Fungal endophytes of Himalayan Cold Desert Induces Heat tolerance in Rice (Oryza sativa L.)

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Abstract

The plants growing in cold desert of western Himalaya have inhabited diversified endophytes. These endophytes can provide fitness to plant under harsh environmental situations. In the current study, 22 fungal endophytes isolated from Artemisia and Xerophytic plants growing in the cold desert were screened for thermo-tolerance at different temperature ranges (28, 30, 32, 34, 36, 38 and 40°C) under in vitro. The only three isolates viz., A2, A7 and X5 exhibited growth up to 40°C and identified as *Penicillium funiculosum* (A2), *Ceriporia lacerate* (A7) and *Endomelanconiopsis endophytica* (X5) using ITS region. These endophytes inoculated to rice seedlings and exposed to elevated temperature (45°C) for 7hr per day for 10 days to study their effect on tolerance of rice to heat stress. The results revealed that endophytes inoculated seedlings showed sustained improvement in shoot and root growth. The *E. endophytica* was chosen to be the best endophyte to impart heat stress as per Fernandez model. This study suggested that cold desert endophytes could induce heat tolerance in plants.

Introduction

The global temperature increases day by day due to change in climate. Frequent heat waves have had serious impacts on rice production (Zhang and White, 2021). Historical data analysis envisaged that 7–8% of rice yield has been decreased due to raise in temperature to 1°C (Baker et al., 1992). International Rice Research Institute (IRRI) demonstrated that the field trials from 1992-2003 showed 10% yield reduction of rice for every raise in one degree of minimum temperature (Peng et al., 2004). High temperature affects all stages of rice plant starting from germination, growth, development, reproduction and yield (Krishnan et al., 2011). The tiller number decreased by 10% when temperature rise from 29/21°C to 37/29°C (Manalo et al., 1994). The synchronism between the emergence of main stem and tiller and also mobilization of nutrients among tillers were affected by high temperature resulting in decreased yield as primary tillers are directly proportional to grain yield in rice (Yoshida, 1981).

Cold deserts are found in high, flat areas, called plateaus, or mountainous areas in temperate regions of the world. Cold deserts have hot summers but extremely cold winters. The Western Himalayan cold deserts have extremes of hot and cold climate combined with excessive dryness. Soil has light grey, poor in fertility and less water holding capacity. Therefore, these desert plants develop some physiological mechanisms like CAM (Crassulacean acid metabolism), modified leaf and also take the advantage of microbial endophytes to survive in hostile environment (Zhang and White, 2021).

The endophytes can colonize the plant tissue without causing any apparent harm and provide fitness under hostile environment. Endophytes can be cultured *in-vitro* and transfer to compatible secondary plants to obtain similar benefits (Redman et al., 2002 and Wang et al., 2021). Endophytes isolated from cold deserts seems to adapt wider range of temperature as cold desert has influenced by fluctuated temperature ranges from -45°C in winter to 40°C in summer (Tewari and Kapoor, 2013). Therefore, we used in our study the endophytes isolated from the cold desert plants to understand induction of thermotolerance temperature sensitive rice variety IR-64.

Materials And Methods

**Screening for thermotolerance of endophytic isolates**

The fungal endophytes isolated from Artemisia and xerophytic plants of Western Himalayan cold desert and preserved at School of Ecology and Conservation Laboratory, University of Agricultural Sciences, Bangalore-560065. The 22 isolates were procured and rejuvenated on potato dextrose agar (PDA) for the present study. The endophytic isolates were screened for temperature tolerance. Isolates were cultured in PDA plates and incubated at different temperature (28°C, 30°C, 32°C, 34°C, 36°C, 38°C and 40°C) for five days. Fungal growth was measured by radial diameter of colony on fifth day of incubation.

