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Screening of Some Hybrid Lines of *Telfaria occidentalis* (F. Hook) for Adaptability to the Rain Forest Ecology and Resistance to Telfaria Mosaic Virus (Temv)

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Authors' contributions

This work was carried out in collaboration between all authors. Author GMU designed the study. Authors IPE and UCT wrote the protocol and author GMU wrote the first draft of the manuscript. Authors IPE, EEI and ISE managed the literature searches. Author GMU performed the analyses of the study and all authors discuss the conclusion. All authors read and approved the final manuscript.

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ABSTRACT

The adaptability to the rain forest ecology and resistance to the Telfaria Mosaic Virus (TeMV) of seven (7) hybrid lines and a local cultivar of *Telfaria occidentalis* were evaluated in Esierebom, Calabar and Uyo in 2014 and 2015 vegetable growing seasons. Hybrid lines of *T. occidentalis* were screened for resistance to TeMV and adaptability to the rainforest ecology using growth and reproductive performance after inoculation. Data for growth performance was taken fortnightly

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while data for reproductive performance were taken at maturity. Data generated were collated and analysed for significant (P<0.05) differences at 5% level of probability according to the analysis of variance (ANOVA) procedures. The results shows that the order of performance and resistance to the virus was EN 2000-19 > EN 2000-6 > EN 2000 – 17 > EN 2000- 9 > EN 2000-1 > EN 2000-24 >EN 2000-X > 'Edem Ubong'. The results reveal significant (P<0.05) differences among hybrids lines and local cultivar for number of primary branches per plant. Biomass weight (kg), vine length (m) and Leaf area (cm^2) also differed (p<0.05) significantly among the hybrid lines and local cultivar. Results showed that the lines EN 2000-19, EN 2000-17 and EN 2000-6 were highly adapted to the rainforest ecology with significant (P<0.05) higher differences in lengths of vines, leaf area, number of branches per plant and number of leaves per plant. EN 2000-24, 'Edem Ubong', EN 2000-x and EN 2000-1 all showed least adaptability with significant (P<0.05) low differences in vine lengths, number of primary and secondary branches per plant, number of leaves per plant and leaf area. Results of lines resistance to TeMV showed that the cultivars, EN 2000-1, 'Edem Ubong', EN 2000-X and EN 2000-24 were highly susceptible to the virus with resultant low growth and reproductive performance while the lines EN 2000-19, EN 2000-17 and EN 2000-9 showed high resistance to the virus through high growth and reproductive performance. Adaptable and highly resistance lines of *T. occidentalis* can be harness for the purpose of increasing the yield of the vegetable in this agro-ecology using marker assisted selection for maintaining stability of hybrid lines after segregation in subsequent generations.

Keywords: Adaptability; resistance; TeMV; hybrid lines; rain forest ecology, Telfaria occidentalis.

1. INTRODUCTION

The importance of leafy vegetables in the nutritional requirements of humans and animals has created a heightened need for seeking for means of increasing it productivity and adaptability especially in the rainforest agro-ecological zone of Nigeria where *Telfaria occidentalis* has remained a household leafy vegetable for the preparation of local delicacies like 'Edikang ikong', 'Ofe ugwu' 'Ekpang Kukwo', Anyan Ekpang among others. *Telfaria occidentalis* is widely eaten in Nigeria especially in the rain forest agro-ecological zone where it is highly cultivated for it edible succulent shoots, tender vines, leaves and seeds.

The genus Telfaria named after the famous Irish naturalist, botanist and plant collector Charles Telfair, is a nutritionally important member of the family Cucurbitaceae and order Telfarieae [1,2], which has over 130 genera and 900 species and is among the most important and wide spread families [3]. It is a tropical dioecious vegetable and perennial herbaceous cucurbit which climbs by means of coiled tendrils [4].

Telfaria occidentalis (F. Hook) is a leafy vegetable commonly found and cultivated in the tropics where it is commonly called fluted pumpkin. The growth and reproductive performance of *T. occidentalis* is dependent on genotypes, prevailing soil conditions, pests and diseases and best agronomic practices [5]. *T. occidentalis i*s a diploid plant with a chromosome number of 2n=22 [6].

