



BIOLOGICAL SCIENCES

Iron Or Zinc Bioaccumulated In Mycelial Biomass Of Edible Basidiomycetes

SUZANA H. UMEO, MARIA GRACIELA I. FARIA, DOUGLAS C. DRAGUNSKI,
JULIANA S. DO VALLE, NELSON B. COLAUTO & GIANI ANDREA LINDE

Abstract: Iron and zinc bioaccumulation in mycelial biomass of different medicinal basidiomycetes was evaluated in order to produce metal-enriched mycelial biomass as an alternative functional food from non-animal sources and based on biotechnology processes. *Pleurotus ostreatus* strain U2-9, U2-11, U6-8, and U6-9, *Pleurotus eryngii* strain U8-11, *Schizophyllum commune* strain U6-7, and *Lentinula edodes* strain U6-11 and U6-12 were grown in malt extract agar with or without addition of 50 mg/L iron or 7.5 mg/L zinc. The mycelial biomass was separated and iron and zinc concentrations were determined in a flame atomic absorption spectrophotometer. Basidiomycete strains presented different growth rates with the presence of iron and zinc; there was no dependence between the metal bioaccumulation and the fungal growth. The fungi presented greater capacity to bioaccumulate iron than zinc. *P. ostreatus* (U2-9) has greater iron bioaccumulation (3197.7 mg/kg) while *P. ostreatus* (U6-8) greater zinc bioaccumulation (440.4 mg/kg) in mycelial biomass. *P. ostreatus* (U2-9), *P. ostreatus* (U2-11), and *S. commune* (U6-7) had the highest metal translocation rates from the culture medium to mycelial biomass. The mycelial biomass enriched with iron or zinc is an alternative to a new functional food from non-animal sources.

Key words: Basidiomycota, bioaccumulation, edible mushroom, mycelial biomass.

INTRODUCTION

Basidiomycetes with biologically active compounds have been used as food and/or as dietary supplement (Wasser 2002, 2017). In 2010 *Pleurotus ostreatus* (Jacq.) P. Kumm. was the most produced basidiocarp in China (5 million t) followed by *Lentinula edodes* (Berk.) Pegler (3.5 million tons) (Yu 2012). *P. ostreatus* has anti-hypercholesterolemic (Bobek et al. 1991) and antidiabetic (Rathore et al. 2017) activities, and antitumor or immunostimulating polysaccharides (Wasser 2002). *L. edodes* has antibacterial (Ishikawa et al. 2001), immune-modulating (Dai et al. 2015), and antitumor - lentinan - (Rasmy et al. 2010, Gao et al. 2018) activities. *Pleurotus*

eryngii (DC.) Quél. has antioxidant, anti-hypertensive, and hipocholesterolemic (Abidin et al. 2017) activities and Japan has improved its production from 6700 t in 2000 to over 37000 t in 2009 (Royse & Sánchez 2017). *Schizophyllum commune* Fr. produces hydrolytic enzymes with biotechnological potential (Tovar-Herrera et al. 2018), antioxidant and immunomodulatory activities and is used as an adjuvant tumor therapy with the antitumor polysaccharide schizophyllan (Lindequist et al. 2005, Sullivan et al. 2006, Asatiani et al. 2010, Wasser 2010).

The production of mycelial biomass is considered safer than basidiocarps because basidiocarps could accumulate pesticides and heavy metals from agroindustrial substrates

(Vieira et al. 2013, Yokota et al. 2016). The bioaccumulation of nutritional metals in the mycelial biomass from edible and medicinal basidiomycetes is an alternative of non-animal mineral source associated with functional and medicinal activities of the basidiomycetes. In addition, ion bioaccumulation is higher in mycelial biomass (Almeida et al. 2015) than basidiocarps (Yokota et al. 2016). Also, mycelial biomass cultivation could easily produce bioactive compounds in a shorter and controlled period than mushroom cultivation (Almeida et al. 2015). Thus, mycelial biomass is an alternative for bioaccumulation of nutritional and pharmaceutical metals such as zinc (Marcante et al. 2014), lithium (Faria et al. 2018, 2019), and iron (Almeida et al. 2015).

For basidiomycetes, the increase of metal concentration in the culture medium increases bioaccumulation capacity (Umeo et al. 2019). In addition, some metals can affect some compound yield such as copper that induces laccase activity (Bellettini et al. 2019, Valle et al. 2015), lithium that reduces mycelial biomass production (Faria et al. 2018, 2019), and iron that reduces mushroom production and antioxidant activity (Yokota et al. 2016). Thus, because addition of metals in culture medium could affect fungal metabolism, case-by-case studies are necessary for the production of fungal biomass and the ion bioaccumulation with the basidiomycete of nutritional and pharmaceutical interest.

