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An Overview of Stress-Tolerant Promoting Endophytic Fungal Isolates from Hiyung Chilli Grown in South Kalimantan

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ABSTRACT

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Endophytic fungi are a unique class of microorganisms that lives in plant tissues. These fungi could spread from roots to shoots and interact with nearly all plants in a symbiotic, mutualistic, or commensal manner. Endophytic fungi are believed to exert several roles related to plant growth, including the ability to tolerate stress, especially in Hiyung chilli plants. This plant is widely cultivated in Indonesia and is a well-known host for endophytic fungi. The Hiyung chilli is a popular variety grown in the South Kalimantan Province, which receives national recognition and is geographically certified by the Indonesian government. This wetland-cultivated chilli plant has a uniquely high capsaicin content and long fruit shelf life compared to other varieties. Although endophytic fungal isolates are prevalently found on chilli plants, their potential to influence the growth of their host remains to be proven beyond a doubt. This review emphasizes the sparse reports on the potential of these antagonistic fungi in protecting chilli plants against other pathogens by producing auxin/Indole-3-Acetic Acid (IAA) and capsaicin. The study hypothesised that the antagonistic abilities of these endophytic fungi against other fungal pathogens are related to their IAA- and capsaicin production that synergistically increase the chilli plants' threshold against biotic and abiotic stress.

Keywords: Anti-pathogenic fungi, Auxin, capsaicin, Indonesian chilli, South Kalimantan

Introduction

Hiyung chilli pepper is a protected variety cultivated in the wetlands of Hiyung village, Tapin Regency, in South Kalimantan Province [1–3]. It is worth noting that this chilli plant is a designated national variety by the Ministry of Agriculture of the Republic of Indonesia [4]. Interestingly, the variant also has its own certificate of geographical indication [5]. The advantages of Hiyung chilli pepper are stated in the attachment to the decree of the Minister of Agriculture No. 031/Kpts/SR.120/D.2.7/4/2016. This high-producing variety produces highly spicy chillies (capsaicin content is

more than 2000 ppm) and has a long shelf life of over 6 days [1].

According to the literature, these spicy peppers grow rather well as they act as hosts to endophytic fungi, which are known for their ability to inhibit the growth of the pathogenic fungus, *Colletotrichum capsici* [6, 7]. Endophytic fungi isolated from chilli plants purportedly have a variety of uses, including supporting plant growth [8–15], and are a source of bioactive compounds, for instance, capsaicin [16]. Endophytic fungi isolated from chilli plant reported so far are the *Tricho-*

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derma sp. [6, 7], *Gliocladium* sp. [7, 17], *Aspergillus* sp. [17, 18], *Curvularia* sp. [19], *Alternaria* sp., *Colletotrichum* sp., and *Cordyceps* sp. [20]. These fungal isolates grow internally in their hosts and are associated with promoting healthy plant tissues. Most importantly, their presence does not interfere with growth [21] or produce unwanted symptoms in the plants [22].

4 It said that endophytic fungi positively affect the growth of the host plant because of the phytohormones they produce, such as auxins [15, 26]. These fungi also secrete the same bioactive compounds as their host plants [27]. For instance, the antagonistic endophytic fungus, *Alternaria alternata*, isolated from chilli plants, was shown to produce capsaicin, which is the very same compound found in chillies, too [16]. In this perspective, the interesting antagonistic feature of endophytic fungi warrants further research by the scientific

Certain endophytic species were discovered as epiphytic or rhizosphere microbiota, though the concept of endophytic fungi was altered as research progressed [24–26].

Endophytic fungi infection on host plants begins with the formation of fungal colonies. The fungus proliferates in tandem with the maturation of the host's root tissues, forming thicker hyphae in differentiated epidermal and cortical cells. After germination, chlamyospores form a network of hyphae in the roots before branching into the host's intercellular space in the sub-epidermal regions. Fungal colonies are formed when single hyphae cells occupy the differentiated young root tissue, where the intercellular hyphae branch and traverse the cell wall [27].

Endophytic fungi are divided into two major groups based on evolution, taxonomy, host plants, and ecological relationships, namely the clavicip-

Table 1. Symbiotic Criteria Used For The Characterization Of Classes Of Endophytic Fungi [24].

