Characterization of *Pleurotus djamor* neohaplonts recovered by production of protoplasts and chemical dedikaryotization

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Abstract

Production of hybrid strains is accomplished by mating monosporic isolates or neohaplonts, obtained either by chemical dedikaryotization or by production of protoplast. However, differences in growth rate among recovered neohaplonts have been reported. The presence of phenotypic and genetic changes among the neohaplonts recovered either by chemical dedikaryotization or by production of protoplast, was evaluated by measuring growth and morphology, and by molecular characterization using six ISSR markers to identify polymorphisms. Neohaplonts recovered by both methods presented variation in growth rate depending on their compatibility type and recovery method. Using ISSR markers, 59.2% polymorphism was established. Neohaplonts recovered by both monokaryotization procedures presented differences in growth rate and polymorphism.

Keywords Hybrid production · ISSR · Monokaryotization · Polymorphism

Introduction

World production of cultivated, edible mushrooms has increased more than 30-fold since 1978–2013, but 85% of the world production results from cultivation of only five

genera: *Lentinula* (22%), *Pleurotus* (19%), *Auricularia* (17%), *Agaricus* (15%) and *Flammulina* (11%) (Royse et al. 2017). *Pleurotus*, with five or six cultivated species, is the genus with the highest diversity of any other cultivated genera of agarics (Singh and Kamal 2017).

From 1997 to 2010, the global production of *Pleurotus* spp. increased from 0.876 to 6.288 million tons, representing a 618% increment (Royse 2014). Despite the increment in production, massive mushroom cultivation has many challenges that can be overcome through genetic improvement. Genetically improved strains can not only increase the quality of cultivated mushrooms and reduce cultivation costs, but they can also rise farmer's revenue in the short term (Avin et al. 2012).

Genetic improvement of mushrooms is based upon hybridization, that is to say, the combination of desirable characteristics from different strains, creating variability within the existing germplasm (Kumara and Edirimanna 2009). Intermating isolated spores is a widely used method for hybridization and has been successfully utilized by scientists to improve production yield and various quality parameters (Eger 1978; Singh and Kamal 2017). Despite its simplicity, hybridization by intermating isolated spores allows genetic recombination to occur during meiosis, thus producing unpredictable phenotypic variation. To overcome

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these limitations, other methods have been developed, such as dedikaryotization, which implies the recovery of the monokaryotic components (neohaplonts) of selected dikaryons for production of hybrids either by intermating (Leal-Lara and Eger-Hummel 1982) or by protoplast fusion (Castle et al. 1987; Wessels et al. 1976).

However, a very important hindrance in every improvement program is natural variability (Singh and Kamal 2017). Mutations and recombination during meiosis can produce variability, specifically mutation expresses its potential by intermating of different genotypes where new combinations arise due to genetic assortment (Singh and Kamal 2017), they can arise naturally or by a mutagen agent.

There is evidence supporting that dedikaryotization methods affect phenotypic characteristics of the monokaryotic components (neohaplonts). Fukamasa et al. (1994) recovered *Lentinula edodes* neohaplonts by protoplast production that exhibited different growth rates. Guadarrama et al. (2014) recovered *Pleurotus djamor* neohaplonts and they reported differences like mycelial morphology, color and growth rate for the monokaryotic components. Taken together, these data suggest that even when genetic recombination is not allowed during dedikaryotization, neohaplonts exhibit phenotypic variations not present in their parental strain, thus, more investigations are needed to establish to what extent this variability diminishes dedikaryotization reliability. Here we analyze phenotypic variation of neohaplonts recovered by dedikaryotization from *Pleurotus djamor*.

Materials and methods

Biological material

In this research, *Pleurotus djamor* wild strain (CC051) was used. Stocks of all strains generated in this study were deposited at the fungal collection of the Cellular Cultures of the Biotechnology Interdisciplinary Professional Unit (UPIBI-IPN).