**Molecular identification of thermotolerant endophytic isolates**

The endophytic isolates of genomic DNA were extracted by Cetyltrimethylammonium bromide (CTAB) method (Vainio et al., 1998). The internal transcribed spacer (ITS) region of genomic DNA was amplified using universal primer ITS1-F (5′- TCCGATTGAACCTGGG 3′) and ITS4-R (5′- TCTCCGGCTTAATGATGC 3′) by polymerase chain reaction (PCR). PCR amplification was performed using Master cycler (Eppendorf, Germany) with a 20μl reaction mixture that comprised 2μl 1X taq buffer with MgCl₂ (1.5mM), 2μl dNTP's (10mM), 0.5μl each primer (10pmol), 0.3μl Taq DNA polymerase (3U) and 1μl template DNA (100ng). The PCR was carried out with an initial denaturation at 94°C for 4min, followed by 35 cycles at 94°C for 30s, 55°C for 1min and 72°C for 30s, and a final extension at 72°C for 12min. The PCR amplified products were sequenced by SciGenome labs, Cochin, Kerala, India. The nucleotide sequences were queried in the NCBI GenBank database using a Basic Local Alignment Search Tool (BLAST). Sequences of each fungal species and corresponding reference sequences from GenBank were subjected to ClustalW analysis. The phylogenetic tree was constructed through maximum likelihood method and Tamura-Nei model, using MEGA X. The recognized sequences were placed in GenBank with accession number.

**Interaction of fungal endophytes with Rice under heat stress**

Evaluation of fungal endophytes on their ability to impart heat tolerance in rice (variety IR-64) was carried out in plant growth chamber at Indian Institute of Horticulture Research (ICAR-IIHR), Hesaraghatta, Bangalore. There were two sets of experiments. 1. Heat stress (45°C for 7hr per day for 10 days) and 2. Without heat stress (normal temperature conditions, 30±0.5°C). Each set comprised with following treatments. 1. Control (uninoculated plants) 2. *Ceriporia lacerate* 3. *Endomelanconiopsis endophytica* and 4. *Penicillium funiculosum*. Rice seeds were surface sterilized using 3 % sodium hypochlorite followed by 70 % alcohol. The surface sterilized seeds were repeatedly washed with sterile water and soaked for overnight. The pre-germinated seeds were sown in pots filled with soil and FYM (1:1w/w). Three seedlings per pot were maintained and grown for fifteen days. The thermotolerant endophytes were inoculated by stem prick method (Bhunjun et al., 2020) and allowed to colonize for 10 days. After colonization, set-1 seedlings were exposed to heat (45°C) for 10 days in growth
stress. Endophytes colonized plants which lead to improved absorption of nutrients and water from soil, resulting in a more vigorous plant and helps to cope of heat stress. The root system plays a vital role in adaptation of whole plant under heat stress (Huang et al., 2017). Endophyte had significantly higher tillers number, dry weight, leaf length and wet weight under drought condition (Jajarmi et al., 2016). This envisaged that these three isolates could sustain heat stress it might be the cold desert of Western Himalaya had extreme hot climate (40°C) during summer (Tewari and Kapoor, 2013). These endophytes were identified using ITS region of rDNA as Epichloë sp. infected rye grass produced more tillers than uninfected plants. The endophyte Penicillium funiculosum colonized plants found superior in increasing plant height, number of tillers and leaves, root volume, fresh and dry weight of shoot and root in normal growth condition. Whereas under stress condition, the E. endophytica and P. funiculosum colonized plants showed significantly (P < 0.01) higher shoot and root growth parameter compared to C. lacerate. The un-inoculated plants produced least growth of rice.

### Categories of treatments based on their performance in normal and stress conditions

The treatments were divided into four categories based on Fernandez (1992) model using stress tolerance index of biomass. The treatment E. endophytica inoculated plants belongs to group A that indicates the production of higher biomass under the both conditions (normal and stress). The P. funiculosum and C. lacerate fall under group B having maximum biomass only under normal growth condition. The uninoculated plants formed group D produced least biomass under both the conditions (Fig. 3).

