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Effect of landraces on *Musa* micropropagation

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Abstract

This work aimed at assessing the performance of two landraces of *Musa* spp in micropropagation. Tissues from Owom and Efol were sterilized by soaking the explants in 70 % ethanol for 30 sec, 8 % NaOCl for 5 min, 5 g/L benlate for 5 min, and in 1.2 g/L $HgCl_2$ for 10 min before being thoroughly washed with distilled water. Tissue exposure to U. V. light for 5 min was done before they were cultured in a shooting MS-based culture medium supplemented with 5.00 mg/L concentration of 6-Benzylaminopurine, and later to a rooting MS-based culture medium supplemented with indoleacetic acid (IAA) of 2.00 mg/l concentration. Data were collected on number of shoots, shoot height, shoot's health, days to rooting, root number, and root length. The result from shooting medium showed that the plants' heights were highly significantly different at $P \leq 0.01$, with Owom producing marginal mean heights of 3.59 cm and 5.05 cm at 3 and 5 weeks respectively, after initiation. A very high significant difference was observed with plant health at $P \leq 0.001$ whereby Owom landrace reduced in health status as the week increased, unlike Efol which increased in health status across the weeks. For rooting medium, significant differences at $P \leq 0.05$ for days to rooting, root length, health performance, and plant height were observed. While high significant difference was observed for the number of roots at $P \leq 0.01$. Owom performed significantly better than Efol and has shown value for commercial production and breeding programs.

Keywords: Micropropagation; Landrace; *Musa*; Tissue**.**

1. Introduction

Micropropagation has been used for the fast production of plantlets and the advancement of progress made in research. Some factors determine the progress in micropropagation. The type of hormone and concentration has been one of the determining factors of attaining progress in micropropagation. An example is seen in the report of [1] which stated that BAP proved effective when used alone in shoot proliferation of cassava. Other observations showed that growth regulators work together to produce the desired effect. According to the report of [2], combination of BAP and Kinetin at 0.75 mg/L produced an average of 7.30 micro shoots per explant.

The genotypic difference is another factor to determine in micropropagation of plants. Plants of different genetic makeup may react differently to the same environment. Increased genetic dissimilarity among plants increases the probability of showing varied responses by those plants to the same medium. Researchers have reported different protocols for different plant species. While most works reported the use of semi-solid medium for micropropagation, [3] reported success in micropropagation of *Musa* acuminata with the use of soil as substrate. However, most reports on *Musa* spp used MS medium [4; 5]. In a study on *Bacopa monnieri* (L.) Pennell plant, a standardized micropropagation method was established using MS medium supplemented with 0.5 mg/l BAP [6].

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Majority of micropropagation research on *Musa* spp is carried out for already established spp that are known with some peculiarities and are fairly clean. Applying such protocols to landraces may not give related results. Landraces have their peculiarities and may behave significantly different to a standardized micropropagation protocol. Therefore, this work aimed at determining the shooting capacity of genetically different landraces in *Musa* micropropagation.

2. Material and methods

2.1. Micropropagation of Identified *Musa* landraces

Musa suckers of field-grown *Musa* landraces were used to generate explants for tissue culture work at the tissue culture laboratory which is located at National Root Crops Research Institute, Umudike.

2.2. Initiation of shoot cultures

Shoot tips of Owom and Efol (40 – 100 cm height) which grew in the *Musa* field germplasm of the Faculty of Agriculture & Natural Resources Management of Ebonyi State University were used as explants. The collected suckers were washed and trimmed to a size of 1.0 – 1.5 cm. The trimmed tissue was surface sterilized by soaking the explants in 70 percent ethanol for 30 sec, followed by soaking of explants in 8 percent NaOCl for 5 minutes. The explants were also soaked in 5 g/L benlate for 5 minutes and in 1.2 g/L $HgCl_2$ for 10 minutes before they were thoroughly washed with distilled water. The rinsed explants were then exposed to U. V. for 5 minutes before final trimming was done to reduce the shoot tips to a size of 3 × 5 mm, which composes the apical dome, few leaves primordia, and a thin layer of corm tissue [7]. The explants were initiated directly on MS-based [8] culture medium contained in reusable Magenta boxes (or 125 ml flask) with each containing 50 ml of culture medium. Commercial grade sugar was used as source of energy, [9] at a concentration of 30 g/l. Also, BAP was applied at a concentration of 0.004 g/L as a shooting regulator and Cefotaxime was added at 500 mg/L to generate clean cultures.