Culture media

Plates with 1.5% malt extract agar (MEA) were employed for propagation of *Pleurotus djamor* strains. For protoplast isolation, strains were cultivated in liquid MYG (2% malt, 0.2% yeast extract and 2% glucose) medium. The same medium supplemented with 0.6 M mannitol and 1.5% (base) or 0.5% (top) agar was used as a regeneration medium (RM) for protoplasts. The dedikaryotization solution (DS) was prepared with 20 g/L anhydrous glucose and peptone P (Oxoid LP0037).

Dedikaryotization

A MEA plate, fully grown with mycelium from strain CC051 was blended (Waring Blender, high speed) for 60 s, and 25 μ L homogenate was inoculated in 100 mL flasks with 50 mL dedikaryotization solution (DS) and incubated at 28 °C. When mycelium growth was noticeable in flasks with DS, they were homogenized with 50 mL sterile distilled water (Waring Blender, high speed) for 60 s; 25 μ L of this homogenate was inoculated on MEA plates and incubated at 28 °C until colonies were formed. Growing colonies were observed under the microscope to identify neohaplonts, characterized by the absence of clamp connections (Valencia del Toro and Leal-Lara 1999, 2002).

Protoplast production

A MEA plate, fully grown with mycelium from strain CC051 was blended (Waring Blender, high speed) for 60 s, and 25 μ L homogenate was inoculated in 50 mL MYG broth and incubated at 28 °C for 2 weeks. The resulting mycelium was homogenized and suspended in 500 mL flask with 25 mL MYG broth and incubated at 28 °C for 4 days. The resulting mycelium was collected by filtration through gauze and rinsed three times with 0.6 M mannitol and then placed in a Falcon tube with 2 volumes of 0.6 M mannitol with 4% lysing enzymes from *Trichoderma harzianum* (Sigma-Aldrich Inc., St. Louis, MO) and incubated at 28 °C for 2 h. Protoplasts were separated from hyphal debris by filtration through gauze, washed twice with 0.6 M mannitol and collected by centrifugation (1450 g for 5 min at 4 °C). For regeneration, 10^3 protoplasts were plated on RM and incubated at 28 °C until colonies were formed. Growing colonies were observed under microscope to identify neohaplonts (Fukamasa-Nakai et al. 1994; Dhitaphichit and Pornsuriya 2005; Selvakumar et al. 2015).

Identification of neohaplonts' compatibility types and production of reconstituted strains

To identify the two types of monokaryotic components, a neohaplont was randomly selected and mated on MEA plates with all remaining neohaplonts. Dikaryotic mycelium, characterized by the presence of clamp connections, was produced when two compatible neohaplonts were mated. With this approach, eight reconstituted strains were produced.

Kinetics of mycelia growth on Agar media with Baranyi model.

Mycelia growth on solid medium (MEA plates) was estimated by measuring colony diameter. For each strain, four replicates were measured. Growth velocity was determined using the following nonlinear regression Baranyi model (Baty and Delignette-Muller 2004):

$$y(t_{\max}) = y_{\max} + \ln \left(\frac{-1 + e^{\mu_{\max} \lambda} + e^{\mu_{\max} t}}{(-1 + e^{\mu_{\max} t}) + e^{(\mu_{\max} \lambda + y_{\max} - y) t}} \right)$$

where λ = lag phase, $\mu_{\max}$ = maximum growth rate, $y_{\max}$ = growth on the last day, y = growth on the first day, t = days, $e = 2.7183$, and $y(t_{\max})$ = growth rate.

Extraction of genomic DNA and PCR conditions

Genomic DNA was isolated with gDNA Plant Kit (Cat no CS 18000, Invitrogen). PCR reactions were performed with 25 μ L reaction mixture containing 22.5 μ L PCR Supermix (Cat no 10572014, Invitrogen), 35 ng template DNA, 20 μ M ISSR primer. ISSR amplification conditions were: 5 min initial denaturation at 94 °C; 35 cycles consisting of 1 min at 94 °C, 1 min from 53.5 to 63 °C and 3 min at 72 °C and a final 10 min at 72 °C.

