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Growth Interactions and Somatic Compatibility of *Daldinia concentrica* (Bolton) Ces. & De Not. Isolates from Edo State, Nigeria

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ABSTRACT: This study investigated the compatibility of selected isolates of the medicinal mushroom, *Daldinia concentrica* of southern Nigeria origin. The pure cultures of the mushroom were prepared on Potato Dextrose Agar. Compatibility was done by pairing the isolates in the Petri dishes. Five millimetre agar block of each of isolate was inoculated in a Potato Dextrose Agar plate in dual cultures with a distance of 2 cm apart. Five replicate plates were prepared for each paired isolates. The controls were the pairs from the same plate of isolate. Daily determination of mycelia extension in millimetre was done until the mycelia of the two inocula merged or repelled each other. It was observed that 60% of the paired isolates were incompatible while the remaining 40% were compatible. There were demarcation zones in between incompatible isolates pairs while total fusion occurred in all the compatible isolate pairs of the mushroom. The differences in the mycelial extension of the paired isolates was found to be significant (p=0.05). The highest mycelial extension (14.83±0.40mm) was found in one isolate with a corresponding value of 4.67±0.33mm for another isolate. The level of incompatibility in the study suggests that there may be more genotypes of the mushroom in the field. This study therefore underscores the need for more investigations on the intra-specific eco-diversity of the mushroom.

Keywords: Chimera, Diversity, Individuality, Mycelial growth, Vegetative compatibility, Demarcation line

Introduction

The population structure and distribution of fungi growing in a particular area can be projected when isolates of some species obtained from a specified location are tested for somatic incompatibility. This will show if a species has non-individualistic mode of behaviour or whether they have the capacity for intra-specific exchange of nuclei (Fries, 1987). The extent of intra-specific gene flow depends on somatic compatibility.

Identical mycelia intermingle freely while mycelia of different genotypes show vegetative incompatibility when confronted with each other (Webster and Weber, 2007). Refai *et al.* (2013) studied the vegetative compatibility of thirteen isolates of *Trichoderma* spp. They recorded a high level (80%) of vegetative incompatibility which was characterized by overgrowth, zones of inhibition, demarcation lines and ridges of conidia among the isolates.

There is now a renewed interest in the study of the *Daldinia concentrica*. The mushroom has been found to contain many substances with potent medicinal values (Lee *et al*., 2006; Qin *et al*., 2006; Qin *et al*., 2008; Shao *et al*., 2008). In Nigeria, the first report of its use among the Igbos was that of Akpaja *et al.* (2003). Akpaja *et al.* (2014) reported its use for treating a variety of ailments ranging from boil to malignant growth on the ear among the Okpameri people of Edo State. The use of the mushroom among the Igala people of Nigeria was reported by Ayodele *et al.* (2011). To the best of our knowledge, the most recent reports on some vital aspects of the vegetative growth of the fungus were those of Akpaja and Okhuoya (2018a,b and c) in Nigeria.

Compatibility systems regulate the vegetative status and mating of an ascomycete. These are: the vegetative compatibility systems which maintains the genetic integrity of vegetative mycelia and, the mating compatibility system which regulates the fusion between gametes or reproductive structure. The potential for outbreeding within species is also controlled by this. Although, many workers have reported on the ecology and compatibility systems of the genus *Daldinia*, attempts have not been made to investigate the behaviour of the fungus in the field, in Nigeria. This is with special reference to host range and intraspecific gene flow. The objective of this study was therefore to determine the extent of genotypic variation of the fungus in the study area.

Materials and method

Collection of fruiting bodies and establishment of pure cultures of Daldinia concentrica: Fruiting bodies of the test mushroom were collected from a two-acre fallowing vegetation at Eko-Abetu community near Benin City. Pure cultures were established on Potato Dextrose Agar following the method of Stamets and Chilton (1983). The pure cultures so established from each fruiting body constituted the isolates used in the study. They were separately transferred into slants in McCartney bottles and kept in the refrigerator (4ºC) until used.

