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# ISOLATED ROOT SYMBIOTIC FUNGI (RSF) FROM *Drynaria quercifolia* L. INDUCED TOLERANCE TO SNAP-PEG 8000 MILD DROUGHT STRESS IN PSB RC10 (PAGSANJAN) RICE (*Oryza sativa* L.)

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***Abstract***

*Rice (Oryza sativa L.) needs greater amount of water for its growth and metabolism as compared to other agricultural crops making it vulnerable to drought. Previous studies have suggested that symbiotic fungi can be utilized as exogenous producers of radical scavenging metabolites that enhance the adaptive ability of their host plants exposed to various environmental stresses such as drought. The actual stress-adaptive mechanisms of these root symbiotic fungal (RSF) isolates were tested on drought-stressed rice. The results of the study showed that the five RSF isolates have different mechanisms in protecting rice seedlings against drought. However, one RSF isolate stood out. The rice seedlings treated with the unidentified Mucoromycotina isolate, F9P2RSF21, appeared to have the tallest shoots, 100% germination rate, highest seedling vigor index (SVI) and drought tolerance index (DTI) together with the lowest drought susceptibility index (DSI). Statistical data also revealed that this isolate approximated the plant shoot length, germination rate, SVI, DTI and DSI of those rice seedlings grown in non-drought conditions. These drought parameters showed that the unidentified Mucoromycotina isolate, F9P2RSF21, was proved best in combating the far-reaching effects of drought in agriculture. With the apparent consistency in the results pertaining the potential drought stress tolerance capacity of RSF isolates on Drynaria quercifolia, this study may be a potential breakthrough in the field of agriculture and rice research.*

**Key words*:*** Drought indices, Drought tolerance, Drynaria quercifolia, Rice and Root symbiotic fungi.

## INTRODUCTION

Rice (*Oryzasativa* L.) is the predominant food for many countries worldwide. In the Philippines, rice production has huge contribution as household income and helps millions of Filipino families. Given the aforementioned significance of rice in the country's economy, it is expected that majority of the nation's agricultural lands are dedicated to rice production (Barba *et al*., 2014). Rice, like any other plants, experiences formation of reactive oxygen species (ROS) in their normal metabolic processes. Rice may experience detrimental damages in its morphology and physiology due to excessive ROS produced by environmental stresses. A very common example of stress experienced by rice is drought. For its normal growth and development, rice needs greater amount of water as compared to other agricultural crops making it vulnerable to drought (Pandey and Shukla, 2015). In relation to drought, the climate change has caused several areas worldwide to experience longer drought periods; while other regions are exposed to heavy rainfalls. The El-Nino phenomenon, an abnormal weather condition that causes drought, has affected countries such as Philippines, Indonesia, Malaysia and Thailand (Fukai and Cooper, 1995). The erratic rainfall pattern and drought conditions caused remarkable cutback in yields of major agricultural crops, particularly rice. If this irregular pattern and drought conditions continue, it may give drastic effect to the rice and agricultural industry due to their dependency on climatic conditions (Barba *et al*., 2014).

To avoid the detrimental damages caused by excessive ROS brought about by various environmental stresses such as drought, plants have evolved mechanisms to control them sustainably. Plant's endogenous ROS-quenching mechanism plays a critical function in keeping the redox balance of its system. For instance, enzymes play a vital role in the reduction of ROS levels as well as avoiding oxidative stress (Sugiharto *et al*., 2016). The activities of these enzymes play an important role as being the primary line of defense in eradicating free radicals (Elavarthi and Martin, 2010). Nonetheless, excessive ROS exposure might cause failure to plant's endogenous radical scavenging system. When this happens, exogenous antioxidants are necessary to maintain homeostasis of metabolic oxidation-reduction reactions.

Previous studies have suggested that symbiotic fungi can be utilized as exogenous producers of radical scavenging and novel bioactive compounds (e.g. phenolic acids, tannins and flavonoids) (Ravindran and Naveenan, 2011; Dhankhar *et al*., 2012; Yadav *et al*., 2014; Ludwig-Müller, 2015; Al-Askar, 2016) that can combat ROS production during stress. Studies even demonstrated that filamentous fungi *Aspergillus* and *Monascus* are good sources of phenolics that are good antioxidants due to the scavenging activity of their hydroxyl groups especially when their hosts are exposed to drought or other related environmental stresses (Manach *et al*., 2004; Arora and Chandra, 2010). Moreover, a variety of fungal strains such as *Penicillium*, *Mortierella* and *Acremonium* were speculated to produce enzymes (e.g. catalase and peroxidase) that counter oxidation and arrest damaging properties of ROS (Chandra and Arora, 2009) brought about by drought.

With the above mentioned benefits that can be acquired from symbiotic fungi, scientists have focused on the feasibility of natural remedies such as fungal symbiosis to plants. Literature showed that the mutualistic relationship of fungi to plants helped them acclimatize with new environmental stresses such as desiccation, increased solar radiation exposure and extreme temperature fluctuations (Selosse and Le Tacon, 1998). Hence, there is a great tendency that symbiotic fungi can provide certain physiochemical mechanisms to their host plants experiencing drought stress. This project was therefore crafted to determine the likelihood of the RSF isolates to provide drought stress tolerance to rice. Holistically, it aimed to combat the far-reaching effects of drought in the agricultural industry.