Electrophoresis analysis

A 1.2% agarose gel and 1 $\times$ TAE buffer was used to separate amplified ISSR. Electrophoresis was run at 100 V for 1 h for ISSR genome-specific ISSR products. Separated band profiles were visualized under UV Transilluminator. The sizes of the amplified fingerprints were determined using 2 log (100–10,000 bp) ladder (New England Biolabs) as a standard molecular weight marker.

Statistical analysis

Growth variations among neohaplonts and reconstituted strains were analyzed by factorial analysis of variance (ANOVA) and the Duncan post hoc test for multiple comparisons of means with SPSS. Data generated from six ISSR primers for each hybrid strain were entered into a binomial matrix. The presence/absence matrix was utilized to calculate Euclidean distance and a dendrogram was constructed with R function *hist*.

Primer banding characteristics were obtained; total bands (TB), number of polymorphic bands (PB) and percentage of polymorphism (PP). Polymorphic information content (PIC), marker index (MI), resolving power (RP) and EMR

(effective multiplex ratio) (Roldán-Ruiz et al. 2000; Varshney et al. 2007) were used to analyze the fitness of the genetic profiles and the performance of the markers.

Results

Recovery of the monokaryotic components

After chemical dedikaryotization of CC051, 11 monokaryotic components were recovered, 5 belonging to compatibility type 1 and 6 to compatibility type 2. Conversely, from protoplast production, eight neohaplonts were obtained, four of type 1 and four of type 2. According to Chi squared test ($p < 0.05$, not shown), recovery of monokaryotic components was symmetrical for both dedikaryotization techniques. Eight reconstituted strains were obtained by mating the recovered neohaplonts from both methods (Table 1).

Mycelia growth

Growth parameters of neohaplonts, reconstituted dikaryons and the parental dikaryon CC051 are shown in Table 1. Mycelium of type I neohaplonts and dikaryotic strains exhibited a cottony texture, different than the slower growing type II neohaplonts with a floccose texture. Statistical analysis indicated significant differences in growth parameters. In general, the monokaryotic strains presented significant differences with respect to dikaryotic strains, and neohaplonts of distinct compatibility type also exhibited significant differences.

The group of dikaryotic strains, i.e., reconstituted dikaryons and the parental dikaryon CC051, exhibited the fastest growth. Neohaplonts of type 1, either recovered by chemical dedikaryotization or from protoplasts, showed an intermediate growth, while type 2 neohaplonts were the slowest (Table 1; Fig. 1). Noteworthy, the standard error showed variability among strains from each group. Major variability between strains was observed in the group of type II neohaplonts, specifically neohaplonts obtained by protoplast production (Fig. 1). The variability of growth among strains of each group is apparently associated with the dedikaryotization method.

A factorial ANOVA indicated differences between groups and with the Duncan test, the different levels of $\mu_{\max}$ were assessed (Fig. 2). Neohaplonts from the two compatibility types were grouped separately, i.e., type I neohaplonts showed higher $\mu_{\max}$ than type II neohaplonts. Dikaryotic strains exhibit higher $\mu_{\max}$ than neohaplonts. Reconstituted dikaryons from neohaplonts by protoplasts showed higher $\mu_{\max}$ than the other reconstituted dikaryotic strains.