Preparation of Potato Dextrose Agar: The method of preparing Potato Dextrose Agar, as described by Aneja (2003) was followed. 200g peeled Irish potato was used for getting potato broth. This was done by adding 1000 ml of water to the peeled potato in a one litre beaker. The mixture was boiled for 1 hour. Filtration using cheese cloth was thereafter done. To the filtrate (potato extract) was added 20g dextrose and 20g agar. The volume was brought to 1000 ml by the addition of distilled water. The whole content in the beaker was then emptied into a 1000 ml conical flask. The mouth of the conical flask was plugged with cotton wool and covered with foil paper. The medium was then sterilized at 121^0C, 15 lb pressure, for 20 minutes in an autoclave.

Somatic Compatibility: This study was carried out in order to determine which of the selected isolates were compatible with each other. This was done by pairing the isolates in the plates. Five millimetre agar block of each of isolate was inoculated in a Potato Dextrose Agar plate in dual culture with a distance of 2 cm apart. Five replicate plates were prepared for each pair. The controls were the pairs from the same plate of isolate. Daily determination of mycelial extension in millimetre was done until the mycelia of the two inocula merged or repelled each other (De Simone and Annesi, 2012).

Analysis of Data: Results were expressed as means and standard error of replicate values. Data obtained were subjected to analysis of variance (ANOVA). Where there were significant differences, means were separated by Duncan Multiple Range F-Test using SPSS 15.0 package.

Results and discussion

The somatic compatibility of the selected isolates used in this study showed that there was some level of compatibility among them (Table 1).

Table 1: Somatic compatibility of paired isolates of *Daldinia concentrica*

	Isolate				
Isolate	i	ii	iii	iv	v
i	+	-	-	-	-
ii	-	+	-	-	-
iii	+	+	+	-	-
iv	-	-	-	+	-
v	+	-	+	-	+

\+ = Compatible, = Incompatible

The number of incompatible isolates was more than the compatible ones. About 60% of the paired cultures were incompatible while 40% were compatible. As shown in Plate (1), there was complete fusion among some isolates while different forms of incompatibility were found among other pairs. Zones of inhibition were observable between some paired isolates (Plate 2).

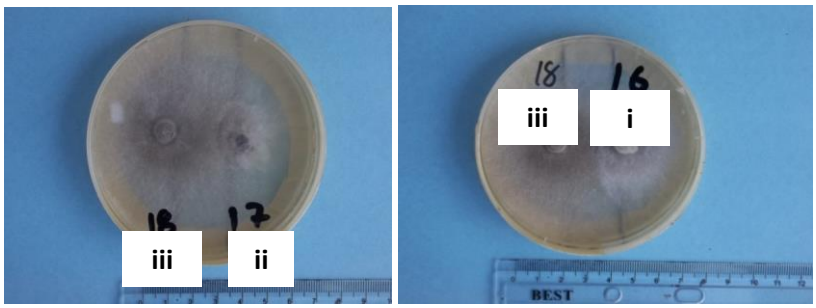

Plate 1: Compatible paired isolates of *Daldinia concentrica*

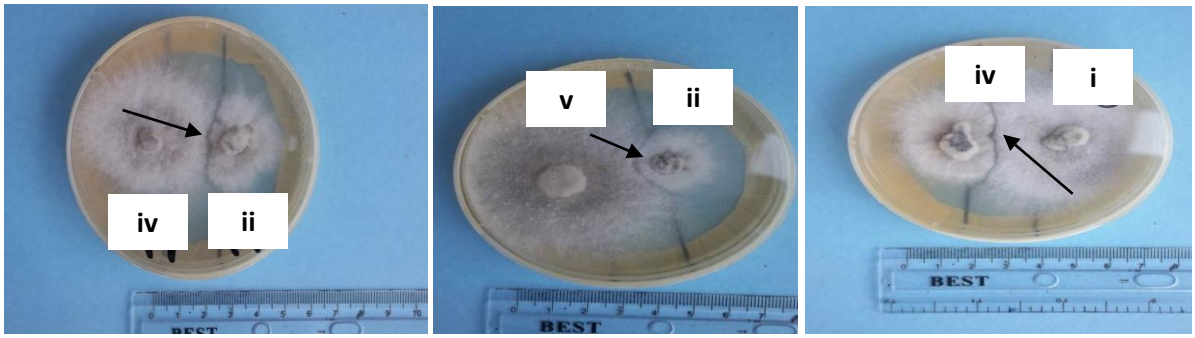

Plate 2: Incompatible paired isolates of *Daldinia concentrica.* Arrows shows lines of demarcation between incompatible isolates. Roman numeral refers to individual isolates