Telfaria occidentalis is of West Africa origin and is grown across the low land humid tropics of West Africa. The crop is partially drought resistant and is tolerant to a wide range of ecology [7]. Soil pH of 6.5 – 7.0 is preferable and in loamy clay texture. The yield is enhanced with the application of nitrogen – based fertilizers or in areas with high organic matter content [8].

T. occidentalis is a perennial vine growing up to ten (10) meters in length [9]. It has branched tendrils with coil tubes. Leaves have three to four leaflets while petiole varies between 2 – 5 cm. Lower pairs of leaflets are asymmetrical, oviate and shortly acuminated. Leaflets are arranged alternately along the vine length and terminal leaflet may be up to 15 cm in length. The plant is diecious bearing male and female flowers in different plants. Male plants produce smaller leaves than the female plants. Male inflorescence projects conspicuously from the stem on petals, which are usually between 5 to 25 cm in length and are white, small and produced in racemes. Female flowers are solitary and short stake and are produce in the axils of leaves. Corolla lobes of female plants are creamy, whitish or purple dark in basal area with short stiff bristles. The fruits are pale green, with waxy whitish deposit, strongly ribbed at maturity, up to 25 cm in diameter, 3 – 36 kg in weight and with light yellow and fibrous mesocarp. The

seeds are flat, reaching between 3 – 6 cm at maturity attached by a slight projection to the placenta. Thirty to seventy seeds may sometimes be produced per fruit (pod) as reported by [10].

Telfaria occidentalis is mostly affected by Telfaria mosaic virus, which has plague the vegetable for centuries and has reduced the yield of the vegetable as much as 75% [11]. The virus has affected the yield of the vegetable for years especially with the continuous cultivation of available local cultivars known as Edem ubong and Edem aran, which are highly susceptible to the pathogen. This has led to reduced yield, vegetable food shortages, high prices of the vegetable and low farmer's income. The virus has remained intractable to local efforts due to unfamiliar nature and unclassified characteristics of the viral pathogen and the absence of planned research efforts by mandate institutes in this direction, thus leading to continuous decline in yield of the vegetable and reduced farmer's income.

It was in the light of this, that this research was carried out to screen and evaluate some hybrid lines for adaptability to the rainforest ecology and examine the inherent resistance of the hybrid lines to the devastating viral pathogen, so the resistant lines can be recommended for use for increase yield of the vegetable, improvement of resistance in susceptible lines and increase adaptive ability of the least adaptable lines.

2. MATERIALS AND METHODS

2.1 Plant Materials

Seven hybrid lines of *Telfaria occidentalis* were obtained from the National Horticultural Research Institute (NIHORT) Ibadan, their identities were as follows;

i. EN 2000 – 24
ii. EN 2000 – X
iii. EN 2000 – 9
iv. EN 2000 – 1
v. EN 2000 – 17
vi. EN 2000 – 6
vii. EN 2000– 19 and a local cultivar obtained locally known as
viii. 'Edem Ubong'

2.1.1 Study area

The laboratory bench work was carried out in the University of Calabar, while the field work was carried out in Esierebom, Calabar, Cross River State and Akpa Ube area of Uyo, Akwa Ibom State respectively.

2.1.2 Sterilization of soil

Soil used for the study was mixed with 1.0kg of cowdung to improve its fertility status. The soil was later put in an open container and later into a wide pan and heated for about an hour. This was aimed at getting rid of soil borne pathogens. The sterilized soil was then allowed to cool properly before been used to filled polybags, which was used for planting the Telfaria seeds.

2.1.3 Propagation

Seeds of the hybrid lines and local cultivar were screened for viability and later sown out in polybags half filled with 500g of sterilized soil. The experiment was laid out in a Completely Randomized Designed (CRD) with three replicates. One seed was planted per polybag and was watered twice daily to avoid rotting of seeds. All agronomic and routine vegetable crop production practices were duly observed and followed.

