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Full Length Article

The Effect of Endophytes on the Oxidation Resistance of Black Rice

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Abstract

Rice endophytes can produce enzymes such as cellulase which improve quality, increase yield, enhance nitrogen fixing capacity and improve resistance. This study aimed to improve the antioxidant capacity of black rice by spraying rice with endophytes at the heading stage. The results indicated that there were significant differences in the effects of different endophytes on the antioxidant capacity of black rice and the effects of endophytes on antioxidant capacity varied amongst different rice varieties. Eight endophytes were found to significantly improve the total antioxidant capacity (T-AOC) of the black rice variety "1C902". The highest increase in antioxidant capacity was 6.3 times, with an increase of 832.838 IU g^{-1}. Six strains were found to significantly improve hydroxyl radical-scavenging capacity (SFRC) of black rice "1C902". The highest increase in SFRC was 1.3 times, with an increased amount of 1855.348 IU g^{-1}. Three strains were found to significantly increase T-AOC in black rice "1C903". The highest increase in T-AOC was 1.1 times, with an increased amount of 946.455 IU g^{-1}. Four strains were found to significantly increase SFRC of black rice "1C903". The highest increase in SFRC was 82.1%, with an increased amount of 848.189 IU g^{-1}. © 2020 Friends Science Publishers

Keywords: Fungi; Cytokinin; Oxidation resistance

Introduction

Plant endophytes refer to fungi or bacteria that live in plants (Bacon and White 2016), which are usually distributed in the interstitial space or inside the cells of the plant. Colonized in different tissues of the plant, plant endophytes can often act on the entire plant body (Hurek *et al*. 1994; Barrow 2003; Verma *et al*. 2004). Most endophytes and host plants have a mutually beneficial and symbiotic relationship. Although endophytes rely on the nutrients of the plant host for survival, most endophytes will not cause harm to the host plant, but can promote the growth of the host plant (Ali *et al*. 2017; Jaber and Enkerli 2017). Plant endophytes can secrete and produce substances such as plant growth hormone, cytokinin and gibberellin. Furthermore, endophytes can fix nitrogen and dissolving phosphorus, helping plants resist environmental stress (Montañez *et al*. 2012; Truyens *et al*. 2015). Different endophytes produce different effects on plants. Certain endophytic fungi will produce toxic compounds like alkaloids, terpenes, polyketones, phenols, which act to protect plants from insects and pathogenic bacteria. For example, the neurotoxic indole-diterpenoid alkaloids produced by fungi can poison herbivorous insects that ingest them (Siegel *et al*. 1990). Some endophytic bacteria can produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which can reduce the content of ACC and 2-oxobutanoat in plants, inhibiting the synthesis of ethylene and delaying plant senescence (Hardoim *et al*. 2015).

A variety of endophytic florae are distributed in rice (Walitang *et al.* 2017). Zhang *et al*. (2019) used the simple sequence repeat method to study genetic diversity in five rice varieties with different genotypes and used a 16S rRNA gene bank to analyze genetic differences of endophytic flora of rice seeds. They found that endophytic abundance distributions were significantly different. Walitang *et al.* (2018) crossbred different rice seeds under different growth environments and carried out multiple generations of repeated inbreeding to study the differences in endophytic communities. The endophytic community composition was highly stable and did not significantly change over time. They then used end-restriction fragment length polymorphism analysis to evaluate the distribution of endogenous bacterial communities, and found significant differences in the diversity of endogenous bacteria between

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the primary generation and the progeny in terms of richness, uniformity, and diversity index.

