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Genetic Diversity Among Strains of *Pleurotus* species (oyster mushroom) Using Morphometric Traits Under Varied Temperature and pH

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Abstract

Genetic diversity in nineteen strains of *Pleurotus* was studied using morphometric traits and growth factors. Ability of the isolates of these strains to tolerate different ranges of temperature and pH were evaluated. Highest mycelial growth rates were obtained at 25 °C (mutants and hybrids) and 30 °C (wild type), while LAU 90 (mutant) performed satisfactorily at all evaluated temperature ranges (15-35 °C). Highest mycelial yields (dry weight) were produced by LAU 90 at different pH regimes (4.0 - 9.0), while hybrids LN 97 and LN 98 maximally produced mycelial yield at pH 5.0 and 7.0, respectively. Analysis od Principal component (PC) revealed that components of these strains accounted for 86.1% of total variations among the strains with first PC recording 44.6%. The dendrogram discriminated nineteen *Pleurotus* genotypes into two major genetic groups with mutants and hybrid strains in Cluster A, separated distinctly from wild parents in Cluster B, indicating genetic diversity. The expression of heterosis can be maximized by information obtained among the hybrid strains and mutant (LAU90) strain. The hybrid (LN98) strain with superior performance may be selected for adoption in commercial mushroom production.

Keywords: Genetic diversity; Morphometric traits; Oyster mushroom; Growth factors; Mycelial growth.

1.0 Introduction

Oyster mushrooms are most commercially cultivated edible mushrooms in the world (Kibar and Perksen, 2008). Generally, mushrooms have been valued as nutritional and medicinal resources for mankind. Mushroom production has translated into multi-billion dollar industry as its consumption continues to increase greatly due to demand by modern consumers seeking health-added benefits to their food. It was reported in year 2005 that mushroom farming, production and utilization increased worldwide, with 10 million metric tonnes of edible and medicinal mushroom produced in various countries (Royse, 2005).

Edible mushrooms are well known and used in dietetics for prevention of atherosclerosis due to high content of fibre, protein, microelements and very low fat. Fresh mushroom contains 3-35% fibre, 3-21% carbohydrate with low calorific value and are excellent sources of minerals and vitamins (Nakalembe *et al.,* 2015). The lipid compounds in mushrooms are generally unsaturated (Ng *et al.,* 1999) with free fatty acids that constitute about 2-8% of dry weight (Breece, 1990). Mineral nutrients in mushrooms include phosphorus, calcium and iron (Adebayo *et al.,* 2014), while the vitamins are thiamine, riboflavin, ascorbic acid, ergosterine and niacin.

In recent years, the trend of research has been directed toward discovering ways of treating and manipulating mushrooms with the possibility of imparting added values. Owing to its various nutritional contents and health benefits, the development of improved and high yielding varieties of mushrooms, especially in terms of biomass yield, fruit body and biochemical substances (Adebayo *et al.,* 2013) is being continuously researched into, with reference to *Pleurotus* species cultivation.

Growth factors, morphological and agronomic traits can be adopted in genetic diversity studied of various plants species (Solak and Gupta, 2001). Genetic diversity of *Pleurotus* species in terms of morphometric traits under different environmental conditions will unravel the potential of the species and enhance selection of genotypes or strains with vigour for yield improvement. Divergent species may have good breeding values, and strains from the same parent with diverse potential may enhance selection of genotypes for better performance in yield (Adebayo *et al.,* 2014).

Utilization of genotypes from different clusters as parents of crosses can be used for maximum variability for selection in the segregating population of plants (Genet *et al.,* 2005). This same principle may not be different for mushrooms being a lower plant. Yu *et al.* (2013) previously reported the genetic diversity of *P. ostreatus* cultivars. Genetic variability and systematics of isoenzymes in *Pleurotus* species was also reported by Georgios *et al.* (2009). However, there is little or no available report on genetic diversity of *Pleurotus* species based on growth responses to various conditions of culture media.

Therefore, the present study was designed to; (1) generate genetic variability through development of mutants and hybrids from wild collections of *Pleurotus* species, and study their growth response under various temperature and pH regimes; (2) assess genetic diversity among the generated mutants and hybrids relative to wild parents with a view to select improved isolates for further development.

2.0 Materials and methods

2.1 Collection of strains

Dikaryotic mycelium of *P. pulmonarius* (LAU 09) and *P. ostreatus* (LAU 10) were isolated and characterized at Pure and Applied Biology Department, Ladoke Akintola University of Technology, Ogbomoso, Nigeria (Adebayo *et al.,* 2016), while *P. citrinopileatus* (NE 01), *P. cornucopiae* (NE 02), *P. djamor* (NE 03), *P. sajor-cajor* (NE 05), *P. sapidus* (NE 07) and *P. ostreatus* (NE 08) were collected from Plant Science Department, NEIST, Jorhat, India. The strains were maintained on potato dextrose agar (PDA) slant at 4°C.