2.3. Multiplication of axillary shoot-tip

Shoot multiplication was done by supplementing the medium with a 5.00 mg/L concentration of 6-Benzylaminopurine. The cultures were kept in growth chamber which had temperature regulation of 28 °C. The research design was a factorial experiment in a completely randomized design (CRD). Explants were observed for many dependent variables which include number of shoots, shoot height, shoot health and number of roots for three and five weeks after initiation. The number of shoots and roots was counted per culture while shoot height was determined using a centimetre calibrated meter rule. Plant health was determined using a 5-point scale following the principle of [10], with modification (Table 1).

Table 1 Cultured Plant Health Rating Scale

Scale	State	Features
1	Not healthy	Dead tissue or dying tissue
2	Fairly healthy	Green-coloured shoots with emerging leaves or yellow-coloured leaves.
3	Intermediately healthy	Green-coloured average heightened shoot, few fully emerged narrow leaves with light green colouration.
4	Healthy	Green-coloured heightened shoot with many fully expanded green leaves
5	Very healthy	The green-coloured shoot which grew to the top of the culture glass, very many broad green leaves that spread and touched the surfaces of the culture glass

Source: Modified from [10]

2.4. Regeneration of plant's root

Regenerated shoots were taken to a nutrient medium which induces root formation. The medium was supplemented with indoleacetic acid (IAA) of 2.00 mg/l concentration. Data were taken on days to rooting, root number, root length, plant height, plant health, and shoot number, in four weeks after culturing. Lengths were measured using a centimetre calibrated meter rule.

2.5. Acclimatization

Plants were acclimatized in a hardening chamber for four weeks on a rooting substrate comprised of river sand (sandy soil) and sawdust on a ratio of 1:1. The plants were gently irrigated with spraying cans possessing small nozzles, as the plantlets were carefully exposed to the environment. Data were collected on plant height, leaf number, leaf length, leaf width, and plant health. For every plant, the leaf with the highest length was measured for its length and width using a centimetre calibrated metre rule. Whereas leaf number was gotten by counting the number of leaves each plant possesses.

3. Result

3.1. Landraces effect at 3 and 5 weeks after initiation.

The effect of varied landraces across different weeks' intervals for shoot height, plant height, plant health, and root number were determined (Figure 1 - 4). At 3 weeks after initiation, the Owom genotype produced a marginal mean of 2.11 shoots, while 3.07 shoots were produced at 5 weeks after initiation (Figure 1). For Efol landrace, marginal means of 1.46 and 2.35 shoots were produced, respectively, for 3 and 5 weeks after initiation.

The variation in plant heights across the weeks for the landraces was highly significantly different at $P \leq 0.01$ (Figure 2). At 3 and 5 weeks after initiation, Owom produced marginal mean plantlet heights of 3.59 cm and 5.05 cm, respectively. While Efol produced plantlets of 2.50 cm and 3.56 cm, respectively, at 3 and 5 weeks after initiation. For plant health (Figure 3), a very high significant difference was observed at $P \leq 0.001$. Plantlets from Owom landrace reduced in health as the number of weeks increased, with a mean of 2.58 and 2.42 at 3 and 5 weeks after initiation. An increase in health status across the weeks was observed for Efol, with values of 1.76 and 1.89 respectively, at 3 and 5 weeks after initiation.

For root numbers (Figure 4), no significant difference was observed at $P \leq 0.05$. However, Owom produced marginal mean of 2.05 roots at 3 weeks after initiation and 2.31 roots at 5 weeks after initiation. For Efol, marginal mean of 1.89 and 2.53 roots were produced respectively, at 3 and 5 weeks after initiation.