## MATERIALS AND METHODS

**Isolation and identification of root symbiotic fungi (RSF):** The epiphytic fern, *Drynaria quercifolia*, often colonizes Acacia (*Samanae saman*) found in the Don Mariano Marcos Memorial State University – North La Union Campus (DMMMSU-NLUC). This state university is found in Bacnotan, La Union, Philippines. These ferns are treated as *valueless* outgrowths in tree barks and are often cut to maintain the aesthetic beauty of DMMMSU-NLUC. This study was therefore conducted to highlight the potential agricultural significance of these seemingly *valueless* plants. Ten *D. quercifolia* were collected. The roots of these ferns were cut into representative segments and grown in Potato Dextrose Agar (PDA) (*TM Media*). Five morpho-species were isolated and transferred in PDA slants. These root symbiotic fungal (RSF) isolates were molecularly identified. Following the protocol of Liu *et al.* (2010), genomic DNA of the isolates were extracted. The DNA of these isolates were amplified using *Vivantis* PCR mixture, *Vivantis* nuclease-free water, and ITS 1 forward (TCCGTAGGTGAACCTGCGG) and ITS 4 reverse (TCCTCCGCTTATTGATATGC) primers

(*IDT Primers*). After isolation, the amplicons were submitted to 1st Base Sequencing Facility in Malaysia for cleaning and subsequent sequencing. The generated sequences of the RSF isolates were published to the National Center for Biotechnology Information (NCBI) GenBank for sequence storage. The following are the identified RSF isolates and their respective GenBank accession numbers: (1) $F_1P_3RSF_3$ - *Meyerozyma guilliermondii* (KY474516), (2) $F_2P_3RSF_5$ - *Trichoderma yunnanense* (KY474517),(3) $F_3P_3RSF_8$ - *Trichoderma simmonsii* (KY474518),(4) $F_5P_1RSF_{16}$ - Unidentified Mucoromycotina (KY474524), (5) $F_9P_2RSF_{21}$ - Unidentified Mucoromycotina (KY474527). These RSF isolates were tested for their actual drought protective mechanisms on rice.

**Drought tolerance capacity assay:** Three replicates of each of the RSF isolates were initially grown in Sabouraud Dextrose Broth (SDB) (*TM Media*) at room temperature for seven days. On the other hand, rice seeds (PSB Rc10 - Pagsanjan) were obtained from the Philippine Rice Research Institute (PhilRice), Munoz, Nueva Ecija, Philippines. This rice variety was chosen because it is an early maturing variety often used for drought tolerance properties testing. Therefore, its exposure to a drought environment would either impede its growth, germination rate, or vigor (Valencia, 2015). The rice seeds were surface sterilized with 2.5% sodium hypochlorite for one hour and rinsed with Type 1 water (*Unichrom*). The seeds were placed in sterile petri plates with sterile absorbent paper. Sterile distilled water was sprayed onto the seeds then allowed to grow in the dark at ambient room conditions for seven days. In the preparation of basal culture media, the protocol of Santos and Ocampo (2005) was used with few modifications. Simple Nutrient Addition Program (SNAP) solution was prepared by adding 1.25 ml SNAP solution A and B to 1000 ml distilled water. Drought stress was induced to the prepared basal growth medium following the procedure of Trachsel *et al.* (2010). One hundred fifty grams of polyethylene glycol (PEG) 8000 (*Sigma*) was added to the prepared 1000 ml SNAP solution. This is equivalent to 15% PEG 8000 (= water potential of -4.0 bars) considered as 'mild' drought (Michel, 1983). Five hundred microliters of the PEG-SNAP mixture was transferred to each of the sterilized 25x200 mm test tubes that were used as growth chambers.

The pre-germinated rice seedlings were moved to the different RSF culture broth suspensions. Fifteen seedlings with equal root/shoot lengths were inoculated with culture filtrate (CF) for 24 hours. Three replicates with five seedlings were prepared per CF. The RSF-inoculated seedling was transferred to sterilized growth chambers and were allowed to grow for 14 days under artificial light (1000 lux) and at ambient room conditions. Two hundred fifty microliters of CF were also added to the previously prepared 500 µl SNAP-PEG growing medium. Positive controls were seedlings immersed to 20 ppm gibberellic acid (99%; *Sigma*), 20 ppm indole-3-acetic acid (99%; *Sigma*), and seedlings grown in SNAP solution without PEG (non-drought condition). Negative controls were seedlings immersed to sterile broth and water.

**Drought stress tolerance calculations**: The number of germinated seeds was recorded to calculate for the germination rate. The shoot lengths of all seedlings that survived in the SNAP-PEG medium were recorded. The germination rate and the mean shoot lengths of the rice plants were computed at 14 DAP (days after pre-germination) for the seedling vigor index (SVI). The SVI was computed using the formula recommended by Amprayn *et al.* (2012):

Seedling Vigor Index (SVI) = shoot length x germination rate. Two drought-stress indices based on the protocol of Kumar *et al.* (2014) with few modifications were calculated based on the mathematical association between yield during drought stress and in normal non-drought settings. For this study, yield was denoted by the computed seedling vigor:

a) Drought Tolerance Index (DTI) = $[Vi_{ND} \times Vi_D] / (Vi_{ND})^2$. This parameter denotes how tolerant rice seedlings are in drought conditions. Values range from 0 to 1 where 0 indicates that rice seedlings are non-drought tolerant and 1 indicates that the rice seedlings are highly tolerant to drought stress; and

b) Drought Susceptibility Index (DSI) = 1- ($Vi_D$ / $Vi_{ND}$). This parameter denotes how susceptible rice seedlings are in drought conditions. Values range from 0 to 1 where 0 indicates that rice seedlings are non-susceptible and 1 indicates that the rice seedlings are highly susceptible to drought stress.