Iron and zinc are essential metals for animal and human health and are important for their participation in the metabolic processes (Dunn et al. 2007). The need for iron intake doubles at six years of age from 0.9 mg/day to 1.8 mg/day, increasing even more during puberty (WHO/FAO 2004). Usually, iron deficiency is followed by the lack of other micronutrients such as zinc (Allen et al. 2006). Therefore, in order to

provide alternative food and/or supplement bioaccumulated with metals it is necessary to search for edible and medicinal basidiomycetes with this capability.

Our study aimed to evaluate the iron or zinc bioaccumulation in mycelial biomass of different basidiomycete species and strains cultivated in liquid malt extract medium added of iron or zinc. The mycelial biomass enriched with minerals could be added to breadsticks (Proserpio et al. 2019) and/or cookies in order to obtain functional foods with high nutritional values (Cornelia & Chandra 2019).

MATERIALS AND METHODS

Biological material

Eight basidiomycete strains of *Lentinula edodes* (U6-11, U6-12), *Pleurotus eryngii* (U8-11), *Pleurotus ostreatus* [U2-9 (also INCQS 40358), U2-11, U6-8, and U6-9], and *Schizophyllum commune* (U6-7) from the Culture Collection of the Molecular Biology Laboratory of Paranaense University were utilized. Cryopreserved mycelia were recovered in 20 g/L malt extract agar (MEA) medium (autoclaved at 121 °C for 20 min) in the dark at 25 ± 1 °C (Mantovani et al. 2012, Linde et al. 2018). Mycelia showing homogenous growth without sectoring were selected as inoculum.

Determination of mycelial biomass

The solid cultivation medium consisted of MEA added with 50.0 mg/L iron from a FeSO₄ solution (Almeida et al. 2015) or 7.5 mg/L zinc from a ZnSO₄ solution (Marcante et al. 2014). MEA without ion addition was used as control. MEA was autoclaved (121 °C for 20 min) and FeSO₄ or ZnSO₄ solutions were previously filtered (0.22 µm diameter pore) and homogenized in autoclaved MEA just before poured into Petri dishes (90 mm). One MEA disk (5 mm diameter) containing mycelia was placed in the middle of

the cultivation medium as inoculum and kept for 28 days at 28 ± 1 °C in the dark.

Colonized cultivation medium was heated up in a microwave oven for 30 s to easily remove the mycelial biomass from the surface of the cultivation medium. The mycelial biomass was transferred to a 500 mL becker containing 200 mL ultrapurified water at 80 °C, and the mixture stirred vigorously for 5 min to remove any residual cultivation medium. Next, the mycelial biomass was separated by centrifugation at 2900 g for 15 min at 4 °C. This process was carried three times. The precipitate biomass was transferred to a 50 mL becker containing 20 mL nitric acid (10%) to remove metals adhered to the mycelial biomass surface. The mixture was stirred vigorously for 5 min, the mycelial biomass separated by centrifugation at 2900 g for 15 min at 4 °C and the precipitated dried at 60 °C until constant mass. Arithmetical mean was calculated from ten replicates to obtain the mycelial biomass (dry basis).

Determination of metals in the mycelial biomass

For ion extraction, 67% HNO₃, 1:12 (mass:volume), was added to dehydrated mycelial biomass and kept at 22 ± 2 °C for 72 h. The mixture was heated at 100 °C and 30% H₂O₂, 1:6 (mass:volume), was added until complete solubilization. The mixture was filtered (14 µm porosity filter paper), washed and the volume adjusted to 10 mL with ultrapurified water (Yokota et al. 2016). The ion concentration of samples was determined by flame atomic absorption spectrophotometry (GBC model 932 plus). For ion quantification, a calibration curve ($R^2 = 0.998$) was used with analytical standard certified and traceable (IAA-203 Lot K00461 ultra scientific analytical solutions) with 0.01 µg mL⁻¹ ion detection limit. In addition, for each batch of analyzes, an internal standard was placed without the analytical

ion and with the certified element standard. Arithmetical mean was calculated from ten replicates to obtain the metal bioaccumulation in mycelial biomass (dry basis).