### Discussion

The numerous studies have been conducted on improvement of crop growth under heat stress using thermotolerant endophytes isolated from harsh environment or wild plants. However, the use of cold desert thermotolerant endophytes were less explored therefore we have analysed the effect of cold desert endophytes on improvement of fitness of rice under heat stress. In present study, the isolates A2, A7 and X5 were observed to be heat tolerant and grown at the range from 28°C to 40°C. This envisaged that these three isolates could sustain heat stress it might be the cold desert of Western Himalaya had extreme of hot climate (40°C) during summer (Tewari and Kapoor, 2013). These endophytes were identified using ITS region of rDNA as P. funiculosum, C. lacerate and E. endophytica. The ITS region of rDNA sequences is widely used to examine phylogenetic positions or relationship of a species because this region are flanked by preserved segments (18S, 5.8S and 28S genes) which provide information about the phylogeny and the taxonomic level, since their evolution is slow and they are highly similar within different taxa (Ramesh et al., 2017). High temperature is one of the most important environmental stresses which severely affect the rice growth by reducing the emergence of leaves and tillers resulting in decreased biomass. In the present study, significant higher tiller number was recorded when the plants inoculated with E. endophytica which might positively influenced the new tillers under heat stress by reducing the effects of heat stress on tiller bud. This is in accordance with Vila-Aiub et al. (2005) who reported that Neotyphodium sp. infected rye grass produced more tillers than uninfected plants. The endophyte P. funiculosum inoculated plants showed highest number of leaves compared to other endophytes which resulted in increased fresh weight of shoot. Similarly Lolium perenne infected with Epichloë endophyte had significantly higher tillers number, dry weight, leaf length and wet weight under drought condition (Jajarmi et al., 2015). The root system plays a vital role in adaptation of whole plant under heat stress (Huang et al., 2012). Significant improved in root growth was observed in endophytes colonized plants which lead to improved absorption of nutrients and water from soil, resulting in a more vigorous plant and helps to cope of heat stress. E. endophytica colonized plants found better in influencing the root growth compared to others. Our results are in agreement with Waqas et al. (2015) who demonstrated that Paecilomyces formosus LWL1 improved root biomass of rice under heat stress. Higher root to shoot ratio was found in plants inoculated with E. endophytica, which indicate that the endophyte could protect the root system.
In conclusion, this investigation explored the possibility of using cold desert endophytes for mitigating the heat stress. The endophyte *E. endophytica* seems to be more effective in imparting heat stress tolerance in rice by improving the growth of shoot and root attributes (IR-64).

**Declarations**

**Acknowledgments**

Authors are grateful to Dr. Uma shaanker, School of Ecology and Conservation laboratory, UAS, GKVK, Bangalore for providing fungal cultures. APS thankful to the Department of Science and Technology (DST), GOI, New Delhi, for awarding INSPIRE fellowship (DST/INSPIRE Fellowship/2016/IF160469).

**References**


**Tables**

**Table 1.** Effects of different temperatures on growth of the fungal colony (diameter in cm)
ophytic fungal isolates

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Hosts</th>
<th>Isolates code</th>
<th>Closest match</th>
<th>Sequence length (bp)</th>
<th>Query coverage (%)</th>
<th>Percent identity (%)</th>
<th>Organisms identified</th>
<th>NCBI Accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Artimisia sp.</td>
<td>A2</td>
<td>Penicillium funiculosum</td>
<td>654</td>
<td>98</td>
<td>99</td>
<td>Penicillium funiculosum</td>
<td>OM368442</td>
</tr>
<tr>
<td>7</td>
<td>Artimisia sp.</td>
<td>A7</td>
<td>Ceriporia lacerate</td>
<td>479</td>
<td>96</td>
<td>98</td>
<td>Ceriporia lacerate</td>
<td>MT899187</td>
</tr>
<tr>
<td>8</td>
<td>Xerophytic plant</td>
<td>X5</td>
<td>Endomelanconiopsis</td>
<td>473</td>
<td>98</td>
<td>98</td>
<td>Endomelanconiopsis endophytica</td>
<td>MT900590</td>
</tr>
</tbody>
</table>

Data shown above are the means of three replication with ± standard error. *A- Artemisia plant X- Xerophytic plant*

**Table 2. Molecular identification of thermotolerant fungal endophytes**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant height (cm)</th>
<th>No. of Tillers (/3plant)</th>
<th>No. of Leaves (/3plant)</th>
<th>Fresh wt. shoot (g/3plant)</th>
<th>Dry wt. s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>33.53±0.72</td>
<td>8.50±0.20</td>
<td>36.50±0.61</td>
<td>5.95±0.07</td>
<td>1.54±0.0</td>
</tr>
<tr>
<td><em>C. lacerata</em></td>
<td>35.51±1.17</td>
<td>10.50±0.20</td>
<td>40.00±0.00</td>
<td>7.92±0.08</td>
<td>2.33±0.0</td>
</tr>
<tr>
<td><em>E. endophytica</em></td>
<td>36.90±0.53</td>
<td>11.00±0.00</td>
<td>45.00±0.12</td>
<td>8.89±0.05</td>
<td>2.26±0.0</td>
</tr>
<tr>
<td><em>P. funiculosum</em></td>
<td>36.62±0.19</td>
<td>14.50±0.20</td>
<td>55.50±0.20</td>
<td>10.36±0.08</td>
<td>4.21±0.05</td>
</tr>
<tr>
<td><em>P</em></td>
<td>3.31</td>
<td>199.33</td>
<td>263.85</td>
<td>660.86</td>
<td>184.85</td>
</tr>
</tbody>
</table>