When plants undergo oxidative stress, they produce a large number of oxidative intermediates, ultimately causing oxidative damage to plants (Yuan *et al*. 2019). Plants can resist stress conditions by using enzymatic and non-enzymatic antioxidant defense mechanisms. Endophytes enhance the defensive capability of host plants by contributing exogenous antioxidant enzymes and non-enzymatic antioxidants. These antioxidants help avoid excessive accumulation of reactive oxygen species (ROS) in plants, reducing oxidative damage that occurs during biotic and abiotic stress (Khan *et al*. 2013). Indeed, Qin *et al*. (2019) isolated 12 endophytic fungi from the roots and leaves of *Myricaria laxiflora*, and found that *Aspergillus fumigatus* strain SG-17 exhibited a strong antioxidant capacity both *in vivo* and *in vitro*. The main antioxidant in *A. fumigatus* is (Z)-N-(4-hydroxystyryl) formamide (NFA), an analogue of coumarin. Plant growth under four different conditions were observed: drought, humid, drought+SG-17 crude extract and drought + proline. Qin *et al*. (2019) found that plants in drought+SG-17 crude extract group could grow normally. The reason is that *Aspergillus fumigatus* can maintain the integrity of plant cell membranes and regulate the content of NADPH oxidase, antioxidant enzymes and Heat Shock Protein (HSP). Where, NADPH oxidase can directly reduce the production of ROS, antioxidant enzymes and HSP can promote the degradation of ROS. Ullah *et al*. (2019) isolated two endophytic strains from *Solanum nigrum*, identified them as *Serum bacillus* IU01 and enterobacteria IU02 by 16S DNA sequencing, and then placed *Brassica juncea* plants inoculated with *Serum bacillus* strains IU01 or IU02 in environments with different Cd concentrations. The results showed that the activities of antioxidant enzymes such as catalase (CAT), polyphenol oxidase (PPO) and peroxidase (POD) in plants exposed to different concentrations of Cd stress were significantly increased when endophytes were not inoculated. However, the antioxidant enzyme activities in plants exposed to different concentrations of Cd stress were significantly reduced and plant tolerance to Cd and antioxidant capacity were significantly improved after inoculation of IU01 and IU02, suggesting that endophytes can reduce the damage of Cd stress on plants.

At the present, most studies on black rice have targeted bioactive substances such as anthocyanins and flavones (Pal *et al*. 2019). There are few studies about the effects of endophyte antioxidant activity on black rice (Verma *et al*. 2017; Greetatorn *et al*. 2019). Furthermore, the endophytic communities have yet to be characterized for black rice with high nutritional value. In previous studies, 8 endophytes (including 5 fungi and 3 bacteria) have been isolated from black rice seeds. In this paper, we aimed to study the effects of these 8 endophytes on the antioxidant capacity of black rice.

Materials and Methods

Experimental materials

Five types of endophytic fungi in black rice (POB15-1, POB15-11, POB15-15, POB14-7, POB14-10), 3 types of endophytic bacteria in black rice (NOB14-2, NOB15-2, NOB15-9), isolated from black rice seeds and kept separately in the laboratory, were used in this study. Two black rice varieties "1C902" and "1C903", planted in a rice test field in Zhaoan County, Fujian Province, China, were used in this study. Beef extract peptone (NA) medium (pH 7.0) was comprised of 3 g L^{-1} of beef extract, 10 g L^{-1} of peptone, 5 g L^{-1} of sodium chloride, and 15 g L^{-1} of agar.

Preparation of endophytes

After activation with the Potato Dextrose Agar (PDA) medium, the 5 types of endophytic fungi were cultured in a 28°C incubator for 7 d, and the fungus lawn was washed with sterile water to dilute the spore fluid concentration to 3×10^6 CUF mL^{-1}. After activation with the NA medium, the 3 types of endophytic bacteria were cultured in a 37°C incubator for 1 d, and the bacterial lawn was washed with sterile water to dilute the bacterial solution concentration to 3×10^6 CUF mL^{-1}.

Endophyte spraying experiment

Black rice varieties "1C902" and "1C903" were planted *via* single seed transplant. Endophytes were sprayed at the heading stage of rice, mainly on rice ears and leaves. Each microbial inoculum was sprayed on three plants of each rice variety, and the same type of rice without endophyte spraying was used as a control. After harvesting, the rice seeds were dried in a 32°C drying oven, and then processed into brown rice, crushed and sifted with an 80-mesh sieve for further use.

