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論文の内容の要約

氏　　名	Ousmane Diene
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【論文の内容の要約】

※課題設定、方法論、実験・解析、結論・考察など、当該論文の全体がわかる形で作成してください。

The overall results of these studies suggest that it is now clear that the population of dark septate endophytic fungi in Japan is richer than previously thought and comprises undiscribed species, which is demonstrated by the description of the endophytic fungal species *Pseudosigmoidea ibarakiensis* recovered from forest soil in Ibaraki, Japan. This endophytic fungal population has a great potential in supporting plant growth in variable abiotic stress conditions, including nitrogen deficiency, tolerance of extra acid pH and cadmium and cesium contamination. The knowledge earned from these will help understand how fungal endophytes can contribute to improve agriculture development through increase land availability.

1. Species richness of fungal endophyte population in Japan

Japan has coniferous rainforests, characteristic of warmer temperate latitudes. Therefore, whether undiscribed species could be found therein was highly questionable. Beside *Heteroconium chaetospira* which is the common fungal endophyte known from Japan, there have been several other new records of endophytes, including *Veronaeopsis simplex*, *Helminthosporium velutinum*, and *Scolecobacidium humicola*, (Narisawa, 2008; Diene *et al.*, 2010; Mahmoud and Narisawa, 2014). Other endophytes already reported from other temperate latitudes were also reported and included *Phaelocephala fortinii*, *Leptodontidium orchidicola*, and *Meliniomyces variabilis*. All these previous reports were related to endophytic status observed for the first time in some fungal species but could not describe any new fungal species. Therefore the description of *Pseudosigmoidea ibarakiensis* (Diene *et al.*, 2013) was to our knowledge the first report a new DSE taxon from Japan. Although it was only one species that was described in this study, it is sufficient enough to prove that other undescribed DSE species can be found in warmer temperate latitudes. There is a great number of fungal species of unknown and/or undescribed endophytes left over the 1 million estimates of the total endophyte species made by Ganley (2004). It is well known that much of the taxonomy and ecology of fungal endophytes particularly are unknown. Therefore, chance of getting new records are real and it will be necessary to continue collecting and identifying fungal endophytes in these warmer areas in order to assess their species richness but also to take advantage of endophytes with respect to the challenging needs of potential isolates to use for restoring non-arable lands existing in different places with a variety of climates for agricultural use. The new species along with *H. velutinum*, possessed the particular and major ability to colonize host plants inoculated root, stem, and leaf parts. *Pseudosigmoidea ibarakiensis* showed colonization rates of 100% on roots and stem, and 93% on leaves of host plant, whereas *H. velutinum* showed 26%, 33% and 73% on leaves, stems and roots, respectively. Fungal endophytes applications are mainly carried out by soil inoculation in the field (Narisawa *et al.*, 1998; Usuki *et al.*, 2002; Ohtaka, *et al.*, 2008) even though spray method has been experimented in laboratory conditions using endophytic entomopathogenic fungal species (Posada *et al.*, 2007; Parsa *et al.* 2013). With this new finding, the recurrent problem of endophyte application in field conditions can be solved. Such quality would be able to provide an easy inoculation method in the field whenever these isolates are set for use in a large scale conditions to increase the rate of colonization of host plants.

2. Tolerance of abiotic stress

Abiotic stresses tested in these studies were related to nutrient deficiency, unfavorable pH, and metals contamination through cesium and cadmium. Under each type of stress, at least one of the tested fungal isolates has been able to support plant growth, suggesting that fungal endophytes offer a starting point for resolving the tremendous abiotic stress problems in a sustainable way.

With the abilities conferred to host plants by *P. ibarakiensis* to stand ultra low pH, and to utilize organic nitrogen and support their growth with no need of nitrogen supply, it is a huge step which has been crossed over. Until now, strategies involved in the mitigation of soil pH such as liming techniques and inorganic amendments (Caires *et al*., 2008; Grabowska, 2011) or use of by-products (Li *et al.*, 2010) have shown to be both difficult to apply, specially at large scale farming, and not sustainable. Fungal endophytes therefore shed a light for a practical and sustainable manner to exploit soils with unfavorable pH conditions in general and extra acid pH particularly, at least under laboratory conditions. Interestingly, the problem of low nitrogen uptake always related to low pH might found a solution with such fungi. Different endophytes seem to play different ecological roles, but based on these findings, *P. ibarakiensis* combines pH tolerance ability and simultaneous organic nitrogen usage capability. The rare properties of capability of using effectively organic nitrogen, tolerance to extra low pH, and ability to colonize root stem and leaves can be exploited for its effective disposition in agriculture production to cope with abiotic stresses.