2.2 Development of mutant isolates of *P. pulmonarius*

Actively growing cultures (7 d old) of *P. pulmonarius* (LAU 09) on PDA plates (90 mm) were exposed to UV light (210 nm, Millipore xx63 70000) for 3 h at 30 min intervals (30 min, 60 min, 90 min, 120 min, 150 min and 180 min) (Adebayo *et al.,* 2012). The mutants were selected using morphological (growth and appearance of mycelium and fruit body) and physiological (responses to temperature and pH) features. Plug (6 mm) from peripheral of Petri dish of mutant strains was subcultured on the PDA with 5% yeast extract agar (YEA), incubated at 25°C for 7 days with wild type as control. All cultures were in triplicates and the diameters of mycelial growth were measured.

2.3 Hybridization of *P. pulmonarius* with other species of *Pleurotus*

Hybridization was carried out between two different dikaryon strains of *Pleurotus* species. Mating compatibilities of *P. pulmonarius* (LAU 09) with seven other species of *Pleurotus ostreatus* (LAU 10), *P. citrinopileatus* (NE 01), *P. cornucopiae* (NE 02), *P. djamor* (NE 03), *P. sajor-caju* (NE 05), *P. sapidus* (NE 07), *P. ostreatus* (NE 08) were determined through interstrain pairing among dikaryon isolates. Cross compatibility of strains was determined by scoring for the presence of clamp connexions and pairing was performed as reported by Adebayo *et al.* (2013). In each plate, the agar plugs of two monocultures to be crossed were placed 20 mm apart and incubated at 25°C until a well-developed contact zone was established 12 days after incubation.

2.4 Mycelium growth rates at different temperature regimes

Optimum temperatures for mycelium linear growth for wild, mutant and hybrid strains over a range of 15–35 °C at 5 °C increase were assessed on PDA with 5% of YEA. Experiments were conducted in Petri dishes in replicates (Zervakis *et al.,* 2001), incubated for 7 d, and linear growth rates were determined using the equation $Y = K_rX + C$ (where K_r is mycelium linear growth, Y is the distance covered by mycelium growth, X is the time taken for mycelium growth and C is constant).

2.5 Effect of pH on mycelium growth

Determination of optimum pH for mycelia growth was carried out in potato dextrose broth (PDB) with 5% of yeast extract powder (YE) over a

pH range of 4.0 to 9.0, adjusted with (0.1 M) HCl and NaOH. Experiments were carried out in 250 ml flasks containing 100 ml substrate. The flasks were inoculated with a plug (6 mm) in replicates and incubated at 25 ℃ for 7 days at 150 rotations. Mycelial mats were harvested and quantified using modified method of Oloke *et al.* (2009).

2.5 Spawn production

Rice grains (100 g/bottle) were washed four times, boiled for 45 min and dried. The dried grains were mixed with 1% w/w of calcium carbonate ($CaCO_3$), dispensed in bottles and sterilized at 121°C for 30 min. The sterile grains were inoculated with 6 plugs (6 mm) of actively growing culture in replicates, incubated at 23 ± 2 ℃ and mycelium running was recorded at three days interval (Adebayo *et al.*, 2012). Ramification rate, ramification days, weight of ramified mycelia and spawn productivity were determined using equation (1);

Weight of ramified Mycelia = Final weight of spawn + substrate – Initial weight of spawn + substrate **(1)**

$$\text{Ramification rate} = \frac{average\ length\ of\ mycelial\ ramification}{avearge\ number\ of\ days} \quad \textbf{(2)}$$

$$Spawn\ Productivity = \frac{weight\ of mycelial\ ramification}{ramified\ days} \times 100 \quad \textbf{(3)}$$

2.6 Statistical analysis for mycelia yield production

Data obtained for linear growth rate and mycelium yield in triplicates were subjected to statistical analyses using SPSS (Version 16). The means of attributes evaluated under different conditions of growth were separated using Duncan's Multiple Range Test (DMRT). A cluster analysis was performed using Ward Clustering Method (Azeez and Morakinyo, 2014) based on average values of morphometric traits and growth factors evaluated.