For both indices, $Vi_{ND}$ denotes the seedling vigor under non-drought conditions and $Vi_D$ denotes the seedling vigor under drought conditions.

**Data presentation and statistical analysis**: All data were presented and statistically analyzed using SPSS software version 20. The mean shoot length and germination rate of RSF-inoculated rice seedlings exposed in SNAP-PEG medium were presented using the Chart Builder bar and line graph with dual Y coordinates where mean shoot lengths are shown as bar graph and the germination rates (%) are presented as interpolated line graph. The SVI was presented in tabular format. The two drought stress indices (drought tolerance index and drought susceptibility index) were presented using Char Builder bar graph with dual Y coordinates. One-way ANOVA with Scheffe post-hoc test was used to determine significant differences on the mean shoot lengths, SVI, DTI and DSI. Error bars displayed represent + standard deviations.

## RESULTS AND DISCUSSIONS

Figure 1 shows the visual appearance of RSF-treated rice seedlings at 14 DAPS in SNAP-PEG (15%) growth medium. The 15% PEG concentration is equivalent to a water potential of -4.0 bars and is considered as ‘mild’ drought (Michel, 1983). In an environment with mild drought, an obviously reduced shoot length is observed in the negative control (broth and water). All RSF-treated rice seedlings appear taller than the negative control. *T. simmonsii*-treated rice seedlings appear to be comparable with the IAA control and the unidentified Mucoromycotina isolate ($F_9P_2RSF_{21}$) appear to match the height of rice seedlings grown in a non-drought environment (SNAP without PEG). Expectedly, 20ppm GA-treated rice seedlings were the longest but also show distinct thin and spindle shoot growth typified by increased exposure to GA. Apparently, shorter rice seedlings grew when inoculated with *M. guilliermondii* and the unidentified Mucoromycotina isolate ($F_5P_1RSF_{16}$) as compared to the positive controls (GA, IAA and SNAP solution without PEG).


*Figure 1. Visual appearance of RSF-treated rice seedlings at 14 DAP in SNAP-PEG (15%) growth medium. Abbreviations: SNAP = Simple Nutrient Addition Program; PEG = polyethylene glycol; DAP = days after pre-germination.*