± indicates standard error of mean (n = 4); the dissimilar letters indicate significant difference at *P* < 0.05 by using Duncan's Multiple Range Test.
Table 4. Influence of fungal endophytes on root traits and total biomass of rice under stress [S] and without stress [WS]

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Root volume (cm³/3plant)</th>
<th>Fresh wt. root (g/3plant)</th>
<th>Dry wt. root (g/3plant)</th>
<th>Root:Shoot Biomass (g)</th>
<th>BSWS</th>
<th>SSWS</th>
<th>WSWS</th>
<th>SWS</th>
<th>S</th>
<th>SWS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.00±0.00b</td>
<td>3.00±0.00c</td>
<td>7.88±0.16c</td>
<td>2.23±0.05d</td>
<td>0.29±0.01d</td>
<td>0.89±0.02a</td>
<td>0.34±0.01c</td>
<td>2.92±0.06c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. lacerata</td>
<td>6.50±0.00d</td>
<td>3.95±0.10b</td>
<td>7.76±0.55c</td>
<td>3.45±0.00c</td>
<td>1.27±0.06c</td>
<td>0.35±0.01c</td>
<td>0.55±0.02d</td>
<td>0.39±0.01b</td>
<td>3.60±0.12b</td>
<td></td>
</tr>
<tr>
<td>E. endophytica</td>
<td>11.50±0.20c</td>
<td>4.25±0.02a</td>
<td>9.88±0.15b</td>
<td>4.03±0.06a</td>
<td>1.46±0.02b</td>
<td>0.47±0.02a</td>
<td>0.64±0.01c</td>
<td>0.44±0.02a</td>
<td>3.72±0.03b</td>
<td></td>
</tr>
<tr>
<td>P. funiculosum</td>
<td>13.90±0.04a</td>
<td>4.00±0.00b</td>
<td>12.73±0.07a</td>
<td>3.64±0.00b</td>
<td>1.98±0.04a</td>
<td>0.39±0.01b</td>
<td>0.75±0.01b</td>
<td>0.36±0.01bc</td>
<td>4.61±0.07a</td>
<td></td>
</tr>
<tr>
<td>(F₃,12)</td>
<td>920.23</td>
<td>111.39</td>
<td>59.85</td>
<td>445.68</td>
<td>46.17</td>
<td>56.97</td>
<td>101.74</td>
<td>15.35</td>
<td>83.62</td>
<td></td>
</tr>
</tbody>
</table>

± indicates standard error of mean (n = 4); the dissimilar letters indicate significant difference at P < 0.05 by using Duncan's Multiple Range Test.

Figures

Figure 1

Maximum Likelihood tree of the identified fungal endophytes (a) Penicillium funiculosum isolate A2) (b) Ceriporia lacerate isolate A7 and (c) Endomelanconiopsis endophytica isolate X5 and their closest ITS rDNA matches from the GenBank. The phylogenetic tree was constructed with bootstrap value of 500 replicates. Number at the node indicates the bootstrap value.
Figure 2
Fungal colony grown on PDA medium and their fruiting body under microscope (lactophenol cotton blue stain) (a) *Penicillium funiculosum* isolate A2 colony and their conidiophore (b) *Ceriporia lacerate* isolate A7 colony and their aseptate mycelia colony and their hyphae (c) *Endomelanconiopsis endophytica* isolate X5 and their mycelia.

Figure 3

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Yp</th>
<th>Ys</th>
<th>STI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.92</td>
<td>1.16</td>
<td>0.25</td>
</tr>
<tr>
<td><em>C. lacerate</em></td>
<td>3.6</td>
<td>1.22</td>
<td>0.32</td>
</tr>
<tr>
<td><em>E. endophytica</em></td>
<td>3.72</td>
<td>1.56</td>
<td>0.42</td>
</tr>
<tr>
<td><em>P. funiculosum</em></td>
<td>4.61</td>
<td>1.44</td>
<td>0.48</td>
</tr>
</tbody>
</table>