Mycelial biomass grown difference between MEA with and without metal addition was calculated by subtracting mycelial biomass grown in MEA without metal addition of mycelial biomass grown in MEA with metal addition, dividing the result per mycelial biomass grown in MEA without metal addition and multiplying by 100 (dry basis).

Metal bioaccumulated in mycelial biomass was calculated by dividing metal bioaccumulated in mycelial biomass grown in MEA with metal addition per metal bioaccumulated in mycelial biomass grown in MEA without metal addition and multiplying by 100 (dry basis).

Translocated metal yield from the cultivation medium (Petri dish) to the mycelial biomass was determined by multiplying the mycelial biomass grown in MEA with metal addition per the metal bioaccumulated in mycelial biomass grown in MEA with metal addition and dividing by 1000 (dry basis).

Statistical analysis

The experimental design was completely randomized, arithmetical mean, and standard deviation were calculated, and the differences determined by Scott-Knott test ($p \leq 0.05$) utilizing Sisvar software, version 5.6.

RESULTS

Iron addition to the cultivation medium reduced the mycelial biomass production in 68.7% (*L. edodes* U6-12) and 15.0% (*P. ostreatus* U6-8) (Table I). However, among *P. ostreatus* strains, the reduction of the mycelial biomass production ranged from 64.1% (*P. ostreatus* U2-9) to 15.0%

(*P. ostreatus* U6-8) (Table I). Thus, when iron was present in the cultivation medium a great variation on the mycelial biomass production occurred within the species and strains. Iron bioaccumulation capacity in the mycelial biomass varied from 467.9 mg/kg (*L. edodes* U6-11) to 3197.7 mg/kg (*P. ostreatus* U2-9); when compared to the control, the addition of iron in the MEA increased the iron bioaccumulating at 6700% for *L. edodes* U6-11 and 36000% for *P. ostreatus* U2-9 (Table I). Among *P. ostreatus* strains the iron bioaccumulation presented variation of 20% and between *L. edodes* strains the variation was of 74%. Thus, the iron bioaccumulation was species and strain dependents.

Translocated iron yield ranged from 17.7 mg per Petri dish (*L. edodes* U6-11) to 152 mg per Petri dish (*P. ostreatus* U2-9, *P. ostreatus* U2-11) (Table I). *P. ostreatus* (U2-9 and U2-11) presented the greatest ($p \leq 0.05$) iron translocation yield among the strains, followed by *P. ostreatus* (U6-8 and U6-9) ($p \leq 0.05$) and then by *P. eryngii* (U8-11) and *S. commune* (U6-7) ($p \leq 0.05$), and finally by *L. edodes* (U6-11, U6-12) ($p \leq 0.05$) (Table I). In this case, the strain with the greatest yield was also the one that had the greatest iron bioaccumulation capacity (*P. ostreatus* U2-9) among the strains (Table I).

The addition of zinc to the cultivation medium altered the mycelial biomass production from

Table I. Translocated iron per Petri dish yield, iron in mycelial biomass, and mycelial biomass (dry basis) grown in malt extract agar with and without addition of 50.0 mg/L iron.

Species/ Strain	Mycelial biomass (mg/ dish)		Variation of mycelial biomass (%)	Iron in mycelial biomass (mg/kg)		Variation of iron in mycelial biomass (%)	Translocated iron yield (mg/dish)
	MEA+Fe	MEA		MEA+Fe	MEA		
<i>Pleurotus ostreatus</i> U2-9	47.96 ± 7.63b	133.77 ± 3.59a	-64.1	3197.68 ±200.38a	8.90 ± 0.14d	35929.0	152.3a
<i>Pleurotus ostreatus</i> U2-11	57.53 ± 2.37b	104.86 ± 22.57b	-45.1	2642.59 ±50.61c	9.04 ± 0.03d	29232.2	152.0a
<i>Schizophyllum commune</i> U6-7	80.50 ± 12.67a	154.57 ± 21.42a	-47.9	1165.73 ± 72.49e	19.43 ± 2.38a	5999.6	93.7c
<i>Pleurotus ostreatus</i> U6-8	48.87 ± 3.86b	57.47 ± 19.55c	-15.0	2562.68 ± 72.49c	15.77 ± 0.49b	16250.3	125.2b
<i>Pleurotus ostreatus</i> U6-9	46.87 ± 2.06b	77.85 ± 25.14c	-39.8	2882.29 ± 236.60b	13.73 ± 1.25c	20992.6	134.4b
<i>Lentinula edodes</i> U6-11	37.56 ± 7.57c	69.77 ± 11.38b	-46.2	467.86 ± 2379f	6.96 ± 0.21e	6722.1	17.7d
<i>Lentinula edodes</i> U6-12	32.52 ± 7.57c	104.00 ± 37.55b	-68.7	1814.07 ± 138.79d	4.12 ± 0.98f	44030.8	59.7d
<i>Pleurotus eryngii</i> U8-11	56.51 ± 7.20b	102.69 ± 19.58b	-45.0	1613.83 ± 107.03d	12.95 ± 1.00c	12462.0	90.7c