2.1.4 Source of viral inoculum

The viral pathogen of Telfaria mosaic virus disease of Telfaria was obtained from heavily infested Telfaria plants in Esierebom in Calabar South, Cross River State and Akpa Ube area of Uyo, Akwa Ibom state where vegetable cultivation is a major source of livelihood activities of the women and showing associated symptoms of mottling and cupping of young leaves, vein clearing, large yellow patches on leaves and mosaic patterns. The virus was isolated from the infected plants according to koch's procedures and used for the study.

2.1.5 Preparation of buffer

A 0.03 M sodium phosphate buffer was used as inoculation buffer. The buffer was prepared by dissolving 4.26 g of disodium hydrogen phosphate salt (Na_2HPO4) in a litre of distilled water to give a 0.03 molar solution with a pH of 9.3. The pH was adjusted by using 0.5 m monosodium dihydrogen phosphate solution (NaH_2PO4) until the desired pH of 8.0 was obtained. The buffer was kept in a refrigerator until it was required.

2.1.6 Mechanical inoculation of *T. occidentalis* with TeMV:

The prepared inoculum was used to mechanically inoculate a number of test plants from the Telfaria lines evaluated. Leaves from each plant in each experimental unit were mechanically inoculated at the 2 to 3 leaf stage at 9 to 10 days after germination. The leaves to be inoculated were first dusted with Carborandum (600 mesh) and a smooth wood used to rub the inoculum gently on the leaves. Hands were thoroughly washed before the inoculation in other to rid off any virus inoculating particles. The inoculated plants were rinsed with water two minutes after the inoculation. The inoculated and uninoculated (control) groups for each hybrid line and the local cultivar were allow to grow in the experimental site for symptom development according to koch's postulate and for data generation and collection for growth and reproductive performance [12].

2.1.7 Virus maintenance

The virus was maintained in infected *Telfaria occidentalis* hybrid lines and local cultivar, which developed symptoms after ten (10) days of inoculation. The inoculated plants were maintained and used in subsequent inoculations and was mechanically transferred periodically to young seedlings of *T. occidentalis* throughout the study.

2.1.8 Symptomatology

Evidence of infection and symptom/disease development was evident on the 7^{th} day following inoculation. This started with the mottling and cupping of young leaves. It was followed by vein clearing and mosaic patterns on the leaves and vines [13]. Prolonged infection brought about severe mosaic symptoms characterized by large yellow patches and dark green areas on the leaf lamina as well as reduction in the leaf size and general plant size [14]. The control plants which were not inoculated with the virus showed or developed no symptoms.

2.1.9 Data collection and analysis

Data for growth performance was taken at monthly interval for vine length, number of primary and secondary branches per plant, number of leaves per plant and leaf area of plant. Data for reproductive traits were taken at maturity for days to flower initiation, pod length, pod weight, hundred seed weight and number of seeds per pod. Generated data was subjected to statistical analysis adopting the analysis of variance (ANOVA) procedures in a complete randomized design in three replicates. Treatment means that were significant was separated using the Fisher's least significant difference test at 5 percent level of probability.

3. RESULTS AND DISCUSSION

The adaptability of hybrid *Telfaria occidentalis* lines in the rain forest ecology was measured in terms of growth and reproductive performance of the vegetable after inoculation with the viral pathogen. Significant ($p<0.05$) differences were observed among the different *Telfaria occidentalis* hybrid lines and local cultivar evaluated. Seven (7) hybrid lines and one (1) local cultivar of *T. occidentalis* used for the study were evaluated for adaptability using growth and reproductive performance while resistance to the TeMV was evaluated based on the hybrid and local cultivar growth and reproductive performance after mechanical inoculation of leaves and vines with the Telfaria mosaic virus.