Table 1 Growth parameters and genetic characteristics of hybrid strains

Strain	Ploidy	CT	MP	Texture	MG (mm)	$\mu_{\max}$ (mm d ⁻¹)	λ (d)
Nh1c	<i>n</i>	I	C	Ct	58.76 ± 0.18b	7.10 ± 0.13b	1.99 ± 0.30d
Nh2c	<i>n</i>	I	C	Ct	60.37 ± 0.52b	6.34 ± 0.18b	1.21 ± 0.30d
Nh3c	<i>n</i>	I	C	Ct	58.41 ± 0.27b	7.13 ± 0.25b	1.99 ± 0.31d
Nh4c	<i>n</i>	I	C	Ct	59.33 ± 0.25b	6.56 ± 0.06b	1.31 ± 0.32d
Nh5c	<i>n</i>	I	C	Ct	58.51 ± 0.24b	7.24 ± 0.16b	1.93 ± 0.20d
Nh1p	<i>n</i>	I	P	Ct	60.03 ± 0.05b	7.22 ± 0.11b	1.66 ± 0.30c
Nh2p	<i>n</i>	I	P	Ct	63.25 ± 0.31b	7.75 ± 0.21b	1.32 ± 0.33c
Nh3p	<i>n</i>	I	P	Ct	61.42 ± 0.22b	7.26 ± 0.06b	1.43 ± 0.26c
Nh4p	<i>n</i>	I	P	Ct	60.46 ± 0.13b	7.63 ± 0.07b	1.82 ± 0.27c
Nh6c	<i>n</i>	II	C	F	52.07 ± 0.27a	4.35 ± 0.06a	0.34 ± 0.06c
Nh7c	<i>n</i>	II	C	F	47.10 ± 0.71a	4.29 ± 0.08a	1.50 ± 0.13c
Nh8c	<i>n</i>	II	C	F	46.43 ± 0.25a	4.35 ± 0.08a	1.78 ± 0.10c
Nh9c	<i>n</i>	II	C	F	44.92 ± 0.57a	4.05 ± 0.07a	1.31 ± 0.11c
Nh10c	<i>n</i>	II	C	F	47.25 ± 0.78a	6.63 ± 1.93a	2.03 ± 0.37c
Nh11c	<i>n</i>	II	C	F	47.66 ± 0.41a	4.63 ± 0.17a	1.51 ± 0.16c
Nh5p	<i>n</i>	II	P	F	47.31 ± 0.83a	4.41 ± 0.16a	1.40 ± 0.27a
Nh6p	<i>n</i>	II	P	F	48.19 ± 0.68a	4.51 ± 0.04a	1.15 ± 0.20a
Nh7p	<i>n</i>	II	P	F	55.85 ± 0.12a	5.31 ± 0.07a	0.33 ± 0.12a
Nh8p	<i>n</i>	II	P	F	39.24 ± 0.31a	2.81 ± 0.04a	0.51 ± 0.18a
Nh1c X Nh8c	<i>n+n</i>	I+II	C	Ct	64.48 ± 0.07c	11.95 ± 0.15c	1.53 ± 0.03b
Nh1c X Nh10c	<i>n+n</i>	I+II	C	Ct	67.30 ± 0.14c	13.38 ± 0.35c	0.99 ± 0.05b
Nh1c X Nh5p	<i>n+n</i>	I+II	CP	Ct	67.61 ± 0.36c	12.82 ± 0.57c	0.92 ± 0.05b
Nh1c X Nh8p	<i>n+n</i>	I+II	CP	Ct	67.61 ± 0.47c	14.27 ± 0.66c	1.10 ± 0.03b
Nh4p X Nh8c	<i>n+n</i>	I+II	CP	Ct	65.82 ± 0.37c	13.05 ± 0.52c	1.13 ± 0.03b
Nh4p X Nh10c	<i>n+n</i>	I+II	CP	Ct	67.21 ± 0.35c	13.17 ± 0.57c	0.98 ± 0.04b
Nh4p X Nh5p	<i>n+n</i>	I+II	P	Ct	75.81 ± 0.17c	13.97 ± 0.28d	1.10 ± 0.01b
Nh4p X Nh8p	<i>n+n</i>	I+II	P	Ct	67.42 ± 0.03c	13.82 ± 0.28d	1.05 ± 0.06b
CC051	<i>n+n</i>	I+II		Ct	67.36 ± 0.25c	13.00 ± 0.53c	0.93 ± 0.02a

Different letters in a column indicate statistically significant differences (Duncan's test $p < 0.05$)

n monokaryotic, *n+n* dikaryotic, *CT* compatibility type, *MP* monokaryotization procedure, *P* protoplast production, *C* chemical dedikaryotization, *T* texture, *Ct* cottony, *F* floccose, *MG* mycelium growth pondered after 21 days incubation, $X \pm SE$, $\mu_{\max}$ maximum growth rate, $X \pm SE$, λ lag phase, $X \pm SE$, $n = 4$