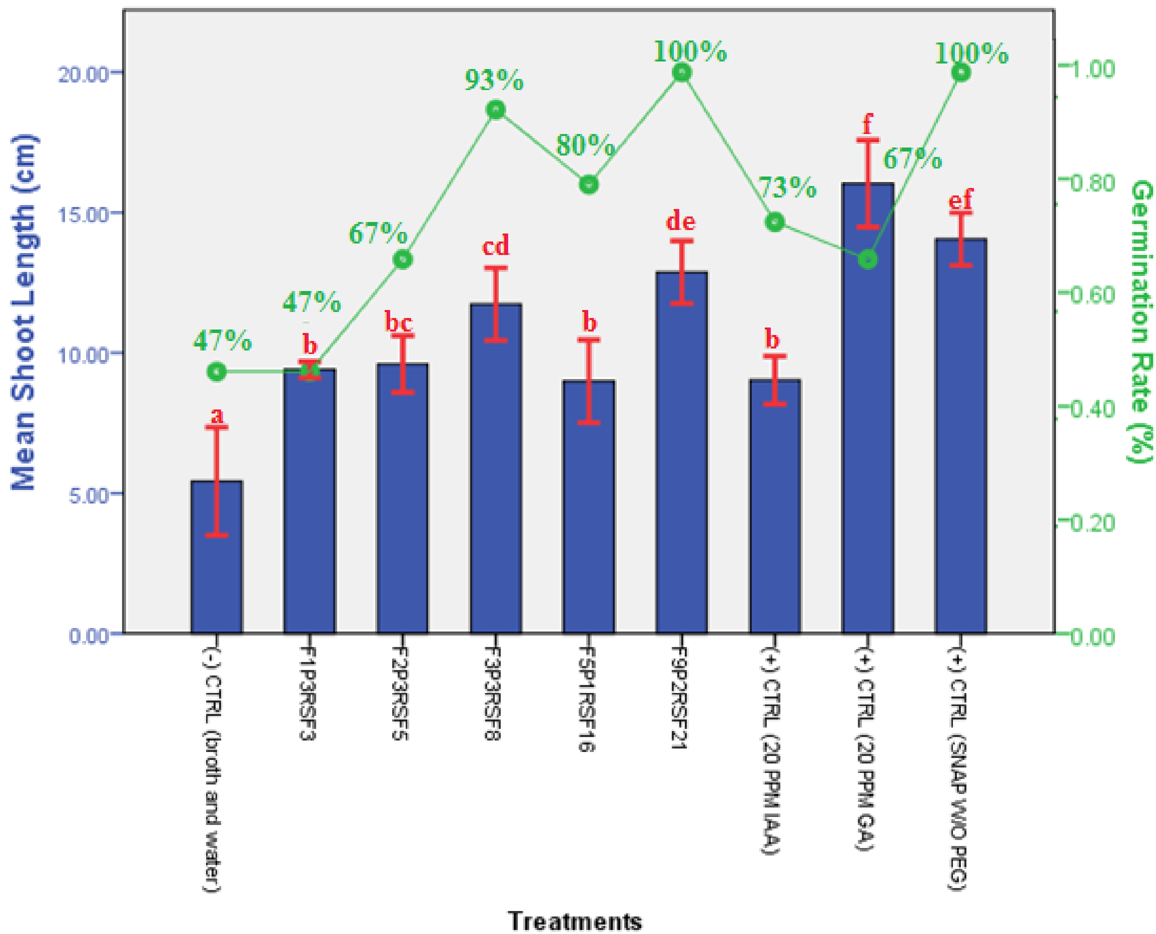


*Figure 2. Mean shoot length (cm) and germination rate (%) of RSF-treated rice seedlings. Control = broth and water, $F_1P_3RSF_3$ = Meyerozyma guilliermondii, $F_2P_3RSF_5$ = Trichoderma yunnanense, $F_3P_3RSF_8$ = Trichoderma simmonsii, $F_5P_1RSF_{16}$ = Unidentified Mucoromycotina, $F_9P_2RSF_{21}$ = Unidentified Mucoromycotina. Means with different letters are significantly different ($\rho$=0.05) n = 9. Mean shoot length (cm) are shown as blue bars while germination rate (%) are shown as interpolated green lines. Error bars are (in red) are $\pm$ standard deviations*

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Increased shoot length and higher germination percentage in a stressful environment (*e.g.* drought) are indications of possible tolerance of plants and can thus be related to the radical scavenging or other stress-adaptive potentials from RSF isolate culture filtrate applied. The mean shoot length and germination rate of RSF-treated rice seedlings are shown in Figure 2. As expected, rice seedlings exposed to 20 ppm GA have the longest shoot length (16.03$\pm$1.55 cm) comparable to rice seedlings grown in SNAP without PEG (14.05$\pm$0.94 cm). Surprisingly, rice seedlings treated with the unidentified Mucoromycotina isolate $F_9P_2RSF_{21}$ also have shoot lengths (12.87+1.12 cm) comparable to that of the SNAP (without PEG) control. This may be an early indication of potential drought tolerance protection that the RSF isolate provides to its host plant. The same $F_9P_2RSF_{21}$-inoculated rice seedlings also have the tallest shoots that are significantly taller than rice seedlings inoculated with the four other RSF isolates, and even to those IAA-treated rice seedlings (9.03±0.86 cm) grown in a drought media (SNAP-PEG solution).In terms of germination rate, only one of the five RSF isolates possibly helped their host seedlings attain perfect germination. Rice seedlings inoculated with the unidentified Mucoromycotina isolate ($F_9P_2RSF_{21}$) have 100% germination similar to those rice seedlings grown in a non-drought growing media attained 100% germination. *T. simmonsii*- and unidentified Mucoromycotina isolate $F_5P_1RSF_{16}$-inoculated rice seedlings also experienced high germination percentage, 93% and 80%, respectively. These rates are higher than the germination percentage of IAA (73%) and GA (67%) -treated rice seedlings exposed to the SNAP-PEG drought growing media.

**Table 1. Mean seedling vigor index (SVI) of RSF treated rice seedlings in SNAP-PEG solution at 14 DAP.**

| RSF Isolate | Description | Mean Seedling Vigor Index | |
|---|---|---|---|
| (-) Control | Broth and Water | $2.53^{a}$ | ±0.90 |
| $F_1P_3RSF_3$ | *Meyerozyma guilliermondii* | $4.39^{b}$ | ±0.13 |
| $F_2P_3RSF_5$ | *Trichoderma yunnanense* | $6.40^{c}$ | ±0.67 |
| $F_3P_3RSF_8$ | *Trichoderma simmonsii* | $10.95^{d}$ | ±1.20 |
| $F_5P_1RSF_{16}$ | Unidentified Mucoromycotina | $7.19^{c}$ | ±1.18 |
| $F_9P_2RSF_{21}$ | Unidentified Mucoromycotina | $12.87^{e}$ | ±1.12 |
| (+) Control 1 | 20 PPM IAA | $6.62^{c}$ | ±0.63 |
| (+) Control 2 | 20 PPM GA | $10.69^{d}$ | ±1.03 |
| (+) Control 3 | SNAP (w/o PEG) | $14.05^{e}$ | ±0.94 |

*Means with different letters are significantly different (ρ=0.05) n = 9. Values are expressed in SVI±S.D. Abbreviations: SNAP = Simple Nutrient Addition Program; PEG = polyethylene glycol; DAP = days after pre-germination.*

Many definitions have been proposed for the meaning of seedling vigor as it affects various plant physiological properties such as seedling growth, aging and senescence, metabolism, germination and growth rate, root, shoot and total plant length, plant biomass, and speed of germination (Gibson, 2008). However, for this study, two growth parameters (shoot length and germination rate) were used for its calculation (Amprayn et al., 2012).