The results as presented are means of two growing seasons in Table 1 showed that significant ($p<0.05$) differences were detected among hybrids lines and local cultivar for some growth parameters such as number of primary branches per plant, for which a total of 10 primary branches were counted from hybrid EN 2000-19 while the least number of 4.00 primary branches were recorded from the local cultivar. Secondary branches were 13.11 in number for EN 2000-6 higher than 4.31 secondary branches counted from the local cultivar. The number of leaves recorded per vine and per plant differed ($p<0.05$) significantly among all lines evaluated. The results showed that 10.07 and 58.39 leaves were recorded per vine and per plant from EN 2000-6 and EN 2000-19 hybrid lines while least number of leaves per vines and per plant of 3.31 and 13.72 were both counted for the local cultivar Edem Ubong. The results further showed that Biomass weight (kg), vine length (m) and leaf area (cm^2) were significantly ($p<0.05$) different among the hybrid lines. As presented in Table 1, the longest vine length of 7.04 m was measured from EN 2000-19 and the shortest vine length of 1.45 m recorded from Edem Ubong. A high biomass weight of 5.74 kg was recorded for EN 2000-19 while the least biomass weight of 1.22 kg was recorded for Edem ubong local cultvar. The results for leaf area for the hybrid lines and

local cultivar showed that the leaves of EN 2000-1 had average area of 102.41 cm 2 compared to the least area of 22.32cm2 obtained from Edem Ubong cultivar.

Results of yield traits performance of seven hybrid lines and one local cultivar presented are means of two seasons trials in Table 2 revealed that EN 2000-X took 63 days to initiate flowering while the local cultivar Edem Ubong required 92 days to initiate flowering under similar agronomic conditions. A larger pod weighing up to 29.4 kg was obtained from the hybrid EN 2000-19 and pod weight of 7.4 kg obtained from Edem Ubong. This local cultivar was highly susceptible to the virus. Some of the local cultivars died and did not reach pod and fruit bearing age. The number of pods produced per plant was significantly (P<0.05) different among the hybrid lines and local cultivar evaluated. En 2000-9 produced 11 pods per plant while the least pods of 2 was counted from Edem Ubong local cultivar. A longer pod measuring 71.43 cm was obtained from EN 2000-x and the shortest pod measuring 24.37 cm obtained from Edem Ubong cultivar. Number of seeds counted per pod also differed ($p<0.05$) significantly among the different lines and local cultivar. Results showed that an average of 72 seeds per pod were counted from EN 2000-19 hybrid and only average of 23 seeds per pod in Edem Ubong cultivar. The weight of 100 seeds measured from each hybrid line and local cultivar detected some significant ($p<0.05$) differences between the treatments means. Weight of 100 seeds of 7.40 kg was weighed from EN 2000-19 hybrid while weight of 100 seeds of 2.09 kg was obtained from Edem Ubong cultivar. It was observed that the bigger pods contain fewer seeds which were bigger and weightier than the smaller pods.

Results revealed that the lines EN 2000-19, EN 2000-17, EN 2000-6 and EN 2000-9 were more adaptable (high growth and yield traits performance compared to other lines under same field conditions) to the rainforest ecology

Table 1. Mean±SE, coefficient of variation and LSD for growth performance of *Telfaria occidentalis* in the rain forest ecology

Telfaria lines	No. of primary branches	No. of secondary branches	No. of leaves/ vine	No. of Leaves/plant	Biomass weight (kg)	Vine length (m)	Leaf area (cm²)
EN 2000 - X	7 .00±00b	10.88±0.65bc	7.11±0.19c	44.88±2.12c	4.43±0.69b	5.33±0.27ab	84.61±0.14b
EN 2000-19	10.00±00a	10.36±1.73bc	8.57±0.15b	58.39±3.67a	5.78±1.10a	7.04±0.21a	99.48±0.16a
EN 2000-9	6.00±00c	5.44±0.77e	4.00±0.43d	29.50±6.21 d	2.96±3.13d	3.00±0.31b	52.55±0.20d
EN 2000-1	9.00±00a	11.09±0.56b	7.81±0.38c	52.63±5.9 4b	4.39±1.10b	6.00±0.22a	102.41±0.11a
EN 2000-17	8.00±00b	9.50±0.19c	8.87±0.19b	43.06±1.36c	3.81±0.8 1c	5.81±0.18ab	72.81±0.09c
EN 2000-6	9.00±00a	13.11±0.17a	10.07±0.15a	43.00±2.38c	3.35±0.40c	6.88±0.13a	82.80±0.06b
EN 2000-24	6.00±00c	7.00±0.19d	4.87±0.22d	25.46±3.43d	2.88±0.2 9d	2.06±0.19bc	76.75±0.09bc
Edem Ubong	4.00±00d	4.31±0.14f	3.31±0.14e	13.12±2.26e	1.22±0.8 3e	1.75±0.16c	22.85±0.04e
CV(%)	0.035	31.10	17.62	36.21	43.15	10.13	13.48
LSD (0.05)	1.98**	1.18**	1.03**	5.87**	1.11**	2.09**	9.19**