Equal letters in the same column do not differ significantly among them by Scott-Knott test ($p \leq 0.05$). MEA = malt extract agar without iron addition; MEA+Fe = malt extract agar added with 50.0 mg/L of iron.

-64.2% (*L. edodes* U6-12) to +7.2% (*P. ostreatus* U6-8) (Table II). *S. commune* (U6-7) practically did not present alteration in the mycelial biomass production compared to zinc addition (Table II). *P. ostreatus* (U6-8) increased the mycelial biomass with zinc addition however, another *P. ostreatus* strain (U2-9) presented reduction of 44.8% on the mycelial biomass growth (Table II). Thus, it was evident that zinc addition in the culture medium affected the mycelial growth depending on species and strains.

The zinc bioaccumulation in the mycelial biomass varied from 180.4 mg/kg (*L. edodes* U6-12) to 440.4 mg/kg (*P. ostreatus* U6-8) which represents approximately an increase of 450% and 1200% compared to each control, respectively (Table II). Translocated zinc bioaccumulation yield ranged from 5.5 mg per Petri dish (*L. edodes* U6-12) to 42.2 mg per Petri dish (*S. commune* U6-7) (Table II). *S. commune* (U6-7) presented the greatest zinc translocation yield ($p \leq 0.05$) among the strains, followed by *P. ostreatus* (U2-9, U2-11, U6-8, U6-9) and *P. eryngii* (U8-11) ($p \leq 0.05$), and finally by *L. edodes* (U6-11, U6-12) ($p \leq 0.05$) (Table II). Although *S. commune* (U6-7) had the greatest yield, *P. ostreatus* (U6-8) was the strain with greatest zinc bioaccumulation making evident that not always the strain that presents the greatest zinc bioaccumulation is the one that can offer the greatest yield of bioaccumulated ions. It suggests that is necessary to consider the capacity to produce mycelial biomass under the ion presence.

Overall, the addition of iron or zinc in the cultivation medium reduced mycelial biomass production (Tables I and II). The strain response to the presence of iron or zinc in the cultivation medium was depending of specie and strain. *P. ostreatus* (U2-9) and *P. ostreatus* (U2-11) were the strains with greater capacity to translocate iron from the cultivation medium to the mycelial biomass. The first one was able to bioaccumulate

a greater amount of iron despite the smallest mycelial biomass production; the second one had greater mycelial biomass production despite smaller bioaccumulation of iron (Table I). It is fundamental that the fungus can grow and produce mycelial biomass in the cultivation medium with high metal concentration, which results in ideal conditions for the high yield of metal bioaccumulation.

DISCUSSION

The strains of wood-decay fungi had distinct capacity to bioaccumulate iron or zinc. Our results demonstrated that *P. ostreatus* strains have greater capacity to bioaccumulate iron while *S. commune* bioaccumulates zinc more effectively. *P. ostreatus* has been utilized to produce mycelial biomass bioaccumulated with metals such as iron (Almeida et al. 2015, Yokota et al. 2016) and lithium (Faria et al. 2018). Basidiomycetes present physiological responses to the environment, generally strain-specific ones. Umeo et al. (2015) observed a high variability for the production of mycelial biomass, exopolysaccharides, and antioxidants among different species and even among strains of the same species, making it essential to evaluate the species and strains separately in order to identify fungi with biotechnological potential. This results are in accordance to our study where different strains of *P. ostreatus* showed different ($p \leq 0.05$) mycelial biomass production and iron or zinc bioaccumulation capacity.

Besides the capacity to bioaccumulate zinc, *S. commune* is also able to produce several enzymes (Asgher et al. 2016) which can explain its high strength to produce mycelial biomass even at high iron or zinc concentrations as shown in our study. Moreover, this fungus is a

Table II. Translocated zinc per Petri dish yield, zinc bioaccumulated in mycelial biomass, and mycelial biomass (dry basis) grown in malt extract agar with and without addition of 7.5 mg/L zinc.