Determination of total antioxidant capacity and hydroxyl radical-scavenging capacity

Exactly 0.1 g of black rice flour was accurately weighed, 10 mL of 80% ethanol was added, and then the solution was diluted to 10 mL after digestion in a water bath at 80°C for 3 h. The solution was shaken well and then left to stand. The supernatant was stored for later use. Total Antioxidant Capability (T-AOC) and Hydroxyl Radical-Scavenging Capacity (SFRC) of black rice were measured using test kits (Nanjing Jiancheng Bioengineering Research Institute Co., Ltd., China), according to instructions. Each measurement was repeated 3 times.

Statistical analysis

The recorded data in this study were statistically analyzed using DPS data processing system.

Results

Effects of spraying endophytes on black rice T-AOC

From Fig. 1 and Table 1, it can be seen that spraying all 8 kinds of endophyte greatly increased the T-AOC of black rice variety "1C902". Increase rate was as follows: 1C902-POB15-15>1C902-POB14-10>1C902-POB15-1>1C902-NOB15-2>1C902-POB15-11>1C902-NOB14-2>1C902-POB14-7>1C902-NOB15-9. The strains POB15-15, POB14-10, POB15-1 and NOB15-2 were found to increase the T-AOC of black rice variety "1C902" by 6.3, 5.6, 4.9 and 4.0 times, respectively, increasing by 832.838, 731.584, 648.786 and 529.187 IU g^{-1}, respectively. These four endophytes had an excellent effect on improving the T-AOC of the black rice variety "1C902", each of which increased the T-AOC by more than 4 times. Furthermore, after spraying the three strains of NOB15-2, POB15-11 and NOB14-2, although there were no significant differences on T-AOC of black rice variety "1C902" (Fig. 1), there were very significant differences between T-AOC in the other five strains.

After spraying 8 kinds of endophytes on black rice variety "1C903", there were very significant differences in the effect on T-AOC (Fig. 2 and Table 1). 3 types of endophytes significantly increased the T-AOC of black rice variety "1C903". Increase rate was as follows: POB15-15> POB14-7> POB14-10. There was a relative increase of 110.2%, 69.6% and 19.7% for respectively, with T-AOC increasing by 946.455, 597.738 and 169.549 IU g^{-1}, respectively. After spraying NOB15-9, no significant difference was observed in the T-AOC and CK of black rice variety "1C903". Furthermore, the other four endophytes (POB15-1, POB15-11, NOB14-2 and NOB15-2) lowered the T-AOC of black rice variety "1C903". That is, spraying these four endophytes was unfavorable for T-AOC of black rice variety "1C903".

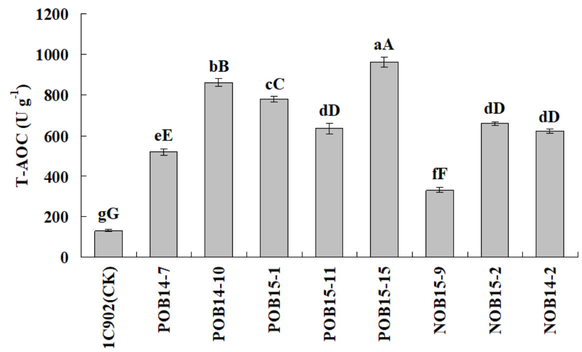

Fig. 1: T-AOC of black rice "1C902" treated with 8 entophytes
Different lower- or upper-case letters in each treatment indicate significant differences ($P < 0.05$ or $P < 0.01$), the same below

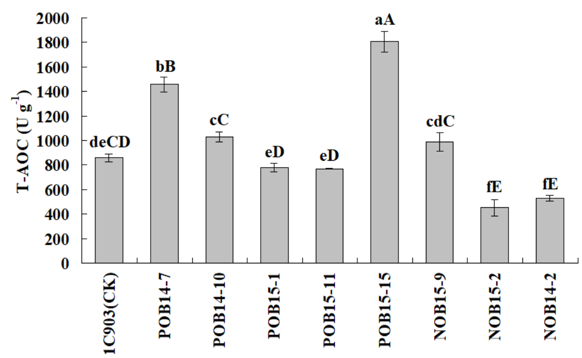

Fig. 2: T-AOC of black rice "1C903" treated with 8 entophytes

Table 1: Variance analysis of T-AOC and SFRC in black rice "1C902" or "1C903" treated with 8 entophytes