Table 1 shows the mean SVI of RSF-treated rice seedlings in SNAP-PEG solution at 14 DAP. As gleaned in the table and figure above, $F_9P_2RSF_{21}$ (unidentified Mucoromycotina) isolate-inoculated rice seedlings exposed to drought stress (SNAP-PEG 15%) were observed to sustain comparable seedling vigor (12.87±1.12 svi) to that of seedlings grown in a non-stressed environment (SNAP without PEG) (14.05±0.94 svi). The $F_9P_2RSF_{21}$ isolate treatment also has an SVI significantly higher than all other RSF isolates treatments, positive controls (GA and IAA) and the negative control (broth and water). On the other hand, *T. simmonsii* isolate treatment was found to have SVI (10.95±1.20 svi) comparable to that of 20 ppm GA positive control treatment (10.69±1.03 svi) and *T. yunnanense* isolate treatment was found to have SVI (6.40±0.67 svi) comparable to that of 20 ppm IAA positive control treatment (6.62±0.63 svi). Despite having the lowest SVI compared to other RSF treatments, *M. guilliermondii* isolate treatment (4.39±0.13 svi) still has significantly higher SVI than that of the negative broth and water control (2.53±0.90 svi).

In experiments where yield is not determined, seedling vigor and drought stress indices are useful tools and have been used as agronomic parameters to measure rice performance exposed to various drought stress conditions (Kumar *et al.*, 2014). In this study, the drought tolerance index (DTI) and the drought susceptibility index (DSI) were used. The seedling vigor and drought stress indices are yield measuring tools to quantify the yield reduction effects of drought (Raman *et al*., 2012).

The mean drought tolerance index (DTI) and the mean drought susceptibility index (DSI) of RSF treated rice seedlings in SNAP-PEG solution at 14 DAP are presented in Figure 3. As gleaned from the table and figure above, rice seedlings exposed to the unidentified Mucoromycotina isolate ($F_9P_2RSF_{21}$) have the highest mean DTI (0.92±0.08 dti) which is significantly higher than the negative control (0.18+0.06 dti), GA (0.76+0.08 dti) and IAA (0.47+0.05 dti) controls, and those rice seedlings inoculated by the four other RSF isolates (*M. guilliermondii*, *T. yunnanense*, *T. simmonsii* and unidentified Mucoromycotina isolate $F_5P_1RSF_{16}$). This isolate's DTI value is, however, comparable to the rice seedlings grown in SNAP without PEG (non-drought environment) which expectedly has a mean DTI of 1.0. This indicates that the rice seedlings inoculated with the $F_9P_2RSF_{21}$ isolate has high tolerance to drought. Similarly, this indicates that the RSF isolate $F_9P_2RSF_{21}$ (unidentified Mucoromycotina) may have exogenously produced antioxidants or radical scavenging metabolites leading to its potential drought tolerance effect to its host rice plant.