Criteria	Clavicipitaceous		Nonclavicipitaceous	
	Class 1	Class 2	Class 3	Class 4
Host range	Narrow	Broad	Broad	Broad
Colonized tissue	Shoots and rhizome	Shoots, roots and rhizoma	Shoot	Root
Colonization on plants	Broad	Broad	Limited	Broad
Biodiversity in plants	Low	Low	High	Unknown
Transmission	Vertical and horizontal	Vertical and horizontal	Horizontal	Horizontal

community. The focus should be to uncover their role in protecting and promoting the growth of Hiyung chilli plants, consequently, improving their biotic and abiotic stress tolerance [5]. However, it is apparent that exploratory studies correlating the positive effects of endophytic fungi growing on Hiyung chillies remain sparse. This is because the fungal's antagonistic properties through auxin and capsaicin production are poorly understood.

Biology of Hiyung Chilli Endophytic Fungi

The term endophytic fungi were first proposed by Christian et al. [25], defined as fungi that can penetrate plant tissues and undergo several life cycles within them. This fungus lives internally on healthy plant tissues without interfering with the plant's growth [21] and does not produce unwanted symptoms [23]. Most mycologists argue that the composition of endophytes (internal microbiota) is different for each host, organ, or tissue.

1 itaceous (C-endophytes) and non-clavicipitaceous (NC-endophytes) groups. The clavicipitaceous (C) and nonclavicipitaceous (NC) endophytes are discerned by their phylogeny and evolutionary characteristics. However, the C-endophytes exists symbiotically with grass, while the NC-endophytes are symbiotic with hosts other than grass [24]. The characteristics of the endophytic fungi classes are shown in Table 1.

As can be seen, endophytic fungi isolated from Hiyung chilli belongs to class 2. Members of this class are more diverse and widely distributed in various ecosystems, comprising the phylum Ascomycota and Basidiomycota, capable of growing in various plant tissues, and transmittable vertically and horizontally. As they can transition between endophytic and free-living fungi, their role in the environment, evolutionarily, physiologically and ecologically, must be viewed with caution.

Table 2. Endophytic fungi of chili plant origin and their potential as anti-pathogenic fungi.

No	Genus	Endophytic Fungi Iso-late	Pathogenic fungi	Reference
1.	<i>Trichoderma</i>	<i>Trichoderma</i> sp.	<i>Colletotrichum capsici</i>	[6, 7]
2.	<i>Gliocladium</i>	<i>Gliocladium</i> sp.	<i>Colletotrichum capsici</i>	[7, 17]
3.	<i>Harmoniella</i>	<i>Harmoniella</i> sp.	<i>Colletotrichum capsici</i>	[7]
4.	<i>Humicola</i>	<i>Humicola</i> sp.	<i>Colletotrichum capsici</i>	[7]
5.	<i>Cunninghamella</i>	<i>Cunninghamella</i> sp.	<i>Colletotrichum capsici</i>	[7]
6.	<i>Aspergillus</i>	<i>Aspergillus flavus</i> , <i>Aspergillus niger</i> <i>Colletotrichum acutatum</i>	<i>Colletotrichum capsici</i> <i>Fusarium oxysporum</i>	[17, 20] [20]
7.	<i>Colletotrichum</i>	<i>Cladosporium gossypicola</i> , <i>Cladosporium colombia</i>	<i>Colletotrichum acutatum</i> ; <i>Fusarium oxysporum</i> ; <i>Phytophthora capsici</i>	[20]
8.	<i>Cordyceps</i>	<i>Cordyceps nutans</i>	<i>Colletotrichum acutatum</i> ; <i>Fusarium oxysporum</i> ;	[20]
9.	<i>Penicillium</i>	<i>Penicillium oxalicum</i> , <i>Penicillium citrinum</i> , <i>Penicillium gossypicola</i> , <i>Penicillium crustosum</i> , <i>Penicillium expansum</i>	<i>Colletotrichum acutatum</i> / <i>Fusarium oxysporum</i> / <i>Phytophthora capsici</i>	[17, 20]
10.	<i>Xylaria</i>	<i>Xylaria</i> sp.	<i>Colletotrichum acutatum</i> and <i>Fusarium oxysporum</i>	[20]
11.	<i>Rhizoctonia</i>	<i>Rhizoctonia</i> sp.	<i>Colletotrichum capsici</i>	[17]
12.	<i>Hormiscium</i>	<i>Hormiscium</i> sp.	<i>Colletotrichum capsici</i>	[17]