3.0 Results

3.1 Nature and identity of the *Pleurotus* genotypes

Part of the data reported in Table 1 has been published by Adebayo *et al.* (2016), especially the wild types and hybrid strains. The isolates consisted of eight wild types (LAU 09, LAU 10, NE 01, NE 02, NE 03, NE 05, NE 07 and NE 08), four mutant strains (LAU 30, LAU 60, LAU 90 and LAU 120) and seven hybrid strains (LAU09 x LAU10 (LL 910), LAU09 x NE01 (LN 91), LAU09 x NE02 (LN 92), LAU09 x NE03 (LN 93), LAU09 x NE05 (LN 95), LAU09 x NE07 (LN 97) and LAU09 x NE08 (LN98). Twelve out of these strains have been characterized to the specie level, with the accession numbers registered at the National Centre for Biotechnology information (NCBI) database. Five uncharacterized strains without accession number were represented by *NIL*. One uncultured strain was characterized, but the nucleotide sequence has no similarity with any strain in National Centre for Biotechnology information after blasting (Table 1).

3.2 Linear growth rates of wild, mutant and hybrid strains of *Pleurotus* species studied

Linear growth rates of wild, mutant and hybrid strains of *Pleurotus* species at different temperatures are shown in Table 2. Appreciable growth was obtained at 20 ℃ with optimum growth temperature of 25 ℃ for all the strains, except NE 02. LAU 90 (mutant) had highest growth rates at all evaluated temperature regimes. Linear growth rates of LAU 90 (mutant) was significantly different ($p<0.05$) from all other strains at evaluated temperatures except at 30℃, while that of LN 92 recorded values that were significantly different from other strains at 15 ℃ and 20 ℃. Highest value of linear growth rates of 2.75 mm/d was obtained in LAU 90 at 25 ℃, followed by 2.74 mm/d obtained in LN 92 at 20℃, while the lowest value of linear growth rates of 0.213 mm/d was obtained in NE 07 at 15 ℃. Highest dry mycelia weight of 0.62 g was obtained in LN 91 (hybrid) at pH 6.0, followed by 0. 59 g, 0.56 g and 0.52 g obtained in LN 92, NE 08 and LN 95 with their respective pH of 6, 4 and 5,while the lowest dry mycelia yield of 0.14 g was produced at pH 9 in NE 02 (wild), LAU 120 (mutant) and LL 910 (hybrid) (Table 3). The mycelia yield were significantly different at pH 4.0, 7.0 and 9.0 in LAU 90 compared to other strains (P<0.05), while LN 91, LN 92 and LN 95 mycelia yield showed significant differences from other strains at pH 5.0. Furthermore, LN 91 produced mycelia yield that was significantly different (P<0.05) at pH 8.0.

Spawn ramification rates of 0. 89 cm/d was recorded in LAU90, followed by 0.587 cm/d in LN97, 0.583 cm/d in LN92 and 0.578 cm/d in LN98. Weight of mycelia ramification was highest in LAU 90 (6.67 g), followed by LN98 (6.4 g) and LN97 (6.10 g). The highest percentage spawn productivity was in LN98 (53.3%), followed by LN97 (50.8%) with the shortest days of ramification (12 days) each. The lowest spawn ramification rates of 0.359 cm/d was obtained in NE 02 while lowest percentage spawn productivity (23.1%), weight of mycelia ramification (3.70 g) and longest days of ramification (16 days) were obtained in LAU 10 (wild) (Table 5). The spawn ramification rates, weight of mycelia ramification and percentages spawn productivity were found to be significantly different (P<0.05) among the strains studied.

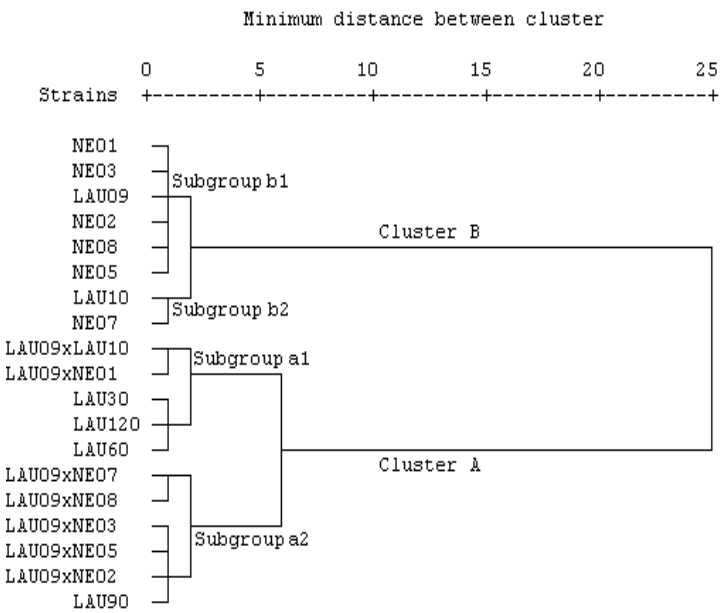

Figure 1: *Dendrogram of relationship among nineteen strains of Pleurotus species studied based on morphometric traits exhibited under different temperature and pH regimes.*