Note; values with same letter in the same column indicate no statistical difference among hybrid lines.
*** indicates significant differences @ P < 0.05*

Table 2. Mean±SE, coefficient of variation and LSD for yield traits performance of *Telfaria occidentalis* in the rain forest ecology

T. oocidentalis lines	Days to flowering	Pod weigth (kg)	No. of pod per plant	Pod length (cm)	No. of seeds per pod	100 seeds weight (kg)
EN 2000 – X	63±0.34	28.2±0.19	6±0.36	71.43± 0.49	46 ±0.44	7.06±1.23
EN 2000-19	67±0.23	25.6±0.62	9±0.41	54.21±0.22	31±0 .34	6.77±0.86
EN 2000-9	66±0.14	29.4±0.50	11±0.21	66.64±0.27	23±0 .20	4.43±0.75
EN 2000-1	89±0.55	13.3±0.28	4±0.76	45.32±0.62	47±0. 35	3.54±0.87
EN 2000-17	77±0.11	15.1±0.11	3±0.64	39.29±0.32	58±0 .41	4.21±1.23
EN 2000-6	86±0.42	9.8±0.23	5±0.91	40.45±0.20	72±0.1 2	5.40±0.93
EN 2000-24	65±0.31	27.6±0.42	8±0.30	38.92±0.51	59±0 .41	5.55±0.60
Edem Ubong	92±0.20	7.4±0.13	2±0-25	24.37±0.43	27±1. 74	2.09±0.93
CV(%)	24.67	14.81	28.09	21.75	23.66	9.23
LSD(0.05)	3.21**	4.97**	2.88**	7.62**	5.11**	NS

***Significant at 5 percent level of probability. NS = Not significant at 0.05%*

with significant ($p<0.05$) differences in average length of vines, leaf area, number of primary and secondary branches and number of leaves per plant respectively. The hybrid lines EN 200-24, EN 2000-X, Edem Ubong, EN 2000-1 showed less adaptability to this ecology with significant ($p<0.05$) differences with low results in vine lengths, number of primary and secondary branches per plant, number of leaves per plant and leaf area (Table 1). Results of hybrid lines and local cultivars resistance to TeMV showed that the cultivars EN 2000-x, Edem Ubong, EN 2000-24 and EN 2000-1 were highly susceptible (poor growth and yield traits performance after inoculation with virus compared to the control) to the virus with resultant low growth and yield performance while the hybrid lines EN 2000-19, EN 2000-6, EN 2000-17 and EN 2000-9 were highly resistance (high growth and yield performance of inoculated lines) to the TeMV showing high growth and yield performance (Table 2).

The research study has been able to show and identify some hybrid lines of *T. occidentalis* that can adapt and show some resistance to the Telfaria mosaic virus in this agro-ecology. Farmers in this area are encouraged and advised to cultivate adaptable and highly resistance hybrid lines for increased growth, yield and income. Adequate breeding methods such as marker assisted breeding, hybridization and cross breeding of resistance hybrid lines with the local variety to improve the genetic resistance of the local cultivar(s) should be carried out by relevant mandate institutes to check the further spread of the Telfaria mosaic virus and secure the sustainable production and availability of this important vegetable for the teeming population of this agro-ecology.

4. CONCLUSION

The current study seeks to address the gap by advocating for the discontinuous use of local cultivars of *Telfaria occidentalis* which was found to be very susceptible to the devastating effect of Telfaria mosaic virus (TeMV) by local farmers in this agro-ecology and to advocate for the use of improved hybrid lines of the vegetable with a view to improving yield, increase farmers income and ensure food security and sustainability for the region.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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