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Research article

Differences in the arbuscular mycorrhizal fungi-improved rice resistance to low temperature at two N levels: Aspects of N and C metabolism on the plant side

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ABSTRACT

We performed an experiment to determine how N and C metabolism is involved in the low-temperature tolerance of mycorrhizal rice (*Oryza sativa*) at different N levels and examined the possible signaling molecules involved in the stress response of mycorrhizal rice. Pot cultures were performed, and mycorrhizal rice growth was evaluated based on treatments at two temperatures (15 °C and 25 °C) and two N levels (20 mg pot^{-1} and 50 mg pot^{-1}). The arbuscular mycorrhizal fungi (AMF) colonization of rice resulted in different responses of the plants to low and high N levels. The mycorrhizal rice with the low N supplementation had more positive feedback from the symbiotic AMF, as indicated by accelerated N and C metabolism of rice possibly involving jasmonic acid (JA) and the up-regulation of enzyme activities for N and C metabolism. Furthermore, the response of the mycorrhizal rice plants to low temperature was associated with P uptake and nitric oxide (NO).

1. Introduction

Arbuscular mycorrhizae (AMs) are among the most widely distributed symbioses throughout the world. More than 80% of terrestrial vascular plants can form an arbuscular mycorrhiza (fungus + root) with arbuscular mycorrhizal fungi (AMF) [1]. A large number of arbuscular and vesicular structures are formed during the colonization of root cortex cells by AMF, aiding the host plant in the uptake of various types of nutrients, such as N, P, and S, through extensive fungal mycelium networks in the soil [2] and improving the stress tolerance of the plants. The host plant, in turn, transfers carbon in the form of photosynthate to AMF to support the fungal life cycle [3].

N is important for the growth of plants because it is one of the basic elements for the synthesis of enzymes and chlorophyll and also because of its direct involvement in cell division and growth. In fact, almost 20–50% of the total N taken up by mycorrhizal roots is due to the uptake of AMF through their vast mycelia in the soil [4]. Furthermore, the colonization and growth of AMF in the soil is often correlated to the N status, whereby high N may inhibit the colonization of AMF in roots and their growth in the soil [5]. In addition, the extent of N transferred from AMF to the host is largely dependent on the status of stored N in the host plant. The symbiotic transfer of N is often more notable in the oligotrophic condition, which suggests that mycorrhizae most likely act as a N sink rather than contributing to N transfer from AMF to the host plant when there is only sufficient N available for the plant [6].

The transfer and metabolism of N in the arbuscular mycorrhizal symbiosis is accompanied by the biosynthesis and flux of carbon from the host plant to AMF. The root cells colonized with AMF are both sites of energy flow and nutrient cycling of the symbiont and the major C storage sink in the soil [3]. In the AM symbiosis, sugar is produced by photosynthesis and transferred to AMF to support their growth. This flux of sugar also acts as a signal to regulate the

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Abbreviations: AMF, arbuscular mycorrhizal fungi; AMs, arbuscular mycorrhizae; AOC, allene oxide cyclase; AOS, allene oxide synthase; CAT, catalase; ERM, extraradical mycelium; GS, glutamine synthetase; IRM, intraradical mycelium; JA, jasmonic acid; MDA, methane dicarboxylic aldehyde; NO, nitric oxide; NR, nitrate reductase; RE, N-use efficiency in recovery; POD, peroxidase; SOD, superoxide dismutase; SPS, sucrose phosphate synthase; SS, sucrose synthase.

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expression of genes involved in C metabolism at the transcriptional and post-transcriptional levels and is coupled with the transfer and metabolism of N in the symbiosis [7]. Carbon, typically in the form of hexoses, is released into the interfacial apoplast between the host plant cell and fungal intraradical mycelium (IRM) and is then transported into the fungal cells for the synthesis of such compounds as amino acids; this process is accompanied by the improved transfer of N from the fungi to the host plant. However, if carbon is absorbed by the fungi directly from the soil, enhanced N transfer and metabolism is not stimulated, as confirmed by gene expression, enzyme activity detection, and ^{15}N-based labeling experiments [7]. Therefore, the N and C metabolism in AMF plants appears to guarantee the maximum benefit for both symbionts to survive environmental stress.

In addition to improving nutrient absorption by the host plant, AMF also enhance the host plant's stress tolerance, including the resistance to low temperature [8]. Low temperatures and other abiotic stresses can damage plant cell membrane permeability due to the generation of reactive oxygen radicals, such as $OH\cdot$ and H_2O_2, which result from membrane lipid peroxidation [9]. AMF can improve the activity of such host plant antioxidant enzymes as catalase (CAT, EC 1.11.1.6), superoxide dismutase (SOD, EC 1.15.1.1), and peroxidase (POD, EC 1.11.1.7) to minimize the injury from reactive oxygen radicals resulting from low-temperature stress [8] and enhance the stress tolerance of the plant due to the change in water uptake through mycorrhizal mycelia [10]. The crosstalk between the transfer and metabolism of N and C in the symbiosis also plays an important role in stress tolerance. It has been suggested that the nitric oxide (NO) generated by nitrate reductase (NR) may act as a signaling molecule for the up-regulation of antioxidant enzyme activities, resulting in enhanced stress tolerance [11]. AMF improve host plant tolerance by modulating the soluble sugar content [8] and also improve themselves resistance by accumulating some metabolites, such as trehalose, which participate in the stress tolerance of AMF [12]. However, the metabolism of N and C and its association with low-temperature tolerance have not yet been clarified, even though this knowledge is necessary for understanding the underlying mechanisms of the stress tolerance of mycorrhizal plants.