ISSR profile

The ISSR products were visualized by electrophoresis, and the banding profile was associated to compatibility type and dikaryotic strains (Fig. 3). Based on ISSR PCR amplification, TB, PB, MB, and PP were obtained. The primers with the highest number of bands were UBC 807 (14 bands) and ISSR-12 (13 bands), while the least amplification was found with primer ISSR 15 and ISSR 12 (both with 7 bands). The size of the amplified fragments varied from 100 bp to 3,000 bp. Six primers amplified 60 fragments with 59.61% polymorphism of 28 strains (19 neohaplonts and nine dikaryotic), and the average per primer was 10 bands.

The PIC, EMR, MI and RP values were calculated and they showed differences for each ISSR primer. The average PIC value was 0.22, the lowest was 0.12 for ISSR 15, and the highest was 0.34 for UBC 807. The maximum value

for EMR, 7.96, was observed with primer UBC 807 and the minimum 1.82 was observed with primer ISSR 15, with an average EMR of 14.39 per primer. The highest MI was observed with primer UBC 807 (2.74) and the lowest with primer ISSR 15 (0.23) with an average MI of 1.19 per primer. The maximum RP value was observed with primer UBC 807 (7.57) and the minimum with primer ISSR 15 (1.29) with an average RP of 3.43 per primer (Table 2). However, if the strains are analyzed by groups, dikaryons and both types of neohaplonts (type I and type II), then the PIC values are different for each group. The group of dikaryons showed the lowest PIC value, 0.06, while type I neohaplonts the highest value, 0.10, and type II neohaplonts an intermediate value, 0.08. (Table 2).

The dendrogram obtained from ISSR profiles showed three clusters (Fig. 4), placing type I neohaplonts in cluster 3, while type II neohaplonts were allocated in cluster 2 and

Fig. 1 Mycelium growth curves of *Pleurotus djamor* strains. Strains were grouped by their compatibility type, monokaryotization method and ploidy: D (parental dikaryon (CC051), dikaryons reconstituted by pairing neohaplonts produced by chemical dedikaryotization or

by protoplasts or by pairing both types of neohaplonts), P TI (type I neohaplonts, protoplast), C TI (type I neohaplonts, chemical dedikaryotization), P TII (type II neohaplonts, protoplast), C TII (type II neohaplonts, chemical dedikaryotization). $n=4$

the dikaryotic strains in cluster 1. Inside the clusters with neohaplonts (cluster I and 2), those obtained by protoplast production were not clearly separated from those obtained by dedikaryotization.

Discussion

Eleven neohaplonts were recovered by chemical dedikaryotization using similar conditions as those reported by other authors. Valencia del Toro and Leal-Lara (2002) recovered 32 monokaryotic components from three *Pleurotus* spp., and they used an homogenization time of 150 s and incubated the dedikaryotization solution for 48–120 h.

Guadarrama-Mendoza et al. (2014) used blending times of 40 and 70 s and incubated for 72 h the dedikaryotization solution with 20 g/L peptone to recover 15 neohaplonts of two *Pleurotus* spp. Valenzuela-Cobos et al. (2017) reported a symmetrical recovery of both types of neohaplonts with 60 and 90 s homogenization times and 20 g/L DS. The number of neohaplonts obtained and the symmetric recovery are directly affected by the dedikaryotization conditions, mechanical sensitivity of the strains, volume of inoculation, blending times and incubation time (Ramírez-Carrillo et al. 2011; Kawasumi et al. 2014).