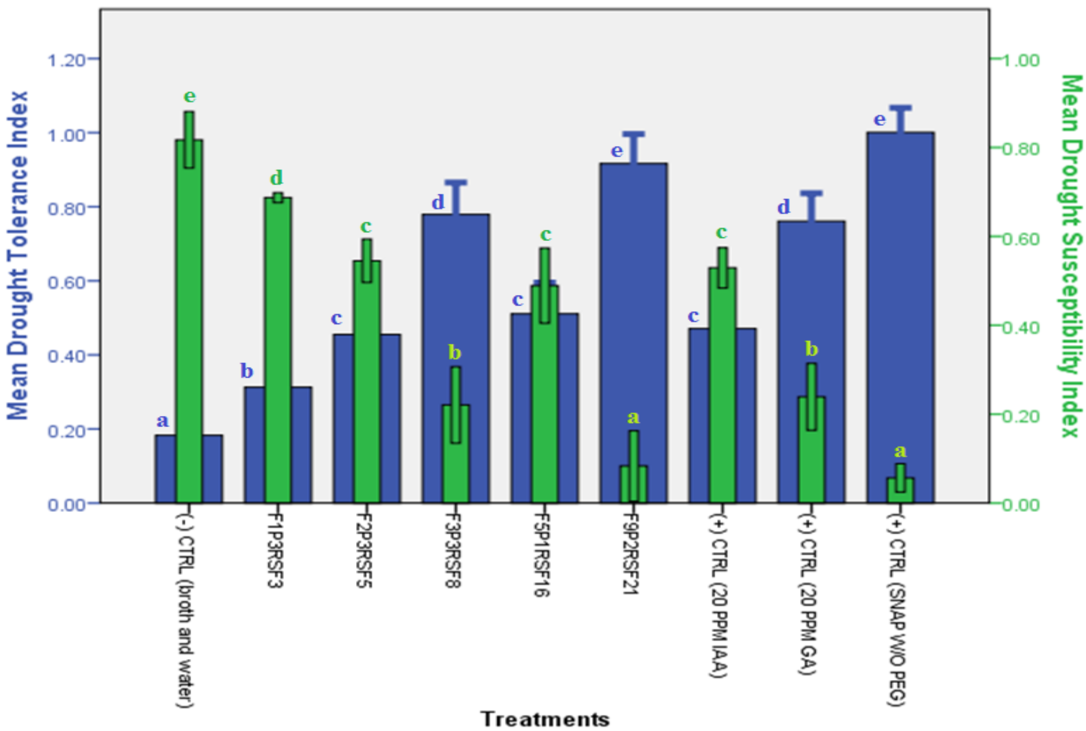


*Figure 3. Mean drought tolerance index (DTI) and mean drought susceptibility index (DSI) of RSF-treated rice seedlings. Control = broth and water, $F_1P_3RSF_3$ = Meyerozyma guilliermondii, $F_2P_3RSF_5$ = Trichoderma yunnanense, $F_3P_3RSF_8$ = Trichoderma simmonsii, $F_5P_1RSF_{16}$ = Unidentified Mucoromycotina, $F_9P_2RSF_{21}$ = Unidentified Mucoromycotina. Means with different letters are significantly different (ρ=0.05) n = 9. Mean DTI are shown as blue bars while mean DSI are shown as green bars. Error bars are + standard deviations*
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The results on the DTI were supported by the results on the mean DSI. The Mucoromycotina isolate ($F_9P_2RSF_{21}$) inoculated rice seedlings acquired the lowest mean DSI (0.08+0.08) which is significantly lower than the negative control (0.82+0.06 dsi), the GA (0.24+0.08 dsi) and IAA (0.53+0.05 dsi) controls, and those rice seedlings exposed to the four other RSF isolates (*M. guilliermondii*, *T. yunnanense*, *T. simmonsii* and unidentified Mucoromycotina isolate $F_5P_1RSF_{16}$). The DSI acquired by rice seedlings treated with the unidentified Mucoromycotina isolate ($F_9P_2RSF_{21}$) is subsequently comparable to those rice seedlings planted on non-drought SNAP medium (0.06+0.03 dsi). This indicates that the rice seedlings inoculated with the $F_9P_2RSF_{21}$ isolate has low susceptibility to drought. Consequently, it means that the RSF isolate

$F_9P_2RSF_{21}$ (unidentified Mucoromycotina) may have produced drought stress-inhibiting metabolites that elicited to the reduced drought susceptibility of its host.

It is also interesting to note that aside from the $F_9P_2RSF_{21}$ isolate, two other isolates have comparable mean DTI and DSI as compared to the positive controls (GA and IAA). The *T. simmonsii*-inoculated rice seedlings have DTI (0.78±0.09 dti) and DSI (0.22±0.09 dsi) comparable to the 20 ppm GA-treated rice seedlings (DTI = 0.76±0.08; DSI = 0.24±0.08). Likewise, *T. yunnanense*-treated rice seedlings have DTI (0.46±0.05 dti) and DSI (0.55±0.05 dsi) comparable to the 20 ppm IAA-treated rice seedlings (DTI = 0.47±0.05; DSI = 0.53±0.05). This indicates that the drought tolerance and drought susceptibility of the rice seedlings treated with these isolates are comparable to the rice seedlings applied with commercial hormones (GA and IAA). On the other hand, the *M. guilliermondii*-treated rice seedlings have the lowest DTI (0.31±0.01 dti)/highest DSI (0.69±0.01 dsi) among the other RSF-inoculated rice treatments. Although it may seem that the rice seedlings inoculated with M. *guilliermondii* have low drought tolerance and high susceptibility to drought stress compared to other RSF treatments, these values are still higher (DTI)/lower (DSI) than the mean values of the broth and water negative control (DTI = 0.18±0.06; DSI = 0.82±0.06).

The results of the study show that the five RSF isolates have different mechanisms in protecting rice seedlings against drought. However, one RSF isolate stood out. The unidentified Mucoromycotina isolate $F_9P_2RSF_{21}$ exhibited the highest SVI, highest DTI, and the lowest DSI. Raman *et al.* (2012) reported that rice seedlings with the highest seedling vigor have the least reduction of yield. Previous rice studies also showed that seedlings which had the highest drought tolerance and the lowest drought susceptibility were drought resistant compared to other rice seedlings (Chauhan *et al.*, 2007; Kumar *et al.*, 2014). The ability of rice seedlings treatments to do practically well during drought indicates production and rice yield stability (Kumar *et al.*, 2014), therefore, the results of this study are crucial as it affects rice production indirectly. However, it is necessary to point out that this study relied on the potential capacity of RSF isolates to provide certain protective mechanisms to their rice plant host experiencing drought stress. Previous studies have shown that symbiotic fungi are excellent producers of antioxidant and other radical scavenging metabolites (Zeng *et al.*, 2011; Govindappa *et al.*, 2013; Kandasamy andKandasamy, 2014; Yadav *et al.*, 2014; Cui *et al.*, 2015; Kumaresan *et al.*, 2015; Smith *et al.*, 2015; Venugopalan and Srivastava, 2015; Pan *et al.*, 2017). The symbiotic fungi has the ability to produce phenolics (Manach *et al.*, 2004; Liu *et al.*, 2007) and antioxidant enzymes (Chandra and Arora, 2009; Barbaneagra *et al.*, 2011; Cristica *et al.*, 2011) that help their host survive in stress conditions especially drought. By affecting plant morphology and growth, as well as biochemical and physiological responses to stress (Prasad *et al.*, 2012), symbiotic fungi can promote channels of radical scavenging, drought avoidance, drought tolerance and drought recovery in their host plants (Malinowski and Belesky, 2000). Hence, the rice seedlings' capability to survive and sustain growth and development under mild drought *in vitro* indicate the ability of their respective fungal symbionts to enhance their performance despite stressful conditions. Nonetheless, the facets of environmental drought tolerance are numerous and cannot simply be attributed to the potential ROS quenching mechanisms of the RSF isolates.