Refai *et al*. (2013) reported a much higher (80%) incompatibility in *Trichoderma* spp. In paired cultures, the growths of some isolates were slowed down by the other isolate. This observation suggests the release of some chemical substances into the medium that retards the growth of the other isolate. There was a significant (p=0.05) difference in the mycelial extension of the isolates as they grew towards each other. In some cases, as shown in Plate 2, there were constriction lines at the meeting points between the two different mycelial colonies. This observation is an indication that the two mycelia are different genotypes (Rayner *et al*., 1984). As stated by Brusini *et al*. (2013), somatic fusion between the soma of two independent multicellular organisms is a mechanism observed in all major multicellular eukaryotic taxa. One characteristic feature that precedes clamp connection in basidiomycetes is the fusion of paired isolates (Carlile *et al*., 2001; Webster and Weber, 2007).

It has been stressed that incompatible genotypes can be identified by a clear zone between the two genets or by an interaction zone of vacuolation, hyphal deformation or death (Rayner *et al*. 1984). This present study sheds light on the types of interactions occurring between the genotypes of the mushroom used in this study.

The growth performance of the paired isolates varied significantly (p=0.05) among them. Significant (p=0.05) variation was observed in isolates ii x v and iv x v pairs (Table 2) while others were not significant. De Simone and Annesi (2012) made a similar observation on *Ganoderma adspersum.* Fries (1987) observed such reactions of mycelia on the cultures of *Suillus luteus*. Fries (1987) stressed that such compatibility test can be used to map the population structure of fungi. In addition, this observation is relevant in the research and development concerning the mushroom where different genotypes of the mushroom are required. Studies are on-going to provide more information on the field ecology and intra-specific genotypic variation of the fungus in Nigeria.

Table 2: Mycelial extension of selected paired isolates of *Daldinia concentrica*

	Isolate	
Dual culture (Isolates)	First	Second
I x I	10.50 ±0.92 [*a]	8.83 ±1.05[ab]
II x II	4.50 ±1.26[a]	15.67 ±1.23[b]
III x III	7.33 ±1.05[a]	12.5 ±1.11[b]
IV x IV	10.33 ±1.73[a]	8.67 ±1.61[ab]
V x V	10.83 ±0.70[a]	9.67 ±0.56[b]
I x II	11.17 ±1.82[a]	8.17 ±1.38[b]
I x III	11.17 ±1.78[a]	8.50 ±1.18[b]
I x IV	10.50 ±1.18[a]	5.17 ±0.91[b]
I x V	9.33 ±0.56[a]	10.17 ±0.40[b]
II x III	6.50 ±1.18[a]	11.50 ±0.85[b]
II x IV	7.17 ±0.98[a]	9.50 ±1.61[b]
II x V	5.17 ±0.75[a]	14.50 ±0.85[b]
III x IV	8.50 ±1.15[a]	8.33 ±0.76[ab]
III x V	13.17 ±0.65[a]	6.67 ±1.09[b]
IV x V	4.67 ±0.33[a]	14.83 ±0.40[b]

[*] Mean of five replicates ± standard error. Values represents mycelial diameter, in mm, 5 days after inoculation. Mean value (± standard error) with different superscript alphabets along the same row are significantly different from each other ($p<0.05$).

This study shows that *Daldinia concentrica* appears to have an inherent recognition mechanism. However, the level of somatic compatibility (40%) observed in this study suggests that the formation of chimera would not be minimal. There is therefore the need to investigate how the fungus maintains its genetic integrity. Over the past few decades, many studies have been conducted on related subject-matter. However, the need to really study the mechanism responsible for incompatibility at the molecular and physiological levels is hereby suggested for this mushroom.

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