3.3 Multivariate analysis of morphometric traits and growth factors

The first five principal component (PC) axes with their Eigen values are as shown in Table 4. The first three principal components accounted for 71.9% of the total variation among the strains in response to various temperature and pH regimes. Most of the variation was explained by the first principal component (44.6%), followed by the second (14.6%) and the third (12.8%). The first component had high positive loadings from ramification rate (RT), seven days of growth (7-G), weight of dry mycelia yield at pH 9.0 of growth (WDMY at pH 9.0), Sixteen, fourteen and five days of growth (16-G, 14-G, 5-G), and spawn productivity (PDT) while only negative loading was recorded from ramification days (RD). Second component had high positive loadings from ramification day (RD) and ten days of growth (10-G) while negative loadings were recorded from Linear growth rate (LGR) at temperature of 20(T-20°C) and 30 (T-30°C). Third component had high positive loadings from weight of dry mycelia yield at pH 9 and 5 of growth (WDMY at pH 9.0 and pH 5.0) only. However, none of the variables were redundant.

Table 1: Strains Identification with Genbank Accession Numbers for Isolates of *Pleurotus* species

Strains	Name	Nature	Genbank accession
LAU 09	*P. pulmonarius*	Wild isolate	JF736658
LAU 10	*P. ostreatus* (Nigeria)	Wild isolate	JF736659
NE 01	*P. citrinopileatus*	Wild isolate	JF736661
NE 02	*P. cornucopiae*	Wild isolate	JF736662
NE 03	*P. djmaor*	Wild isolate	NIL
NE 05	*P. sajor-caju*	Wild isolate	JF736663
NE 07	*P. sapidus*	Wild isolate	JF736664
NE 08	*P. ostreatus* (India)	Wild isolate	NIL
LAU 30	*P. pulmonarius*	Mutant isolate	NIL
LAU 60	*P. pulmonarius*	Mutant isolate	JF736660
LAU 90	*P. pulmonarius*	Mutant isolate	Unculture
LAU 120	*P. pulmonarius*	Mutant isolate	NIL
LAU09 x LAU10 (LL910)	*Pleurotus spp.*	Hybrid isolate	JF680988
LAU09 x NE01 (LN91)	*Pleurotus spp.*	Hybrid isolate	JF680989
LAU09 x NE02 (LN92)	*Pleurotus spp.*	Hybrid isolate	JF680990
LAU09 x NE03 (LN93)	*Pleurotus spp*	Hybrid isolate	NIL
LAU09 x NE05 (LN95)	*Pleurotus spp.*	Hybrid isolate	JF680991
LAU09 x NE07 (LN97)	*Pleurotus spp.*	Hybrid isolate	JF680992
LAU09 x NE08 (LN98)	*Pleurotus spp.*	Hybrid isolate	NIL

NIL = Not determined
Unculture = the nucleotides sequences have no similarity with any strain in Genbank database after blasting

Ward clustering method was used to generate dendrogram in Figure 1, which was able to discriminate among the nineteen strains, dividing them into two main genetic group (Cluster A and B). Cluster A was subdivided into subgroups '1' and '2', with subgroup '1' containing five strains (LL 910, LN 91, LAU 30, LAU 120 and LAU 60) that are made up of two strains of hybrid and three strains of mutant. Subgroup '2' contained six strains of which five are hybrids and one is a mutant (LN 97, LN 98, LN 93, LN 95, LN 92 and LAU 90). Cluster B was subdivided into subgroup

'1' and '2', with subgroup '1' containing six strains, which are all wild type (NE 01, NE 03, LAU 09, NE 02, NE 08 and NE 05).

All strains in this subgroup except LAU 09 were collected from North East of India. Subgroup '2' contained two species, which are also wild types (LAU 10 and NE 07). Considering the morphometric traits and growth factors, the dendrogram at minimum distances of 5 grouped the strains into three distinct groups such as I (NE 01, NE 03, LAU 09, NE 02, NE 08, NE 05, LAU 10 and NE 07), II (LL 910, LN 91, LAU 30, LAU 120 and LAU 60) and III (LN 97, LN 98, LN 93, LN 95, LN 92 and LAU 90).