With the apparent consistency in the results pertaining to the potential drought stress tolerance capacity of RSF isolates on *D. quercifolia*, this study might be a potential breakthrough in the field of agriculture and rice research. The results on the plant shoot length, germination rate, seedling vigor index (SVI), drought tolerance index (DTI) and drought susceptibility index (DSI) reveal that one of the five isolates has induced potential drought tolerance effects in rice. The rice seedlings treated with the unidentified Mucoromycotina isolate $F_9P_2RSF_{21}$ appear to have the tallest shoots, 100% germination rate, highest SVI and DTI together with the lowest DSI which proves that might be the best and sustainable remedy for combating the extensive effects of drought in agriculture.

On top of these values, statistical data also revealed that the unidentified Mucoromycotina isolate $F_9P_2RSF_{21}$ approximated the plant shoot length, germination rate, SVI, DTI and DSI of those rice seedlings grown in non-drought conditions. The isolate also appeared to be statistically higher to all other RSF isolate treatments, positive controls (GA and IAA), and negative control (broth and water) with all the aforementioned parameters in the measurement of drought stress tolerance capacity. Based on these experimental results, indeed, the unidentified Mucoromycotina isolate $F_9P_2RSF_{21}$ is the best candidate for agricultural applications, particularly as the best contender for drought.

## CONCLUSION

The increasing tendencies of drought affecting agriculture also make the knowledge and practical applications on fungi-plant symbiosis an utmost priority. For this study, the best isolate (unidentified Mucoromycotina isolate $F_9P_2RSF_{21}$) to provide drought-tolerance effects on rice was identified. With this isolate's ability to provide drought-protective mechanisms to rice seedlings *in vitro*, its potential use in agriculture is undeniable. Overall, the results of this study are promising particularly in the agricultural industry. It is therefore recommended that more species of epiphytic ferns be investigated to discover other RSF isolates with drought-combatting potentials. In addition, confirmatory studies are hereby recommended to fully understand the physiological and biological mechanisms that helped the rice seedlings survive in drought-stressed conditions to facilitate in the screening and selection RSF isolates with potential drought tolerance impact to the world's most important agricultural produce, rice.

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## REFERENCES

Al-Askar, A. A. 2016. Bioactive compounds produced by *Trichoderma harzianum* 1-SSR for controlling *Fusarium verticillioides* (Sacc.) Nirenberg and Growth Promotion of *Sorghum vulgare*. Egyp. J. Biol. Pest Cont., 26(2):379-386.

Amprayn, K., M. Rose, M. Kecskes, L. Pereg, H. Nguyen and I. Kennedy. 2012. Plant growth promoting characteristics of soil yeast (*Candida tropicalis* HY) and its effectiveness for promoting rice growth. Appl. Soil Ecol., 61:295-299.

Arora, D.S. and P. Chandra. 2010. Assay of antioxidant potential of two *Aspergillus* isolates by different methods under various physio-chemical conditions. Braz. J. Microbiol., 41:765–777.

Barba, R.Jr, N. Marquez and R. Tablizo. 2014. Screening for drought-tolerant and low-input responsive upland rice landraces. Am. J. Pla. Sci., 5:3432-3439.

Barbaneagra, T., A. Manoliu, M. Cristica, E. Ciornea and E. Tutu. 2011. The analysis of catalase and peroxidase activity in saprophytic fungus *Rhizopus nigricans* grown on medium with different concentration of grinded corn caryopsis. Genet. Mol. Biol., 12(3):83-88.

Chandra, P. and D. S. Arora. 2009. Antioxidant activity of fungi isolated from soil of different areas of Pubjab, India. J. Appl. Natur. Sci., 1(2):123-128.

Chauhan, J.S., M. K. Tyagi, A. Kumar, N. I. Nashaat, M. Singh, N. B. Singh, M. L. Jakhar and S. J. Welham. 2007. Drought effects on yield and its components in Indian mustard (*Brassica juncea* L.). Plant Breed., 126:399-402.

Cristica, M., A. Manoliu, T. Barbanegra and E. Coimea.

2011. Compared analysis of catalase and peroxidase activity in cellulolytic fungus *Trichoderma reesei* grown on medium with different concentrations of grinded wheat and barley straws. Genet. Mol. Biol., 12(3):89-93.

Cui, J.L., T.T. Guo, Z.X. Ren and M.L. Wang. 2015. Diversity and antioxidant activity of culturable endophytic fungi from alpine plants of *Rhodiola crenulala*, *R. angusta*, and *R. sachalinensis*. PLoS ONE., 10(3):1721-1728.

Dhankhar, S., S. Kumar, S. Dhankhar and J. Yadav. 2012. Antioxidant activity of fungal symbionts isolated from *Salvadora oleoides* Decne. Int. J. Pharm. Pharm. Sci. 4(2):380-385.

Elavarthi, S. and B. Martin. 2010. Spectrophotometric assays for antioxidant enzymes in plants. Methods Mol. Biol., 639: 273-281.

Fukai, S. and M. Cooper. 1995. Development of drought-resistant cultivars using physio-morphological traits in rice. Field Crops Res., 40:67-86.

Gibson, M.S. 2008. Measurement of vigor in seeds or seedlings. Sugarbeet Res. Ext. Rep., 11:143-147.

Govindappa, M., R. Channabasava, K. R. Sunil Kumar and K. C. Puspalatha. 2013. Antioxidant activity and phytochemical screening of crude endophytes extracts of *Tabebuia argentea* Bur. & K. Sch. Am. J. Pla. Sci., 4:1641-1652.