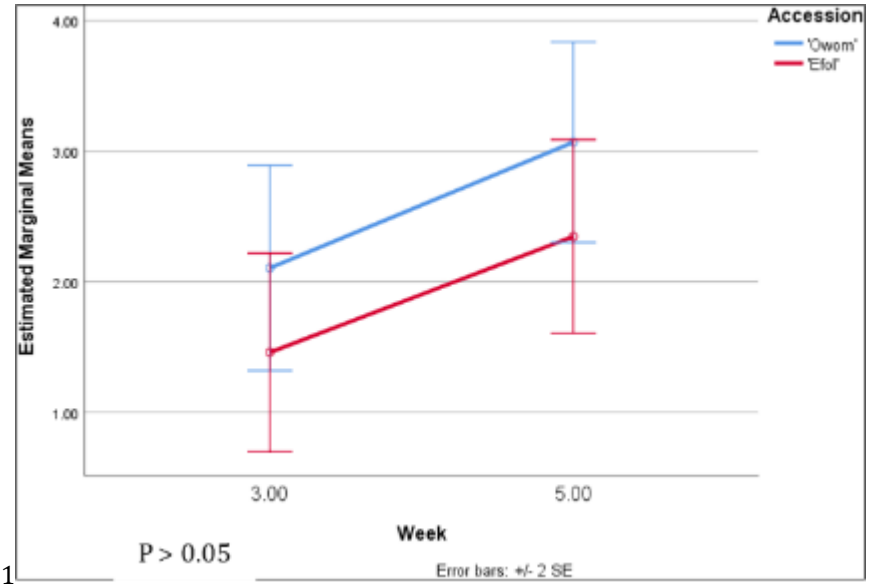
3.2. Effect of landraces on rooting media

At four weeks after culturing plantlets in rooting media, the statistical results on data taken for days to rooting, root length, health performance, and plant height indicated that the *Musa* landraces showed significant differences at $P \leq 0.05$ (Table 2). A significant difference was observed for the number of roots at $P \leq 0.01$. Across the parameters measured, Owom performed significantly better than Efol.

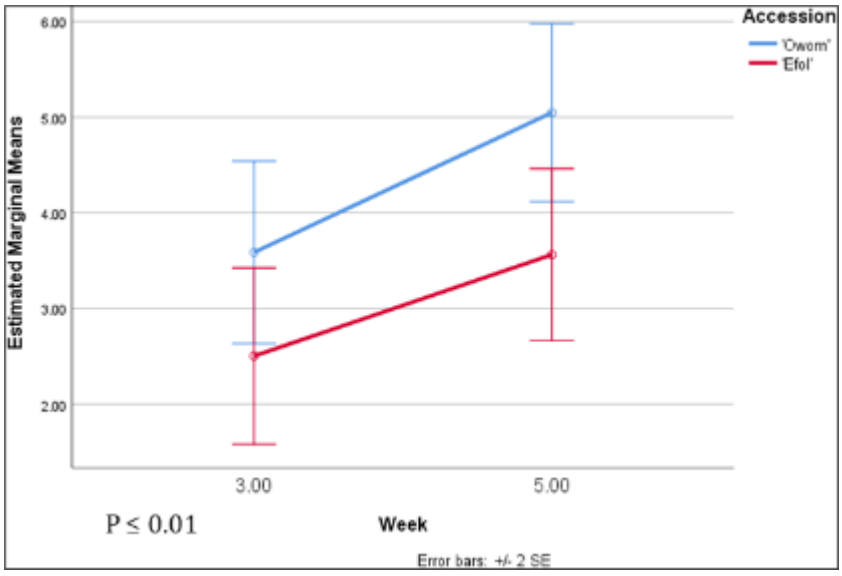
4. Discussion

The study evaluates the effects of varied landraces over different weekly intervals on shoot height, plant height, plant health, and root number in *Musa* landraces, specifically comparing Owom and Efol genotypes. The findings reveal distinct growth patterns and health indicators over 5 weeks, highlighting the differential performance of these two landraces. Three weeks after initiation, the Owom genotype produced an average of 2.11 shoots, which increased to 3.07 shoots by the fifth week. In comparison, the Efol landrace generated fewer shoots, with a marginal mean of 1.46 at three weeks and 2.35 at five weeks (Figure 1). This suggests that Owom has a more robust shoot proliferation capacity over time than Efol [11].