Trait	df	Mean squares			
		T-AOC of "1C902"	T-AOC of "1C903"	SFRC of "1C902"	SFRC of "1C903"
Between groups	8	201145.3***	558778.8***	1533621.6***	657858.5***
Within group	18	262.8	2820.3	7879.7	2779.0
Total variation	26				

***, significant at $P \leq 0.001$; ns: Non significant

Effects of spraying endophytes on black rice SFRC

After spraying 8 different kinds of endophytes, there were very significant differences in the SFRC of black rice variety "1C902" (Fig. 3 and Table 1). Where, 6 strains were found to significantly increase the SFRC of black rice variety "1C902", as follows: NOB15-2>POB15-11>NOB14-2>POB14-10>POB15-15>NOB15-9. The first four strains had increase rates above 50%, the highest of which was 1.3 times, showing increase amounts of 1853.348, 1069.956, 862.725 and 807.013 IU g^{-1}, for respectively. These four endophytes significantly improved the SFRC of black rice variety "1C902". However, spraying the strains POB14-7 and POB15-1 were found to lower the SFRC of black rice variety "1C902", indicating that the spraying of these two endophytes is unfavorable for this rice variety.

Spraying 8 kinds of endophytes had significantly different effects on SFRC of black rice "1C903" (Fig. 4 and Table 1). Where, 4 strains can significantly increase SFRC of black rice "1C903", whose increase rate follows the order of: POB15-1> NOB15-2> POB15-11> NOB14-2. The first three strains have an increase rate of above 40%, the highest of which is 82.1%, and the increase amounts are 848.189, 566.398 and 444.608 IU g^{-1}, respectively. These three endophytes were better for improving the SFRC of black rice variety "1C903" than all other varieties. Spraying the remaining 4 strains (NOB15-9, POB14-10, POB15-15 and POB14-7) were found to decrease the SFRC of the black rice variety "1C903", and spraying strain POB14-7 will significantly lower SFRC of black rice "1C903".

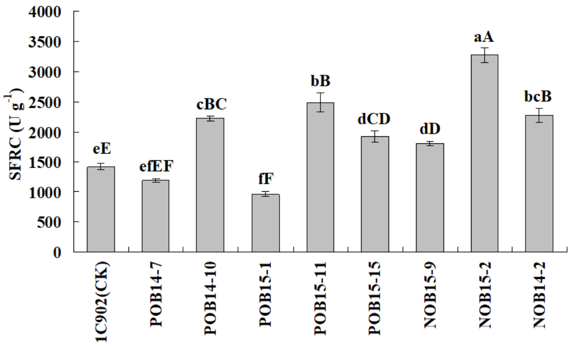

Fig. 3: SFRC of black rice "1C902" treated with 8 entophytes

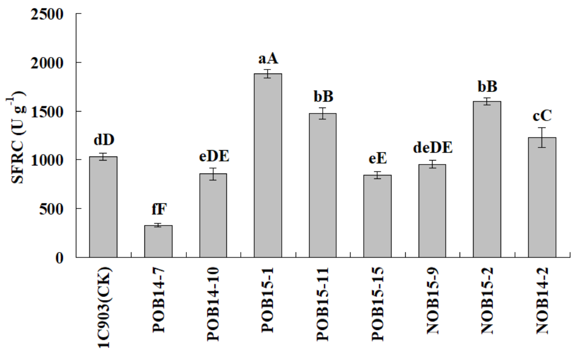

Fig. 4: SFRC of black rice "1C903" treated with 8 entophytes

Discussion

In this study, 8 endophytes were sprayed to two varieties of black rice at the heading stage, some of these endophytes obviously improved the T-AOC and SFRC of black rice. This indicated that spraying the black rice with endophytes can promote their production of antioxidants and their ability to resist stress.

The interaction between endophytes and host plants is primarily used for three purposes: 1) mitigation of the abiotic stress of host plants (such as drought and heavy metals), 2) protection of host plants against the threat of biotic stress (pathogens and herbivores), and 3) promotion og the growth of host plants by adding inorganic substances such as nitrogen, phosphorus and iron (Sessitsch *et al*. 2012).