4.0 Discussion

Information about the *Pleurotus* species (mutant and hybrid) strains reported in this study was registered in (NCBI) GenBank database (https://www.ncbi.nlm.nih.gov/nuccore/accessionnumber). Nature and identity of the strains used in this study, especially the hybrids have been reported by Adebayo *et al.* (2016), while mutants have never been reported before in the literature. The present investigation has clearly shown that strains of *Pleurotus* employed in this study elicited varietal differences under various growth temperature and pH regimes. LAU 90 (a mutant strain) with unmatched nucleotide sequences from GenBank database exhibited exceptional performance. This is an indication that mutation event has led to alteration of genetic arrangement resulting in generation of new strain (Sandhya *et al.*, 2006).

Satisfactory linear growth rates were obtained at 20 °C and optimum growth temperature at 25 °C for all strains. Higher growth rates by LAU 90 (mutant) at temperatures 15 and 35 °C compared to wild strains suggests better temperature tolerance potential of mutant over its wild type LAU 09. Highest linear growth rate (2.74 mm/d) was obtained in LN 92 (hybrid) at 20 °C, compared to its corresponding wild types LAU 09 and NE 02 (1.461 mm/d and 2.11 mm/d respectively). Generally, the optimum growth rates for all strains were observed at 25 °C, with the least growth rates at 15 °C. In previous studies, the optimum temperature for mycelia growth rate for *Pleurotus* species was established at 25 to 30 °C, with no growth observed at 35 °C (Zervakis *et al.*, 2001; Kibar and Perksen, 2008). However, in the present study, considerable higher growth rates were recorded at 35 °C by the mutant strains, a proof that the mutant strains have inherent ability to tolerate high temperature range.

The mutant strains evaluated produced mycelia mats from pH 4 to 9 and satisfactorily from pH 4.0 to 7.0 with highest yield recorded by LAU 90 (mutant). This probably depicted the potential of mutant strains to adapt well to wide pH regimes in comparison to its wild type (LAU 09). The optimum pH varied between 5.0 and 7.0 for both wilds and hybrids, with exception of NE 08 (wild), which produced its highest yield at pH 4.0,

while LN 91, LN 92 and LN97 (hybrids) produced satisfactorily at pH 8 and pH 9. The broad ranges of pH 3.5 to6.0 were previously reported as optima pH for fungal growth and products yield (Restaino *et al.*, 1983; Membre *et al.*, 1999) contrary to the present. Ability of some hybrid strains (LAU09xNE01 'LN91', LAU09xNE02 'LN92' and LAU09xNE07 'LN97') to satisfactorily produce mycelia mat at pH greater than 6.0 is an indication of strains improvement over their wild types. Highest WMR (6.40 g), shorter RD (12 days) with highest PDT (53.3%) were obtained in hybrid (LN 98), followed by mutant strains

Table 2: Linear Growth Rates (LGR in mm/day) of Wild, Mutant and Hybrid Strains of *Pleurotus* Species at Different Temperature Ranges

	Temperature				
Strains	15°C	20°C	25°C	30°C	35°C
LAU 09	0.455±0.05^{fgh}	1.461±0.11^{e}	2.500±0.31^{abc}	1.892±0.48^{abcdef}	0.857±0.08^{e}
LAU 10	0.909±0.39^{bcdef}	0.962±0.22^{bcde}	2.031±0.27^{bcde}	1.622±0.26^{cdefg}	0.905±0.08^{de}
NE 01	1.288±0.32^{abc}	2.481±0.30^{abc}	2.010±0.44^{bcde}	1.972±0.13^{abcde}	1.001±0.07^{cde}
NE 02	0.818±0.02^{cdef}	2.111±0.05^{bcd}	1.281±0.17^{fgh}	1.919±0.03^{abcdef}	0.480±0.16^{f}
NE 03	0.227±0.00^{gh}	1.000±0.25^{cde}	2.500±0.50^{abc}	2.600±0.20^{f}	0.196±0.02^{f}
NE 05	0.813±0.16^{cdef}	1.103±0.07^{ef}	1.340±0.14^{fgh}	2.323±0.23^{abc}	0.156±0.03^{f}
NE 07	0.213±0.01^{gh}	1.056±0.04^{ef}	1.026±0.01^{h}	1.030±0.01^{g}	0.146±0.04^{f}
NE 08	0.133±0.04^{h}	1.253±0.04^{abcd}	1.120±0.05^{gh}	1.220±1.08^{efg}	0.150±0.03^{f}
LAU 30	0.818±0.03^{cdef}	2.296±0.12^{abcd}	2.625±0.17^{ab}	1.838±0.03^{a}	0.951±0.37^{de}
LAU 60	1.227±0.06^{abcde}	2.036±0.37^{cd}	2.656±0.14^{ab}	1.946±0.02^{abcdef}	1.571±0.05^{b}
LAU 90	1.409±0.02^{a}	2.667±0.02^{a}	2.750±0.04^{a}	1.657±0.69^{fg}	1.928±0.03^{a}
LAU 120	1.318±0.04^{ab}	2.259±0.11^{abcd}	1.594±0.08^{defgh}	1.189±0.03^{cdefg}	0.905±0.04^{de}
LAU09 x LAU10	0.826±0.04^{cdef}	2.062±0.08^{cd}	1.860±0.06^{cdef}	1.457±0.11^{defg}	1.050±0.07^{cde}
LAU09 x NE01	1.252±0.10^{abcd}	2.126±0.09^{bcd}	2.244±0.07^{abcd}	2.198±0.06^{abcd}	1.253±0.12^{bcd}
LAU09 x NE02	1.552±0.18^{a}	2.737±0.04^{a}	2.484±0.05^{abd}	2.003±0.01^{abcd}	1.565±0.03^{b}
LAU09 x NE03	0.790±0.06^{def}	2.576±0.10^{ba}	2.509±0.08^{abc}	2.391±0.03^{ab}	1.619±0.08^{ab}
LAU09 x NE05	1.145±0.03^{abcdef}	1.921±0.02^{d}	2.597±0.07^{ab}	2.328±0.06^{abc}	1.547±0.04^{b}
LAU09 x NE07	0.768±0.06^{ef}	1.218±0.05^{ef}	1.490±07^{efgh}	1.566±0.06^{cdefg}	1.322±0.05^{bc}
LAU09x NE08	0.638±0.04^{gf}	1.089±0.07^{ef}	1.764±0.18^{defg}	1.575±0.08^{cdefg}	1.262±0.09^{bcd}