Jasmonic acid (JA) generally fluctuates according to the process of N and C exchange in the symbiosis, and it is known that the colonization of various higher plants with AMF leads to increased endogenous JA levels in the root [13]. It has been postulated that the increase in JA during symbiosis is due to the osmotic stress caused by increased carbohydrate influx from the host shoot to the mycorrhizal root [13]. In addition to the AMF-affected JA levels via N metabolism, it has also been suggested that JA may affect mycorrhizal colonization in the host plant by modifying the expression of genes involved in carbohydrate metabolism [14]. Jasmonates have been implicated in the control of N partitioning, particularly the control of the mobilization and accumulation of the N reserves [15]. All these findings indicate the important role of JA in arbuscular mycorrhizal symbiosis. Furthermore, JA largely contributes to AMF-enhanced biotic and abiotic stress resistance, including pathogen, drought, low temperature, and salt tolerance. Therefore, we sought to ascertain whether JA has a positive effect on improving the low-temperature tolerance of mycorrhizal plants by participating N and C metabolism in the symbiosis, as this has not been investigated to date.

Rice (*Oryza sativa*) is one of the most important crops worldwide. However, rice is more sensitive to low-temperature stress in comparison to other cereals, with damage occurring even at approximately 15 °C [16]. Another crucial factor affecting rice growth and yield is N fertilization, the application of which is generally unempirical and exceeds the rice requirements, resulting in a negative effect on the global N cycle and constituting a major concern of scientists [17]. As a mycorrhizal crop, rice can obtain benefits from the symbiotic AMF [18]; thus, increasing the stress tolerance of rice through mycorrhizal inoculation is a promising topic for researchers. Accordingly, we performed an experiment based on different N supplementation and temperature treatments to elucidate whether AMF can improve rice resistance to low-temperature stress by accelerating the N and C metabolism and the corresponding responses to different N levels. The growth parameters of the host plant and AMF colonization were evaluated, the soluble sugar and total N, P, JA, and NO contents were assessed, and the activities of enzymes involved in N and C metabolism were measured.

2. Results

2.1. Effects of N supplementation and temperature treatment on the growth of rice plants and mycorrhizal colonization

Different N supplementation had various effects on rice growth (Tables 1 and 2). High N resulted in improved growth compared to the control, and the most significant increase was observed with the plants treated with high N at a normal temperature (25 °C). Compared to the non-mycorrhizal rice plants, AMF enhanced growth, resulting in increases in height, root length, and weight. In contrast, low temperature (15 °C) negatively affected rice growth, resulting in decreased height, root length, and weight of the above- and below-ground parts. In comparison to non-mycorrhizal rice, the mycorrhizal rice exhibited notable growth improvement, though the mycorrhiza-induced increases in shoot growth (MSR) and mycorrhiza-induced increases in root growth (MRR) were different between the low and high N levels. When the plants were colonized with AMF, MSR (Fig. 1A) and MRR (Fig. 1B) at low N were significantly higher than at high N; however, the MSR and MRR effects with high N reduced rapidly when the plants suffered from low-temperature stress, a situation that did not occur under the low N condition.

It was found that the different temperatures and N levels affected mycorrhizal colonization (Fig. 2): high N supplementation inhibited colonization compared to low N at both normal and low temperatures, though low-temperature stress decreased AMF colonization. Interestingly, as indicated by the colonization frequency (F%), the decrease in colonization was significant in the plants treated with high N, and the plants treated with low N could compensate for the negative effect of low temperature on AMF colonization (Fig. 2A). No difference in the arbuscular abundance (A %) was observed in our experiment (Fig. 2B).

Table 1

Effects of different N supplement and temperature treatment on the growth of rice. "AM" and "NM" represent mycorrhizal and non-mycorrhizal rice respectively, while the arbuscular mycorrhizal fungi (AMF) was *G. mosseae* 25 °C and 15 °C were set, and N1 and N2 represent the addition of N with 20 mg pot^{-1} and 50 mg pot^{-1} respectively. 4 treatments were as the following: NM N1, NM N2, AM N1, and AM N2. Values are means ± standard errors of five biological replicates. Different lowercase letters following values within a column represent significant difference ($P < 0.05$) based on Duncan's test. Means followed by the same letter are not significantly different.

Treatments		Plant height (cm)	Root length (cm)
25 °C	NM N1	26.44 ± 0.82 bc	8.75 ± 0.52 bc
	AM N1	27.50 ± 0.29 ab	9.19 ± 0.40 ab
	NM N2	29.06 ± 0.94 a	9.67 ± 0.33 a
	AM N2	29.11 ± 1.09 a	9.92 ± 0.55 a
15 °C	NM N1	26.25 ± 0.25 c	8.50 ± 0.35 c
	AM N1	27.50 ± 1.26 ab	8.94 ± 0.34 b
	NM N2	27.23 ± 0.42 b	9.58 ± 0.43 ab
	AM N2	28.25 ± 0.83 ab	9.67 ± 0. 47 a