Plant endophytes can produce antioxidants such as PPO, POD, CAT and superoxide dismutase (SOD), which can help host plants improve their ability to resist stress (Ullah *et al*. 2019). In addition to exogenous antioxidant enzymes, endophytes can also produce some non-enzymatic antioxidants, such as polysaccharides (Chen *et al*. 2011), and lectins that can regulate the activity of antioxidant enzymes (Alen Kina *et al*. 2018). Chen *et al*. (2011) isolated a exopolysaccharides homogenate (As1-1) consisting of mannose and a small amount of galactose from the medium of mangrove endophytic fungus *Aspergillus* Y16. Through *in vitro* experiments, they found that like vitamin C and other antioxidants, As1-1 could remove DPPH free radicals and superoxide radicals. Alen kina *et al*. (2018) studied the ability of two lectins (Sp7 and Sp245) produced by *Azospirillum brasilence* to regulate the activities of POD, CAT and SOD in wheat seedling roots under short-term, low and high temperature environmental stress conditions. Results demonstrated that these two lectins could significantly change the activities of related enzymes within the first few minutes under stress conditions. Both lectins could increase the activities of POD and SOD in wheat roots under stress, thereby enhancing the wheat's adaptability to severe environments.

Although the endophytes could improve the antioxidant capacity of black rice, there is no static law concerning the effects of endophytic bacteria and endophytic fungi on the antioxidant properties of rice. Indeed, the effects of endophytes on antioxidant properties of different varieties of rice appear to vary. This is similar results found by Afridi *et al*. (2019), who planted two varieties of wheat (Khirman and Pasban 90) under salt stress and inoculated endophytes the *Kocuria rhizophila* (14ASP) and *Cronobacter sakazakii* (OF115). They found that Pasban 90 had significantly higher antioxidant activity (POD, SOD and CAT) than Khirman, indicating that the effects of endophytes on the antioxidant properties of different wheat varieties differ.

Spraying different endophytes to black rice had different variation in T-AOC and SFRC in this study. After spraying with the 8 endophytes, the T-AOC of black rice variety "1C902" was greatly improved, the highest increase of which was 6.3 times. However, the black rice variety "1C903" had less of an increase in T-AOC than variety "1C902", as the highest increase was only 1.1 times. Furthermore, 4 kinds of endophytes reduced the T-AOC of black rice variety "1C903" after spraying. The reason for this may be that black rice variety "1C903" has much higher T-AOC (859.063 IU g^{-1}) than "1C902" (130.589 IU g^{-1}). Therefore, the improvement effect of T-AOC after spraying endophyte is obviously inferior in the former, and T-AOC may even be reduced. SFRC of black rice also exhibits a similar response pattern. Except for when strain POB15-1 was applied, black rice "1C902" generally had higher increase in SFRC than "1C903" after endophyte spraying. This demonstrates two things. First, different endophytes have significantly different effects on the antioxidant capacity within the same rice variety. Second, the same endophyte can have very different effects on the antioxidant capacity in different varieties of rice.

Conclusion

Spraying endophytes to black rice at the heading stage can improve the antioxidant capacity of black rice. But there are significant differences in the effects of different endophytes on the antioxidant capacity of black rice and there are significant differences in the effects of endophytes on the antioxidant capacity of different rice varieties. In this study, eight endophytes were found to significantly increase the T-AOC of the black rice variety "1C902" (up to 6.3 times higher than in untreated rice). Furthermore, 6 strains could significantly increase the SFRC of the black rice variety "1C902" (up to 1.3 times higher than in untreated rice). Three strains could also significantly increase the T-AOC of the black rice variety "1C903" (up to 1.1 times higher than in untreated rice). Finally, 4 strains could significantly increase SFRC of the black rice variety "1C903" (up to 82.1% higher than in untreated rice). So, in order to improve the antioxidant capacity of different variety of black rice, different endophytes should be chosen to infect it.

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Author Contributions

JZ and ZH designed the study; YX and YZ performed the experiments, conducted the analysis, and wrote the paper; QW assisted in analyzing the data and interpreted the results; XC assisted in performing the experiments. All authors read and approved the final manuscript.

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