The mean values on the same column followed by different superscripts are significantly different at 0.05 probability level according to Duncan Multiple Range Test

(LAU90) with RT (0. 889 cm/d), WMR (6.67 g), shorter RD (14 days) and the highest PDT (47.6%), while their wild types recorded lesser values. Highest value of WMR, RT and PDT at shorter RD obtained inhybrid strains over their wild types are of commercial importance in mushroom production.Growth factors, morphological and agronomic traits can be adopted in genetic diversity studied of various plants species (Solak and Gupta, 2001). From principal components, we have three groups based on reducing the number of variables. Group one, RT has the largest loading, and was taken as best, followed by 7-G and pH 9-G from component one. RD was the best choice in the group two, followed by 10-G and pH 4-G, while 14-G was the best, followed by 16-G and WMR in the group three. Hence, the nine identified variables could be used to replace forty eight original variables, because they are weighted combinations of the original variables and they are independent of each other (Adebayo *et al.*, 2014). Spatial distribution of the nineteen strains used in this study showed high level of genetic variability considering the dendrogram of relationships among the strains of *Pleurotus* species based on morphometric traits and growth factors. Ward clustering method was

able to differentiate among the nineteen strains by dividing them into two main genetic groups (Clusters A and B).

Table 3: Weight of Dried Mycelia Yield (WDMY in g) of Wild, Mutant and Hybrid Strains of *P. pulmonarius* at Different pH Values