Biodiversity and Potential of Chilli Plant Endophytic Fungi

Table 2 enlists the known symbiotic relationship between several types of endophytic fungi with different varieties of chilli plants. The endophytic fungal species isolated from chilli plants include *Trichoderma* sp. [6], *Botrytis* sp., *Gliocladium* sp., *Harmoniella* sp., *Humicola* sp., *Cunninghamella* sp. [7], *Aspergillus flavus*, *Nigrospora* sp., *Coniothyrium* sp. [18], *Cercospora nicotianae*, *Curvularia* sp., and *Fusarium* sp. [19]. There are several fungal genera isolated from chilli plants of Korean origin, namely *Alternaria*, *Aspergillus*, *Bionectria*, *Chaetomium*, *Cladosporium*, *Colletotrichum*, *Cordyceps*, *Fusarium*, *Geomyces*, *Gnomonia*, *Heterobasidium*, *Irpex*, *Paecilomyces*, *Paparspora*, *Penicillium*, *Peyronellaea*, *Phanerochaete*, *Phialophora*, *Phlebospsis*, *Plectosphaerella* and *Xylaria* [20]. Other species also reported include *Rhizoctonia* sp., *Aspergillus niger*, and *Hormiscium* sp. [17]. Most of these endophytic fungi are isolated from root tissue, stems, and leaves [20].

Specifically, the endophytic fungi can intracellularly and intercellularly enter plant tissues in the rhizosphere and phyllosphere regions through wound openings in roots or stomata on the leaves [21, 23]. Their growth in plant tissue thus affects the anatomical structure [24, 25] and growth of the host plant [26].

The Role of Endophytic Fungi in Sustainable Hiyung Chilli Farming in Swamplands

Swamp land is naturally inundated, either periodically or continuously, due to obstructed drainage caused by tide, river overflow, and rain [32, 4]. Chilli farming in Hiyung village is done in lowland swamp lands [4]; in which the soil is very acidic (pH 3.84). The soil also has high organic carbon content and moderate sodium, phosphate, and potassium levels [4]. However, the ever-changing environmental conditions in Hiyung village can unfavorably impact plant development and growth [33]; thus, forcing the plants to adapt to their surroundings. Abiotic stress in plants may include water content, acidity, and soil organic matter content. And biotic stress such as pathogen

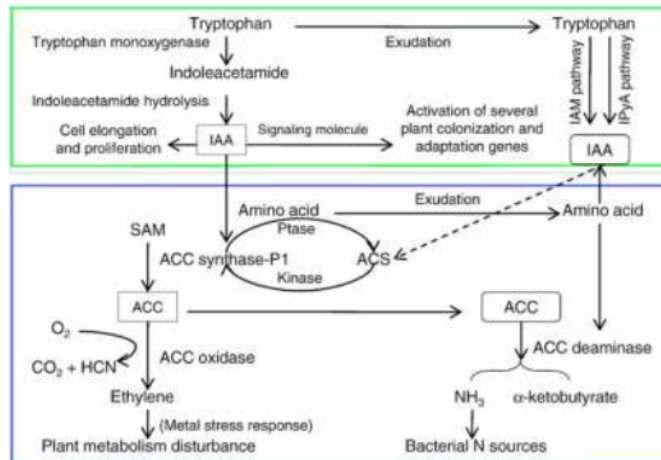


Figure 1. The mechanism of endophytic microbial growth hormones in supporting plant growth. Image adapted from Bae et al. [39].

attack. Plants employ a variety of stress-tolerant mechanisms comprising physiological or molecular ones to adapt to environmental changes [4] successfully. These changes are often assisted by microorganisms, such as endophytic fungi [33].

Environmentally friendly and safe farming approaches are the focus of current research as the demand for agricultural products increases. Various strategies are implemented to support sustainable agriculture and increase crop production while minimizing environmental damage and improving human welfare. As a matter of fact, the symbiotic nature of endophytic fungi-plants is an alternative in sustainable agriculture because of their potential for increasing plant tolerance to biotic and abiotic stress, in addition to improving growth [34]. Several studies reported the crucial role of endophytic microbes in supporting plant growth. They found that these endophytic microbes were lost during domestication and the long-term cultivation of certain crops. However, the issue was solvable by transferring endophytes from related wild plants to cultivated ones [34]. Hence, it can be construed that sustainable agriculture is not possible without these antagonistic endophytic fungi.

Another vital contribution of endophytic fungi is their ability to affect their host plants to adapt to varying abiotic and biotic stress. Certain fungi improve plant fitness by producing certain chemicals that enhance the host's tolerance to biotic and abiotic stress, for instance, inhibiting pathogens and producing phytohormones [24].