The growing use of biofuel worldwide has raised concerns about the competition for land and water of such crops with food crops. Finding land where to grow biomass needed to produce bioenergy has become one of the most limiting factors biofuel production is facing. Results are promising in terms of measures to support assignment of such biomass production in abandoned poor nutrient conditions. Sweet sorghum which is praised for its potential as bioethanol plant has shown significant improved biomass production when inoculated with *H. velutinum* fungal endophyte. *Pseudosigmoidea ibarakiensis* isolate I.4-2-1, in spite its good properties described earlier has not been able to promote sweet sorghum plant growth in the conditions used in the study. However, we can expect that under ultra low pH conditions, it might promote sweet sorghum growth as it is the case with Chinese cabbage; the principle of DSE conferred host tolerance being more related to habitat than host plants (Rodriguez, 2008; Redman *et al.*, 2011). These results also suggest that fungal endophytes offer potential to significantly increase land availability for food crop production. Definitely, land expansion for Buofuel production is a reality all around the world. In major biofuel producing countries like Brazil, the USA, Indonesia, South, and so on, an increase of 25 million ha in the biofuel land use has occurred between 2000 and 2010, whereas agricultural land has decreased by 9 million ha (Langeveld *et al.*, 2013). Shifting the fuel biomass production from the food crop production lands, as it is the mostly the case nowadays to abandoned lands where it will be supported basically by potential fungal endophytes for nutrients provision, can help save agriculture land availability, and furthermore increase the availability. Fertilizers will only be applied to complement the use of the fungi, where necessary. When all 385-472 million ha of abandoned lands, according to estimates made by Campbell *et al.* (2008), are taken into consideration, concerns about agricultural land might by far overtaken.

For the two other abiotic stress inducers, cesium and cadmium, they are reported as one of the today's most significant environmental problems (Chand *et al.*, 2012). In addition to being plant growth limiting factors, they particularly represent serious human and animal health threats associated with risks of their accumulation in food chain (Zurera-Cosano *et al*., 1989; Mohajer *et al*., 2012). The management of such stress inducers seems apparently and certainly more difficult. The results suggest that fungal endophytes have potential to clean up lands contaminated with such pollutants using an endophyte-assisted phytoremediation process, just as plant growth promoting rhizobacteria (Belimov *et al*., 2005; Tak *et al*., 2013). This would be specific to Cs contamination removal by endophytes (*H. velutinum*, *P. ibarakiensis*, *V. simplex*)-inoculated Chinese cabbage offering improvement of both plant growth and Cs accumulation capacities. This advantage could be also extended to removal of Cd contamination applied with tomato plants.

But fungal endophytes offer also in a more practical way the outstanding very unique possibility to directly grow food crops in Cs contaminated lands with subsequent low risk of contaminating the food chain. The influence of DSE fungal symbiosis has suggested a low Cs risk transfer on the food chain as a result of high and negative plant reactions rather than high and positive reactions. The combination of endophyte-assisted phytoremediation and the direct cultivation of plants on Cs contaminated lands when exploited can improve land availability and safe food production.

Studies has reveled that metal contaminants specially Cd concentration in soil has been reduced from different crops by use of different commonly used methods, including inorganic amendments (Cattani *et al*., 2008), fertilization with the use of Zn to reduce uptake by plants (Moustakas *et al*., 2011; Dar *et al*., 2012) and even agronomic practices such as crop rotation (Islam *et al*., 2007). With fungal endophytes, crop rotation can be improved in order to augment the capacity of biofuel plants such as sweet sorghum to uptake Cd from contaminated lands. Fungal endophytes did not show ability to reduce Cd uptake but in contrast showed increased accumulation ability. The fungal isolate *H. velutinum* 41-1 which improved the biomass production of the bioethanol plant, sweet sorghum, and also conferred to tomato plants higher Cd accumulation ability, suggesting that the use of such combination can be a viable alternative in bioremediation of Cd contaminated soils.

3 Conclusion

The described fungal species *P. ibarakiensis* has been of capital interest as it showed along with *H. velutinum* intrinsic qualities that are not very common to fungal endophytes, mainly the ability of colonizing whole host plants parts. Our research furthermore pointed out new fields of application of fungal endophytes, including plant biomass production for biofuel production and the mitigation of abiotic stresses induced by low pH, metal contaminants, etc. that are today serious constraints for agricultural production land availability worldwide, and for which techniques commonly used did not come out with reliable sustainable management practices. Remarkably, endophytes came out with a amazing capability of direct cultivation of editable crops on Cs contaminated soils, which if correctly exploited can reduce considerably risks related to the contamination of the food chain with radionuclides. If endophytes can limit the translocation of metal contaminants to the edible plants parts, at least under low contamination conditions, while simultaneously supporting cultivated plant growth, it is huge step with no precedent that is achieved in the food safety.

We believe that such essential properties cannot be a simple response of nutrients provision and positive host response but migh go beyond, suggesting a multifunctionality of DSE-plant symbiosis as suggested by Mandyam and Jumpponen (2005).

With the potential shown by fungal endophytes in this study, challenges to agricultural production such as low pH and its related metal contamination problems may be amenable to simple solutions in the future if the use of fungal endophytes allows farmers to convert lands affected by these abiotic stress problems into productive arable lands.

The achievement of such ambitious objectives might necessitate the assessment of the species richness of fungal endophytes for finding various potential candidates within the vast endophytes population existing in the natural environments and which have not yet been discovered in order to fully understand their ecological roles, their specific properties in relation to abiotic stress, and their adaptability to different climatic conditions.

The practical aspects of this use will also have to be researched so farmers can learn how to integrate these species within pre-existing ecologically sound agricultural methods so as to ensure continuity in the approach to sustainability. The method of seeds coating with a pool of endophytes developed by Adaptive Symbiotic Technologies Company (Seattle, WA, USA) can serve as a model for the practical usage of fungal endophytes.

It will also be necessary to build awareness of this new field of research among decision makers and farmers to improve interactions and collaboration with scientists working in different fields, thereby encouraging the integration of endophytes in programs being developed for contaminated site management in different countries, and their adoption in agriculture and maximizing their benefits.