Strains	pH4	pH5	pH6	pH7	pH8	pH9
LAU 09	0.39±0.03^{bcd}	0.38±0.01^{cde}	0.39±0.02^{defg}	0.37±0.02^{bcdef}	0.48±0.04^{abcde}	0.30±0.02^{b}
LAU 10	0.26±0.01^{cde}	0.32±0.01^{efg}	0.28±0.02^{gh}	0.26±0.02^{fgh}	0.24±0.02^{defg}	0.20±0.02^{b}
NE 01	0.24±0.04^{de}	0.28±0.01^{fgh}	0.26±0.02^{gh}	0.28±0.03^{efgh}	0.26±0.03^{cdefg}	0.22±0.01^{b}
NE 02	0.20±0.02^{de}	0.24±0.01^{h}	0.22±0.01^{h}	0.22±0.03^{h}	0.20±0.01^{efg}	0.14±0.02^{b}
NE 03	0.39±0.05^{bcde}	0.42±0.00^{cdef}	0.40±0.03^{abc}	0.34±0.02^{bcdefg}	0.30±0.00^{bcdef}	0.24±0.01^{b}
NE 05	0.30±0.06^{cde}	0.35±0.02^{cdef}	0.41±0.01^{fgh}	0.42±0.01^{abcd}	0.32±0.00^{abcdef}	0.26±0.00^{b}
NE 07	0.34±0.04^{bcde}	0.50±0.03^{ab}	0.50±0.03^{bc}	0.44±04^{ab}	0.43±0.03^{ab}	0.38±0.06^{b}
NE 08	0.56±0.05^{b}	0.38±0.04^{cde}	0.34±0.03^{efg}	0.36±0.02^{bcef}	0.37±0.01^{abcd}	0.32±0.02^{b}
LAU 30	0.32±0.04^{cde}	0.32±0.02^{efg}	0.30±0.04^{efg}	0.30±0.03^{defgh}	0.36±0.16^{abcd}	0.33±0.03^{b}
LAU 60	1.30±0.05^{cde}	0.36±0.02^{cdef}	0.38±0.03^{defg}	0.40±0.06^{abcdef}	0.41±0.06^{abc}	0.38±0.02^{b}
LAU 90	1.40±0.24^{a}	0.44±0.01^{bc}	0.48±0.07^{cd}	0.52±0.02^{a}	0.48±0.04^{ab}	1.44±0.40^{a}
LAU 120	0.18±0.01^{de}	0.21±0.04^{h}	0.22±0.03^{h}	0.23±0.04^{gh}	0.18±0.00^{fg}	0.14±0.02^{b}
LAU09xLAU10	0.16±0.01^{e}	0.26±0.01^{gh}	0.30±0.01^{efg}	0.24±0.01^{gh}	0.14±0.01^{g}	0.14±0.00^{b}
LAU09 x NE01	1.26±0.01^{cde}	0.54±0.03^{a}	0.62±0.06^{a}	0.58±0.02^{ab}	0.54±0.02^{a}	0.52±0.01^{b}
LAU09 x NE02	1.47±0.00^{bc}	0.54±0.02^{a}	0.59±0.00^{ab}	0.50±0.06^{a}	0.47±0.01^{ab}	0.46±0.01^{b}
LAU09 x NE03	0.28±0.02^{cde}	0.36±0.01^{cdef}	0.34±0.03^{efg}	0.30±0.03^{defgh}	0.28±0.02^{bcdefg}	0.16±0.02^{b}
LAU09 x NE05	0.28±0.03^{cde}	0.52±0.04^{a}	0.44±0.01^{cde}	0.39±0.04^{bcde}	0.37±0.02^{abcd}	0.37±0.01^{b}
LAU09 x NE07	0.36±0.02^{bcde}	0.38±0.02^{cdef}	0.41±03^{efgh}	0.44±0.02^{abc}	0.42±0.00^{bc}	0.42±0.03^{b}
LAU09 x NE08	0.36±0.03^{bcde}	0.37±0.01^{cd}	0.38±0.01^{defg}	0.32±0.02^{cdefgh}	0.32±0.04^{abcdef}	0.30±0.0^{b}

The mean values on the same column followed by different superscripts are significantly different at 0.05 probability level according to Duncan Multiple test

Cluster A contained mutant and hybrid strains without the presence of wild type, which indicate that mutants and hybrids may be morphologically distinct but share similar genetic features. Cluster B contained only wild strains (both India and Nigeria species), suggesting similarity in genetic background of the strains, since their genetic materials were not tampered with, though they differ in physiological responses. This finding established genetic variability among wild, mutant and hybrid strains studied based on morphometric traits and growth factors. Information here may enhance selection for better performance of mutant and hybrid strains over the wild types in different environmental conditions, which can also enhance yield improvement. In previous study, Adebayo *et al.* (2014) opined that good breeding values could be obtained as a result of strains divergent and member of one heterotic group can be represented by strains in the same cluster. Genet *et al.* (2005), Azeez and Morakinyo (2011) reported that the utilization of genotypes from different clusters as parents of crosses can be used for maximum variability for selection in the segregating population of plants. Hence, available information from the results obtained from the current study could be adopted in breeding for improved yield performance.

5.0 Conclusions

From all indications, results obtained in the present study showed that nineteen strains of *Pleurotus* species are genetically diverse based on the morphometric traits and growth factors, under different temperature and pH regimes. Strain LAU 90 (mutant) with the highest mycelia yield, ramification rate, weight of mycelia ramification, and LN 98 (LAU09 x NE08) with the high mycelia yield and percentage spawn productivity within minimum of 12 days may be adopted for commercial mushroom cultivation.

6.0 Conflict Of Interest

Authors declare no conflict of interest.

Authors Contribution

EAA and JKO designed and executed the experiment; MAA and ONM provided the data and involved in the discussion of results.

Table 4: Eigenvectors and Percentage Explained Variation by the First Five Principal Components of Morphometric Traits and Growth Factors of the Nineteen Strains of *Pleurotus* Species Studied.