Monokaryotization by protoplast production with different enzymes for cell wall degradation (Zhao and Chang 1993; Fukumasa-Nakai et al. 1994) has been reported

Fig. 2 Maximum growth rate ($\mu_{\max}$) values of *Pleurotus djamor* strains. P TII (type II neohaplonts, protoplast), C TII (type II neohaplonts, chemical dedikaryotization), P TI (type I neohaplonts, protoplast), C TI (type I neohaplonts, chemical dedikaryotization), CC051 (parental dikaryon), DRC (reconstituted dikaryons from neohaplonts produced by chemical dedikaryotization) DRP (reconstituted dikary-

ons from neohaplonts produced by protoplasts) and DRC + P (reconstituted dikaryons from neohaplonts produced from both monokaryotization procedures). Different letters indicated significant difference among the parameter values at level $P < 0.05$, according to Duncan test, $n = 4$ for strain

Fig. 3 Polymorphism profile of neohaplonts and dikaryotic strains with ISSR 02

Table 2 Marker parameters calculated for each ISSR primer used for neohaplonts and dikaryotic strains

Primer	Sequence	T (°C)	Dikaryotic strains and neohaplonts								D	NT I	NT II
			TB	PB	MB	PP (%)	PIC	EMR	MI	RP	PIC	PIC	PIC
ISR 11	(CAC) ₃ GC	56.3	7	4	3	57.14	0.21	3.37	0.72	2.21	0	0	0.07
ISR 12	(GAG) ₃ GC	56.3	13	5	8	38.46	0.16	4.40	0.72	3.14	0	0.03	0
UBC 807	(AG) ₈ T	55.0	14	12	2	85.71	0.34	7.96	2.74	7.57	0.33	0.24	0.24
UBC 811	(GA) ₈ C	55.0	10	7	3	70	0.22	5.53	1.24	3.07	0.03	0.10	0.09
ISR 15	(GCA) ₃ CT	53.2	7	2	5	28.57	0.12	1.82	0.23	1.29	0	0	0
ISR 02	(CAG) ₄	63.8	9	7	2	77.78	0.26	5.72	1.50	3.29	0	0	0.08
Total	60	37	23	61.67									
Avg./primer	10	6.17	3.83	59.61	0.22	4.80	1.19	3.43	0.06	0.10	0.08		

D Dikaryotic, NT I neohaplonts type I, NT II neohaplonts type II, TB total bands, PB polymorphic bands, MB monomorphic bands, PP polymorphism, PIC polymorphic information content, EMR effective multiplex ratio, MI marker index, RP resolving power)

Fig. 4 Dendrogram constructed from euclidean distance. Strains were separated into three groups; dikaryotic strains: (Nh4p×Nh8c, Nh1c×Nh8p, Nh1c×Nh5p, Nh1c×Nh8p, CC051, Nh4p×Nh8c, Nh4p×Nh5p, Nh4p×Nh10c and Nh1c×Nh10c), neohaplonts with compatibility type II (Nh9c, Nh8c, Nh6c, Nh8p, Nh10c, Nh11c, Nh6p, Nh7c, Nh5p and Nh7p) and neohaplonts with compatibility type I (Nh5c, Nh1c, Nh2c, Nh4p, Nh2p, Nh4c, Nh3c, Nh1p and Nh3p)

for different species (*Coprinus cinereus*, *Flammulina velutipes*, *Lentinula edodes*, *Lentinus tigrinus*, *P. florida* and *P. sajor-caju*). Production of inter-species hybrids by protoplast fusion has also been reported between *P. djamor* and *P. ostreatus* (Selvakumar et al. 2015). Nevertheless, this is the first report about the variability of neohaplonts recovered by protoplast production.

Mycelial growth is a distinctive property for each strain. The growth rate values allowed to separate dikaryotic strains (parental and reconstituted) and neohaplonts (depending their compatibility type). The differences between neohaplonts of type I and type II are understandable, because each nucleus has a different genotype associated with compatibility type. However, differences among neohaplonts with the same compatibility type were present, such differences were major for type II neohaplonts and they increased for the neohaplonts recovered by protoplast production (Fig. 1). Differences in growth rates among neohaplonts with the same compatibility type, either recovered by chemically dedikaryotization or protoplast production, have been previously reported (Fukumasa-Nakai et al. 1994; Guadarrama et al. 2014). Moreover, Guadarrama et al. (2014) reported differences regarding texture, density, growth, color and growth rate for neohaplonts with the same compatibility type, suggesting a wider variability in characteristics among neohaplonts. The results in this study indicate that the monokaryotization procedures can influence the production of neohaplont isolates with different growth rates. Growth parameters (GM, $\mu_{\max}$) not only allowed to separate strains according to ploidy and compatibility type, but differences among strains of the same group were also found.