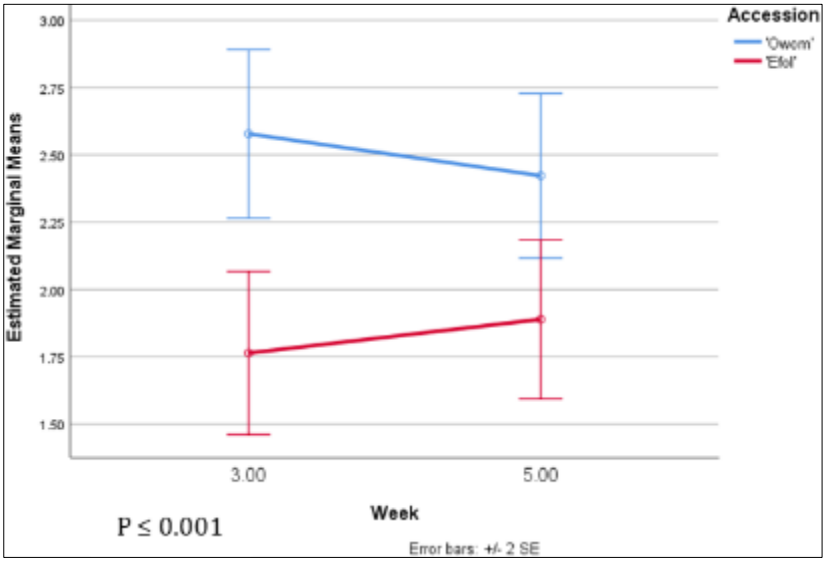
Significant differences in plant heights were observed between the landraces across the weeks ($P \leq 0.01$). At three weeks, Owom plantlets had an average height of 3.59 cm, increasing to 5.05 cm at five weeks. Efol plantlets, on the other hand, were shorter, with heights of 2.50 cm at three weeks and 3.56 cm at five weeks (Figure 2). This indicates a faster growth rate in Owom compared to Efol, which could be crucial for breeding programs aiming at faster vegetative growth [12].


Accession
'Owom'
'Efol'
Estimated Marginal Means
4.00
3.00
2.00
1.00
3.00
5.00
Week
P > 0.05
Error bars: +/- 2 SE

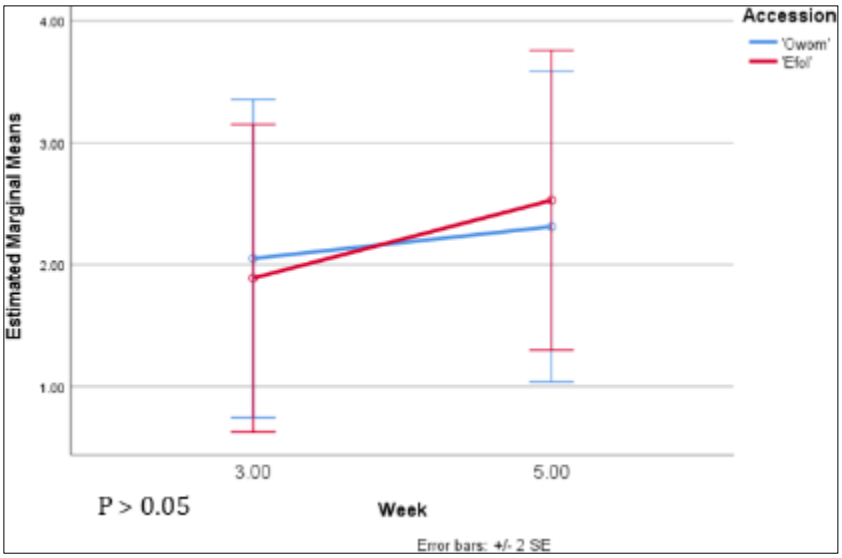
1


Accession
'Owom'
'Efol'
Estimated Marginal Means
6.00
5.00
4.00
3.00
2.00
3.00
5.00
Week
P ≤ 0.01
Error bars: +/- 2 SE

2


Accession
'Owom'
'Efol'
Estimated Marginal Means
3.00
2.75
2.50
2.25
2.00
1.75
1.50
3.00
5.00
Week
P ≤ 0.001
Error bars: +/- 2 SE

3



4

Figure 1-4 Performance of *Musa* Landraces on Micropropagation at 3 and 5 Weeks after Initiation.