Principal Components					
Variables	Prin1	Prin2	Prin3	Prin4	Prin5
3-G	0.603	-0.388	-0.106	-0.429	0.215
5-G	0.747	-0.459	-0.232	-0.262	0.132
7-G	0.823	0.149	-0.250	-0.055	0.371
10-G	0.510	0.634	0.338	0.165	-0.022
12-G	0.696	0.281	0.128	-0.348	0.285
14-G	0.753	0.136	0.434	0.137	-0.318
16-G	0.763	0.168	0.395	0.161	-0.348
RT	0.959	0.130	0.182	-0.098	0.023
RD	-0.555	0.681	0.187	0.313	0.094
WMR	0.673	-0.107	0.360	-0.263	-0.249
PDT	0.734	-0.436	0.108	-0.364	-0.230
LGR at T-15^{o}	0.506	-0.435	0.236	0.184	0.117
LGR at T-20^{o}	0.319	-0.484	0.188	0.573	0.383
LGR at T-25^{o}	0.525	-0.325	0.166	0.676	-0.100
LGR at T-30^{o}	0.151	-0.425	-0.216	0.256	0.090
LGR at T-35^{o}	0.763	-0.462	0.230	0.045	-0.099
WDMY at pH4	0.733	0.536	0.230	0.020	0.252
WDMY at pH5	0.565	0.162	-0.728	0.142	-0.143
WDMY at pH6	0.634	0.121	-0.732	0.111	-0.024
WDMY at pH7	0.709	0.277	-0.582	0.099	-0.059
WDMY at pH8	0.703	0.328	-0.495	0.154	-0.290
WDMY at pH9	0.775	0.419	0.281	0.051	0.309
Eigen value	9.806	3.206	2.811	2.040	1.085
Individual percentage	44.575	14.571	12.776	9.271	4.933
Cumulative percentage	44.575	59.146	71.922	81.193	86.126

Prin1= principal component 1, Prin2 = principal component 2, Prin3 = principal component 3, Prin4 = principal component 4, Prin5 = principal component 5, G= growth, RT = ramification rate, RD = ramification days, WMR = weight of mycelia ramification, PDT = spawn productivity, T= temperature, PV = percentage of variance, PC = percentage cumulative, LGR = Linear growth rate, WDMY = Weight of dry mycelium yield.

Table 5: Spawn Ramification Rates (cm/day) of Wild, Mutant and Hybrid Strains of *P. pulmonarius.*

Strains	RT (cm/d)	RD (day)	WMR (g)	PDT (%)
LAU 09	0.412±0.19^{b}	16	4.900±0.11^{e}	30.62±0.72^{f}
LAU 10	0.425±0.10^{b}	16	3.700±0.17^{e}	23.12±1.08^{f}
NE 01	0.415±0.20^{b}	16	4.700±0.05^{e}	29.37±0.36^{f}
NE 02	0.359±0.03^{b}	16	5.100±0.17^{e}	31.87±1.08^{f}
NE 03	0.400±0.14^{b}	16	4.600±0.11^{e}	28.75±0.72^{f}
NE 05	0.446±0.08^{b}	16	5.400±0.23^{d}	33.75±1.44^{f}
NE 07	0.437±0.02^{b}	16	4.200±0.15^{e}	26.25±0.95^{f}
NE 08	0.437±0.10^{b}	16	5.000±0.17^{e}	31.25±1.08^{f}
LAU 30	0.478±0.04^{b}	14	5.670±0.17^{cd}	40.50±1.23^{f}
LAU 60	0.561±0.05^{b}	14	6.000±0.11^{bc}	42.85±0.82^{f}
LAU 90	0.889±0.13^{a}	14	6.670±0.05^{a}	47.64±0.41^{bc}
LAU 120	0.394±0.05^{b}	14	5.670±0.11^{cd}	40.50±0.82^{f}
LAU09 x LAU10	0.402±0.08^{b}	12	4.200±0.17^{e}	35.00±1.44^{f}
LAU09 x NE01	0.506±0.04^{b}	12	4.400±0.17^{e}	36.66±1.44^{f}
LAU09 x NE02	0.583±0.07^{b}	12	5.700±0.05^{cd}	47.50±0.48^{bc}
LAU09 x NE03	0.571±0.08^{b}	12	5.300±0.20^{d}	44.16±1.73^{e}
LAU09 x NE05	0.478±0.02^{b}	12	5.500±0.20^{d}	45.83±1.66^{d}
LAU09 x NE07	0.587±0.02^{b}	12	6.100±0.05^{bc}	50.83±0.48^{ab}
LAU09 x NE08	0.578±0.05^{b}	12	6.400±0.20^{ab}	53.33±1.73^{a}

RT = ramification rate, RD = ramification days, WMR = weight of mycelia ramification, PDT = spawn productivity

The mean values on the same column followed by different superscripts are significantly different at 0.05 probability level according to Duncan Multiple Range Test

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