Species/ Strain	Mycelial biomass (mg/ dish)		Variation of mycelial biomass (%)	Zinc in mycelial biomass (mg/kg)		Variation of zinc in mycelial biomass (%)	Translocated zinc yield (mg/dish)
	MEA+Zn	MEA		MEA+Zn	MEA		
<i>Pleurotus ostreatus</i> U2-9	73.89 ± 24.88b	133.77 ± 3.59a	-44.8	263.34 ±27.26c	28.21± 1.69a	933.5	19.5b
<i>Pleurotus ostreatus</i> U2-11	56.12 ± 7.43b	104.86 ± 22.57b	-46.5	322.43 ±11.25b	24.21 ± 5.93a	1331.8	18.1b
<i>Schizophyllum commune</i> U6-7	153.29 ± 33.34a	154.57 ± 21.42a	-0.8	277.22 ± 39.81c	33.51 ± 4.23a	827.3	42.2a
<i>Pleurotus ostreatus</i> U6-8	61.61 ± 7.07b	57.47 ± 19.55c	+7.2	440.376 ± 34.23a	37.06 ± 1.12a	1188.3	27.0b
<i>Pleurotus ostreatus</i> U6-9	59.31 ± 33.34b	77.85 ± 25.14c	-23.8	330.11 ± 50.08b	39.31 ± 5.27a	839.8	18.6b
<i>Lentinula edodes</i> U6-11	37.85 ± 6.11b	69.77 ± 11.38b	-45.8	294.83 ± 81.57c	50.30 ± 20.03a	586.1	12.6c
<i>Lentinula edodes</i> U6-12	37.20 ± 11.62b	104.00 ± 37.55b	-64.2	180.39 ± 10.67d	40.13 ± 7.50a	449.5	5.5c
<i>Pleurotus eryngii</i> U8-11	53.23 ± 3.35b	102.69 ± 19.58b	-48.2	360 ± 40.86b	38.16 ±7.33a	943.4	19.1b

Equal letters in the same column do not differ significantly among them by Scott-Knott test ($p \leq 0.05$). MEA = malt extract agar without zinc addition; MEA+Zn = malt extract agar added with 7.5 mg/L iron.

producer of the polysaccharide schizophyllan with antitumor and immune modulating activity (Smith et al. 2003), increasing the nutritional and functional importance of it when associated with metals bioaccumulated in the mycelial biomass.

Our study showed that the concentration of iron bioaccumulated in the mycelial biomass was higher than the one of zinc for all strains. Ebenso et al. (2013) verified that the metal concentration in commercially produced mushrooms of *P. ostreatus* ranged from 118.40 to 89.30 mg/kg iron, and from 23.41 to 16.44 mg/kg zinc. This indicates that these basidiomycetes have natural capacity to translocate greater amounts of iron than zinc. Almeida et al. (2015) reported that *P. ostreatus* mycelial biomass

grown in solid cultivation medium added of 250 mg/L iron, bioaccumulated up to 3616 mg/kg iron. This value is close to the one found in our study for *P. ostreatus* (U2-9) mycelial biomass of 3197.7 mg/kg iron.

Marcante et al. (2014) reported zinc bioaccumulation in *Agaricus subrufescens* Peck (U2-1) - also *Agaricus blazei* Murrill ss. Heinemann and *Agaricus brasiliensis* Wasser et al. - mycelial biomass in solid cultivation medium with addition of 7.5 mg/L zinc of 414 mg/kg. In our study, a similar result to zinc bioaccumulation of 440.4 mg/kg was obtained for *P. ostreatus* (U6-8) mycelial biomass grown in a cultivation medium added with 7.5 mg/L zinc. Matute et al. (2011) reported that *Ganoderma lucidum* mycelial biomass also accumulated

zinc in a dose-dependent manner and that zinc concentration was strongly inhibitory for mycelial biomass growth.

The greatest basidiomycete capacity to bioaccumulate iron compared to zinc can be related to the high iron demand in metabolic processes. Although iron is essential to fungal metabolism, at high concentrations it can release free radicals that cause enzymatic inhibition, displacement or substitution of ions, and membrane rupture that affect the fungal metabolism and reproduction (Gadd 2007). Moreover, an energetic orientation of the metabolism can occur to produce protective compounds to iron and, therefore, reduce the energy utilized for fungal growth (Haas 2003), which would explain the mycelial biomass growth reduction in the cultivation medium with addition of iron or zinc.