Dependent factors: 1 = shoot number; 2 = plant height; 3 = plant health; 4 = root number. Values were significant at P ≤ 0.05.

Table 2 Effect of Different Genetic Landraces on Rooting Performance in *Musa* Micropropagation.

Landrace	Days to Rooting*	Number of Root**	Root Length*	Health Performance*	Plant Height*
Owom	5.60 ± 1.14	6.80 ± 4.97	9.64 ± 3.81	3.60 ± 1.52	5.98 ± 2.66
Efol	7.33 ± 2.31	4.33 ± 3.79	5.25 ± 3.88	2.67 ± 1.53	4.80 ± 2.01
Total	14.93 ± 3.45	11.13 ± 8.76	14.89 ± 7.69	3.27 ± 3.05	10.78 ± 4.67
Mean	7.47 ± 1.73	5.57 ± 4.38	7.45 ± 3.85	1.64 ± 1.53	5.39 ± 2.34

Value represents Mean ± Standard deviation; Significance determined at P ≤ 0.05; * = P ≤ 0.05; ** = P ≤ 0.01.

Plant health varied significantly between the landraces (P ≤ 0.001). For Owom, a decline in health was noted over the weeks, with health scores dropping from 2.58 at three weeks to 2.42 at five weeks. Conversely, Efol showed an improvement in health status, increasing from 1.76 at three weeks to 1.89 at five weeks (Figure 3). This divergence in health trends suggests that Efol may have a better adaptation or resistance to the conditions provided over time [13]. Root numbers did not show significant differences at P ≤ 0.05, although Owom produced a marginal mean of 2.05 roots at three weeks and 2.31 roots at five weeks. Efol produced slightly fewer roots initially, with 1.89 roots at three weeks, increasing to 2.53 roots at five weeks (Figure 4). Despite the lack of statistical significance, the increase in root number for both landraces over time indicates steady root development, which is vital for overall plant stability and nutrient uptake [14]. Across all measured parameters, Owom consistently outperformed Efol, indicating its superior overall growth and development performance under the given conditions

The research findings after four weeks of culturing plantlets in rooting media revealed significant differences among *Musa* landraces across various parameters such as days to rooting, root length, health performance, plant height, and number of roots. According to the statistical analysis presented in Table 2, these differences were found to be statistically significant at the P ≤ 0.05 level for days to rooting, root length, health performance, and plant height. Moreover, the number of roots showed an even higher level of significance, with differences observed at P ≤ 0.01.

Specifically, the *Musa* landrace known as Owom exhibited superior performance compared to Efol across all measured parameters. This outcome suggests that Owom is more efficient in terms of rooting speed, root length development, overall health performance of the plantlets, plant height growth, and root formation compared to Efol under the conditions tested. With the stated result on root development, Owom will have an advantage over Efol by establishing faster and healthier in the field than Efol [15]. Furthermore, the study by [16] has emphasized the importance of genetic selection and breeding programs to enhance desirable traits such as rooting ability and growth vigor in *Musa* species.

5. Conclusion

The study underscores the superior performance of the Owom genotype in terms of shoot production, plant height, and overall robustness, despite a decline in health over time. Efol showed less impressive growth metrics but improved health status over time, suggesting different adaptive strategies between the two genotypes. These findings are crucial for selecting and breeding *Musa* landraces for optimized growth and health in micropropagation. Also, Owom has shown value for commercial production and breeding programs aimed at improving root development and overall growth efficiency in *Musa* species.

Compliance with ethical standards

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Disclosure of conflict of interest

The author does not have any relevant financial or nonfinancial interest to express.

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