Kandasamy, S. and K. Kandasamy. 2014. Antioxidant activity of the mangrove endophytic fungus (*Trichoderma* sp.). J. Coast. Life Med., 2(7):566-570.

Kumar, S., S. Dwivedi, S. Singh, S. Jha, S. Lekshmy, R. Elanchezhian, O. Singh and B. Bhatt. 2014. Identification of drought tolerant rice genotypes by analysing drought tolerance indices and morpho-physiological traits. SABRAO J. Breed. Genet., 46(2):217-230.

Kumaresan, S., V. Karthi, V. Senthilkumar, B. S. Balakumar and A. Stephen. 2015. Biochemical constituents and antioxidant potential of endophytic fungi isolated from the leaves of *Azadirachta indica* A. Juss (Neem) from Chennai, India. J. Acad. Indus. Res., 3(8): 355-361.

Liu, D., S. Coloe, R. Baird and J. Pedersen. 2010. Rapid Mini-Preparation of Fungal DNA for PCR. J. Clin. Microbiol., 38(1):471.

Liu, X., X. Dong, M. Chen, X. Jiang and G. Yan. 2007. Antioxidant activity and phenolics of symbiotic *Xylaria* sp. from *Ginkgo biloba*. Food Chem., 105: 548-554.

Ludwig-Müller, J. 2015. Plants and endophytes: equal partners in secondary metabolite production? Biotechnol. Lett., 37:1325–1334.

Malinowski, D and D. Belesky. 2000. Adaptations of symbiont-infected cool-season grasses to environmental stresses: mechanisms of drought and mineral stress tolerance. Crop Sci., 40:923-940.

Manach, C., A. Scalbert, C. Morand, C. Remesy and L. Jimenez. 2004. Polyphenols: Food sources and bioavailability. Am. J. Clin. Nutr., 79:727-747.

Michel, B.E. 1983. Evaluation of the Water Potentials of Solutions of Polyethylene Glycol 8000. Plant Physiol., 72:66–70.

Pan, F., T.-J. Su, S.-M. Cai and W. Wu. 2017. Fungal endophyte-derived *Fritillaria unibracteata* var. wabuensis: diversity, antioxidant capacities *in vitro* and relations to phenolic, flavonoid or saponin compounds. Sci. Rep., 7:42008. http://doi.org/10.1038/srep42008.

Pandey, V. and A. Shukla. 2015. Acclimation and tolerance strategies of rice under drought stress. Rice Sci., 22(4):147-161.

Prasad, R., S. Kamal, P.K. Sharma, R. Oelmüller and A. Varma. 2012. Root endophyte *Piriformospora indica* DSM 11827 alters plant morphology, enhances biomass and antioxidant activity of medicinal plant *Bacopa monniera*. J. Basic. Microb., 53:1016–1024.

Raman, A., S. Verulkar, N.P. Mandal, M. Varrier, V.D. Shukla, J.L. Dwivedi, B.N. Singh, O.N. Singh, P. Swain, A.K. Mall, S. Robin, R. Chandrababu, A. Jain, T.R. Ram, S. Hittalmani, S. Haefele, H.S. Piepho and A. Kumar. 2012. Drought yield index to select high yielding rice lines under different drought stress severities. Rice., 5(31):1-12.

Ravindran, C. and T. Naveenan. 2011. Adaptation of marine derived fungus *Chaetomium globosum* (NIOCC 36) to alkaline stress using antioxidant properties. Process Biochem. 46(4):847-857.

Santos, P. J. and E. T. Ocampo. 2005. SNAP hydroponics: Development & potential for urban vegetable production. Phillip. J. Crop Sci., 30(2):3-11.

Selosse, M. and F. Le Tacon. 1998. The land flora: A phototroph-fungus partnership? Trends Ecol. Evolut., 13(1):15-20.

Smith, H., S. Doyle and R. Murphy. 2015. Filamentous fungi as a source of natural antioxidants. Food Chem., 185:389–397.

Sugiharto, S., T. Yudiarti and I. Isroli. 2016. Assay of antioxidant potentials of two filamentous fungi isolated from the Indonesian fermented dried cassava. Antioxidants., 5(1):215-223.

Trachsel, S., P. Stamp and A. Hund. 2010. Growth of axile and lateral roots of maize: Response to desiccation stress induced by polyethylene glycol 8000. Maydica ,55:101-109.

Valencia, C. 2015. Philippine Rice brings out drought-resistant rice varieties. Philippine Star Global. Retrieved March 30, 2017 from http://www.philstar.com/agriculture/2015/09/06/1496503/philippine-rice-brings-out-drought-resistant-rice-varieties.

Venugopalan, A and S. Srivastava. 2015. Endophytes as *in vitro* production platforms of high value plant secondary metabolites. Biotechnol. Adv., 33:873–887.

Yadav, M., A. Yadav and J. Yadav. 2014. *In vitro* antioxidant activity and total phenolic content of symbiotic fungi isolated from *Eugenia jambolana* Lam. Asian Pac. J. Trop. Biomed., 7(1):256-261.

Zeng, P., J. Wu, L. Liao, T. Chen, J. Wu and K. Wong. 2011. *In vitro* antioxidant activities of symbiotic fungi isolated from the liverwort *Scapania verrucosa*. Genet. Mol. Res., 10(4):3169-3179.