Potential of Endophytic Fungi as Anti-Pathogenic Fungi

Literature has shown that the mechanism of pathogen inhibition by endophytic fungi can indirectly support plant growth and inhibit the growth of pathogenic fungi through antibiosis, competition, or mycoparasitism. These mechanisms were observed in endophytic fungi from Galam (*Melaleuca cajuputi*) [35] and rice (*Oryza sativa* L.) [36]. A *Trichoderma* sp. isolate employed the above-said mechanisms to inhibit the growth of pathogenic *P. capsici* [37] and *Colletotrichum* sp. [6]. Table 2 denotes various endophytic fungi isolated from the chilli plant, which exhibited inhibitory ability against several pathogenic fungi (Table 2.) [17, 20, 38]. The fungal species isolated from the roots of Hiyung chilli plants, including *Harmoniella* sp., *Cunninghamella* sp., *Humicola* sp., were effective in inhibiting *C. capsici* [7].

The Potential of Endophytic Fungi in Helping Plant Growth through the Production of Indole-3-Acetic-Acid (IAA)

It is worth noting that endophytic fungi also produce phytohormones, which elevates the plant host's ability to tolerate environmental stresses [29, 38, 39] (Figure 1). Phytohormones isolated from these microorganisms include auxins (IAA), cytokinins, and gibberellin. The hormones support germination, growth, and reproduction and protect plants from biotic and abiotic stress [41]. In fact, IAA is one of the most studied phytohormones



Figure 2. Hiyung chili farm (a, b), chilli fruits (c) and chilli-bearing mature plants (d).

produced by endophytic fungi [15, 39], which include cell elongation and division, and is a part of the plants' defence system. It was documented that different IAA biosynthetic pathways regulate different types of plant-microbial interactions [42].

A mutualistic symbiotic microorganism-plant interaction is linked to the synthesis of IAA through the indole-3-pyruvate (IPyA) pathway. Conversely, pathogens mainly use the indole-3-acetamide pathway or (IAM). Also, the amount of IAA liberated by plants affects the efficacy of plant-microbial interactions. Literature has shown that at high IAA concentrations, root elongation is inhibited, and this outcome is related to 1-aminocyclopropane-1-carboxylic acid (ACC) synthesis. Through ethylene synthesis, plant growth is regulated by direct or indirect hormonal balance [43–45].

Another reported interesting observation is that plants inoculated with endophytic fungi grow better than uninoculated ones [8, 10, 15, 42, 43]. This is because the introduced endophytic fungi act as growth-promoting agents through the production of IAA [11, 15, 47] and auxin. In particular, the latter promotes cell differentiation and elongation in host plant tissues and subsequently improves the number of lateral roots and shoot length [44].

Endophytic Fungi from Hiyung Chilli as a Source of Capsaicin

Certain endophytic fungi produce metabolites similar to their hosts. The compounds comprise alkaloids, terpenoids, quinones, steroids, phenols, shikimates, xanthenes, and isoprenoids [21]. These compounds are known for their anti-inflammatory and anti-cancer properties [45]. Interestingly, endophytic fungi isolated from *Capsicum annum* were also found to produce capsaicin [16]. For brevity, capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) is an alkaloid of the capsaicinoid group in the vanilloid family, following the presence of a vanillyl group in its structure. The compound also has a benzene ring endowed with a long hydrophobic carbon chain and a polar amide group. The lowly volatile capsaicin is aromatic solvent-soluble but is insoluble in water [46].

There are consistent reports on the role of capsaicin as a defence mechanism in chilli plants against pathogens [48]. Another fascinating observation on the use of this compound, is that the external application of capsaicin to chilli seeds inhibited seed germination. On that premise, capsaicin from chili peppers might also be valuable in inhibiting weed, i.e., a herbicide [52]. Additionally, the compound enhances the resistance of chilli plants to fungal and bacterial pathogens and insects [51, 52]. The higher concentration of capsaicin in chilli

plants grown in humid environments was also found to reduce the plants' susceptibility to pathogenic fungal infections. Contrariwise, studies showed that chilli plants grown in arid conditions have lower spiciness levels due to lower capsaicin content. The reduced capsaicin content in these chilli plants was due to lower incidences of pathogenic fungal attacks on plants in dry conditions, which do not support their growth. In the case of Hiyung chilli plants, their observably higher capsaicin content is likely an evolutionary adaptation of the plant's defence mechanisms for growth in wetlands.

However, certain pathogenic fungi have developed novel mechanisms to nullify the effects of surplus capsaicin secreted by chilli plants. These counter-mechanisms include the production of multiple enzymes that specifically degrade capsaicin [56], which renders the surplus capsaicin useless in protecting the chilli plants.