Despite abundant in nature, iron is normally found as an insoluble salt (Philpott 2006). Fungus developed several strategies to capture iron such as acidification of medium, reduction of ferric iron to ferrous form, and secretion of iron chelating molecules as hydroxamates (Haas 2003). Siderophores such as rhodotorulic acid, fusarinines, coprogens, and ferrichromes are high-affinity iron-chelating compounds secreted by fungi to transport iron across cell membranes and could catalyze oxygen reactive species that are harmful to cells (Haas 2003). Manoproteins contribute to the retention of siderophore-iron chelates in the cell wall and increase iron uptake by fungi. In addition, ferric iron-binding proteins are also capable of capturing iron (Philpott 2006). Thus, basidiomycetes have different molecules that bind with metals in order to bioaccumulate high concentrations of metals.

Considering that fungi are more efficient to concentrate metals or minerals than plants (Dursun et al. 2006), that iron and zinc are essential metals in animal and human health

(WHO/FAO 2004, Allen et al. 2006, Dunn et al. 2007), and that the main food sources consumed by the population have low iron (Blanco-Rojo & Vaquero, 2019) and zinc (Peres & Koury, 2006) concentration, the mycelial biomass bioaccumulated with these metals could be an alternative functional food with benefits against metal nutritional deficiencies. Comparing our results to beans, which are daily consumed in Brazil, 1 g iron or zinc bioaccumulated mycelial biomass has 32-fold more iron and 22-fold more zinc than 1 g bean (Tan et al. 2008).

CONCLUSIONS

Strains and species of basidiomycetes have distinct responses to the presence of iron or zinc in the cultivation medium and there is no dependence between the metal bioaccumulation capacity and mycelial biomass production. In general, there is a greater capacity to bioaccumulate iron than zinc. *P. ostreatus* (U2-9) has greater iron bioaccumulation (3197.7 mg/kg) while *P. ostreatus* (U6-8) presents greater zinc bioaccumulation (440.4 mg/kg) in mycelial biomass. Also, *P. ostreatus* (U2-9) and *P. ostreatus* (U2-11) have the highest yield to translocate iron (152 mg per Petri dish) from the cultivation medium to the mycelial biomass and *S. commune* (U6-7) has the highest yield to translocate zinc (42 mg per Petri dish) from the cultivation medium to the mycelial biomass. The mycelial biomass enrichment with iron or zinc is an alternative for the development of new functional food and/or food supplement from a non-animal source.

Acknowledgments

The authors thank Universidade Paranaense, Graduate Program in Biotechnology Applied to Agriculture (Probiot), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) -finance code 001-

and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the financial support and fellowship

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How to cite

UMEO SH, FARIA MGI, DRAGUNSKI DC, VALLE JS, COLAUTO NB & LINDE GA. 2020. Iron Or Zinc Bioaccumulated In Mycelial Biomass Of Edible Basidiomycetes. *An Acad Bras Cienc* 92: e20191350. DOI 10.1590/0001-3765202020191350.

*Manuscript received on November 1, 2019;
accepted for publication on March 6, 2020*

SUZANA H. UMEO¹

<https://orcid.org/0000-0003-2895-0123>

MARIA GRACIELA I. FARIA¹

<https://orcid.org/0000-0001-5363-2894>

DOUGLAS C. DRAGUNSKI²

<https://orcid.org/0000-0001-7870-097X>

JULIANA S. DO VALLE¹

<https://orcid.org/0000-0002-9463-5378>

NELSON B. COLAUTO¹

<https://orcid.org/0000-0003-4390-8302>

GIANI ANDREA LINDE¹

<https://orcid.org/0000-0003-1220-2032>

¹Universidade Paranaense, Laboratório de Biologia Molecular, Programa de Pós-graduação em Biotecnologia Aplicada à Agricultura, Praça Mascarenhas de Moraes, 4282, 87502-210 Umuarama, PR, Brazil

²Universidade Estadual do Oeste do Paraná, Centro de Engenharias e Ciências Exatas, Rua da Faculdade, 645, 85903-000 Toledo, PR, Brazil

Correspondence to: **Maria Graciela Iecher Faria**

E-mail: gracielaiecher@prof.unipar.br

Author contributions

Paper conceived, designed and written by: Umeo, SH; Faria, MGI; Linde, GA. Acquisition, analysis and interpretation of data; drafting of the manuscript: Umeo, SH; Faria, MGI; Dragunski, DC; Valle, JS; Colauto, NB. Critical revision of the manuscript for important intellectual content: Colauto, NB. Coordinating the research project: Linde, GA.

