Mean	0.082	0.16	0.07	990.2	0.31
CV (%)	20.41	85.53	45.77	29.25	46.0
S.E.	± 0.017	± 0.14	± 0.03	± 289.63	± 0.14
L.S.D.	0.03	0.27	0.06	578.7	0.29

Values in the same column with similar letters are not significantly different.

of leaves differs significantly ($p \leq 0.01$) among the seedlings from the four sources. The effect of seed source, age, replicate as well as the interaction of sources x age and source x replicate were significant on leaf production in seedlings. The interaction between age of seedlings x replicate had no significant effect on the number of leaves among the seedlings (Table 7).

Biomass production

***Root dry weight (RDW)**:* The effect of seed sources was significant ($p \leq 0.05$) on root dry weight (RDW) of *E. chlorantha* seedlings (Table 8). Seedlings from Omo-1 had the highest mean RDW of 0.11 g followed by seedlings from Lafe (0.10 g) and Okitipupa (0.07 g) and the lowest value of 0.04 g was obtained in seedlings from Omo-2. The mean RDW of seedlings from Omo-1 was significantly different from those of Okitipupa and Omo-2 but not significantly different from that of Lafe (Table 9).

***Stem dry weight (SDW)**:* Seed sources and replicate had no significant effect on stem dry weight (SDW) of seedlings from the four sources (Table 8). Seedlings from Lafe had the highest mean SDW of 0.23 g followed by Omo-2 (0.19 g) and Okitipupa (0.15 g), while Omo-1 had the lowest mean value of 0.07 g. The mean SDW of seedlings from all the sources were not significantly different from each other (Table 9).

***Leaf dry weight (LDW)**:* The effect of seed source and replicate were not significant on leaf dry weight (LDW) of the seedlings (Table 8). Seedlings from Omo-1 had the highest mean value for LDW (0.10g) followed by seedlings from Lafe (0.09 g), Okitipupa (0.05 g) and Omo-2 (0.04 g). The mean LDW of seedlings from the four sources were not significantly different from each other (Table 9).

***Total dry weight (TDW)**:* Seed source and replicate had no significant effect on total dry weight (TDW) of seedlings from four sources (Table 8). Among the sources, seedlings from Lafe had the highest mean TDW of 0.42 g followed by seedlings from Omo-1 (0.40 g) and Okitipupa (0.27g) while Omo-2 had the lowest mean value of 0.16 g. The mean TDW of seedlings from the four sources were not significantly different from each other (Table 9).

Discussion and Conclusions

E. chlorantha is a medicinal tree with established market structure for its products. The high demand for its parts underscore the importance of good vegetative growth rate. A provenance with superior vegetative growth will reduce the gestation period and capital tie-down on investment as well as offers a good competitive ability against weeds in plantation. The variations observed in seed size and seedling vigour of *E. chlorantha* from the different sources offers a unique opportunity for selection and improvement of this species. Seeds from Omo-1 were the biggest in terms of length, breadth and weight; they were also the first seedlot to germinate in addition to having the second highest percentage germination. Usually, seeds contain food reserves, which are depleted during germination to produce shoot and root components of the seedlings. When a seed is big and heavy, it is an indication of large food reserve, which may be manifested in vigorous seedling [3]. This makes Omo-1 a very good seed source for the production of the seedlings of the species for plantation establishment. Also, seedlings from Omo-1 gave the best performance in terms of height, collar diameter and number of leaves per seedling. This agreed with the findings of Oni and Bada [4] and Oni and Gbadamosi [5] that seed size and seedling vigour are positively related.

Seed morphological traits showed a high degree of correlation with seedling characteristics. Seed weights were highly correlated with seedling height, diameter and number of leaves. This is an important selection criterion as higher number of leaves may be indicative of high photosynthetic ability and consequently a better growth rate of the seedlings. Dangasuk et al. [6] made a similar observation on twelve African provenances of *Faidherbia albida.*

What is harvested as forest products in the form of fruits, nuts, leaves, barks and roots for various end uses are biomass produced by the living tree. Hence, the rate of biomass accumulation by a tree is crucial to its growth rate vis-à-vis the quantity of its product that can be available per unit time. Also, pattern of assimilate partitioning in a tree species can affect product availability. Seedlings from Lafe had the highest total dry weight (TDW) and stem dry weight (SDW) within the experimental period. This may be a good indication of the performance of the adult tree produced from seedlings from this source, thus reducing the number of tree to be exploited to get the same quantity of product thereby reducing pressure on the available stand in the forest. In the same vein, the stem bark of *E. chlorantha* is mostly in use, therefore, this trait can be manipulated in the hybridization of the species to enhance its quality.

E. chlorantha is slow growing in nature with intermittent profuse flushes. This may qualify it as a good agro-forestry species, which can allow the co-existence of food crops for some years. With increasing pressure on the limited land area for various economic activities, trees that can tolerate agronomic crops stands a better chance in the competition for land.

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