Hiyung Chilli Local Variety of South Kalimantan, Advantages and Potential of Endophytic Fungi

As the name implies, the chilli comes from the Hiyung village of the Tapin Regency in South Kalimantan (Figures 2(a) and (b)). Morphologically, it has a greenish-purple fruit when young, which gradually turns bright red when matured. The mature fruit is an elongated tapered shape (Figure 2c), and the plant is a shrub with tapered leaves [3]. The similarity coefficient between the Hiyung chilli variety with Nirmala was the highest after morphological observations on 14 chilli varieties [57]. The Nirmala variety is a hybrid cayenne pepper that is relatively resistant to *Ralstonia solanacearum* [58].

Other traits of the Hiyung chilli are its high fruit yield per plant, its extreme spiciness reaching up to 2333.05 ppm, and its high vitamin C content of 66.85 mg / 100 g [3, 5]. However, the spiciness level reduces when it is grown elsewhere. The fruit

also has a long shelf life and could be stored for long durations, up to 10-16 days, at room temperature [3-5].

Based on the literature, IAA phytohormones secreted by endophytic fungi through ethylene metabolism [39], are also produced by plants under duress [60]. For instance, the *Cunninghamella* sp. endophytic fungi produce phytohormones, including IAA, that affect germination [7]. The unusually high capsaicin content in Hiyung chilli compared to other varieties from Mexico, Saudi Arabia, and the USA, also points to its potential for other applications, for example, as an ingredient in pharmaceutical preparations (Table 3). However, the capsaicin content in Hiyung chilli is lower than the Bhut Jolokia variety from Russia.

The high capsaicin content in Hiyung chilli also conveys essential information on the possible metabolites produced by the plant's endophytic symbionts. This is consistent with several reports on these fungi producing metabolites similar to their symbionts [60]. However, further investigations are needed to clarify the facts regarding the association between the fungi's ability to produce capsaicin and their antagonistic behaviour. Also, earlier research discovered that the dry weight of the Hiyung chilli implies that the variant is suitable for cultivation in swamplands. Furthermore, the highly acidic wetlands can inhibit plant growth [62]. That said, this variety of chilli plants' tolerance of acidic environments warrants further examination to prove their synergistic existence with the endophytic fungi they harbour [63, 64].

Based on the literature, the unique stress-tolerant Hiyung chilli plant was likely linked to its role as a host for endophytic fungi, which invariably inhibited colonisation of other pathogenic fungi. The symbiont also produces IAA and auxin, which protect the plant host. However, further efforts are needed to prove this hypothesis pertaining to the Hiyung chilli endophytic fungal isolates. Figure 3

Table 3. Capsaicin content of some varieties of hot peppers.

No	Variety name	As long as	Capsaicin (ppm) content	Source
1	Bhut Jolokia	Russia	3.041 - 25.944	[61]
2	Cipanas	Indonesian	9,23	[62]
3	Lembang-1	Indonesian	7,79	[62]
4	Branang	Indonesian	7,44	[62]
5	Gold	Indonesian	4,3	[62]
6	Gantari	Indonesian	7,12	[62]
7	Hiyung	Indonesian	2.333,05	[5]
8	Red-pepper	USA	228	[63]

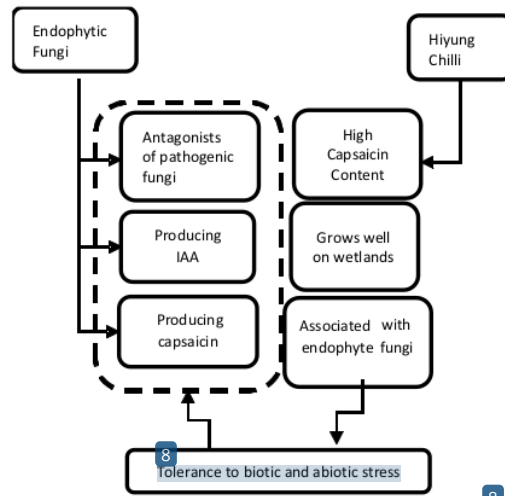


Figure 3. A schematic showing the mechanism by endophytic fungi to enhance biotic and abiotic stress tolerance in their host plants.

illustrates the mechanisms adopted by endophytic fungi to enhance biotic and abiotic stress tolerance in their host plants.

Conclusion

As can be seen, the superior quality of Hiyung chilli is seen to be synonymous with their existence as a symbiont of certain endophytic fungi. So far, their function as antifungal agents and their ability to produce auxin and capsaicin are hypothesised to contribute to the higher stress tolerance in Hiyung chilli plants over other varieties. Nevertheless, future exploratory studies are needed to fully comprehend the above-said mechanisms induced by these endophytic fungal isolates on their Hiyung chilli symbiont, which produces more robust plants.

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