Values of $\mu_{\max}$ for neohaplonts have not been reported so far, however, all dikaryons exhibited a significantly higher $\mu_{\max}$ than all neohaplonts, and type II neohaplonts were slower than type I neohaplonts.

Clark and Anderson (2004) transferred populations of *Schizophyllum commune*, of each ploidy state for 18 months (13,000 generations). They reported variation in growth rates, which increased to a much greater extent among the dikaryotic lines than among the monokaryotic lines. In the dikaryotic mycelia, the two component nuclei coexist in the common cell environment, raising the possibility of coadapted gene combinations between haploid genomes. Artificial selection for growth rate in monokaryons (Connolly and Simchen 1968; Simchen 1996) and dikaryons (Simchen and Jinks 1964) indicates that growth rate has a polygenic basis, but that the phenotypic expression of the polygenes is different in each ploidy phase.

Furthermore, growth rate is a selection criterion that contributes considerably to the success of the whole cultivation procedure. The period of substrate invasion has economic importance, since the media that are non-thoroughly impregnated with the hyphae are sensitive to fungal and bacterial infections resulting in reduced yield (Zervakis et al. 2001).

The differences in the number of bands is related to dissimilar genome sequences for primers of repeated sequences (Mallick and Sikdar 2014). The 59.61% polymorphism reported considering all strains (neohaplonts and dikaryotic) with six markers is related to the presence or absence of bands between strains and it is also associated to ploidy in the strains and to their compatibility type. Therefore, the three groups observed in the dendrogram are also associated to ploidy and compatibility types. Higher rates of

polymorphism have been previously reported, i.e., 74.8% polymorphism in 15 Chinese dikaryotic cultivars of *Pleurotus pulmonarius* was reported by Yin et al. (2013) and Aguilar et al. (2018) reported 99.3% polymorphism in hybrids produced by mating neohaplonts from parental *Pleurotus djamor* strains of different colors. Castle et al. (1987) mentioned that DNA from homokaryotic strains of *Agaricus* spp. exhibited less bands than DNA from heterokaryotic strains. The differences in the PIC values considering all strains or separated in groups, i.e., dikaryons and the two types of neohaplonts, point to the larger variability.

Stressful conditions, such as changing environments, is another factor that has been suggested to increase mutation rate, recombination, genetic polymorphism and gene conversion and thus may ensure higher levels of genetic diversity, providing greater potential for genetic adaptation (Korol et al. 1994; Nevo 2001; Kis-Papo et al. 2011). The monokaryotization procedures are stressful, particularly for the conditions of recovery and regeneration, and under such circumstances, mutation rate and recombination might increase, resulting in the genetic polymorphism shown by the ISSR profiles.

Three parameters, i.e., polymorphic information content (PIC), marker index (MI) and resolving power (RP) have traditionally been used as informative markers (Varshney et al. 2007). RP takes into consideration the capacity of a given primer to discriminate the genotype reported, in the same manner as the marker index (MI), which is confirmed by the high correlation between these two indices (Silva et al. 2013). For this investigation, the primer has the major capacity to discriminate UBC 807, which shows the highest values of these three informative parameters.

Conclusions

The monokaryotization procedures are very important for yielding genetic material that facilitates genetic improvement of mushrooms. However, either protoplast production or chemically dedikaryotization generate monokaryons that exhibited different growth rates, $\mu_{\max}$ and λ . The resulting genetic polymorphism allowed to separate three distinct groups associated to ploidy and compatibility type, although polymorphism was present among strains within the same group. DNA polymorphism can be attributed to the stressful conditions of the monokaryotization procedures that increase mutation rate, recombination and genetic polymorphism. This can also contribute to the differences in the growth parameters observed in this study.

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Compliance with ethical standards

Conflict of interest The authors confirm that there are no known conflicts of interest associated with this publication.

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