

## Characterization of Endomycorrhizal Strains from *Casuarina equisetifolia* to be Used for Reforestation and Rehabilitation of Degraded Ecosystems in Senegal

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**Abstract** In recent decades, the loss of ecosystem productivity has been accelerated in the Sahelian zone. This decrease is caused by human and natural effects, which result in deforestation and degradation of tropical lands. It has been demonstrated that fast-growing trees such as Australian species could promote the rehabilitation of degraded West African lands. Similarly, *Casuarina equisetifolia* an Australian native tree was established in Senegal. *Casuarina* plantation plays an important role in stabilizing coastal dunes, helping to protect adjacent agricultural areas by acting as a windbreak. *C. equisetifolia* develops nitrogen-fixing nodules in association with the soil actinomycete *Frankia* and a mycorrhizal symbiosis in association with arbuscular mycorrhizal fungi (AMF). It has been previously demonstrated that actinorhizal and AMF symbiosis enhance *Casuarina* species development. The symbiotic relationship between AMF and *Casuarina* species improves phosphate and nitrogen uptake. Fungal symbionts significantly improve the development of *C. equisetifolia*. The aim of this study was to assess the effects of fungal strains collected in Australia, the *C. equisetifolia* origin zone and strains collected in Senegal, where *C. equisetifolia* was introduced. *C. equisetifolia* plants were inoculated with Senegalese, Australian strains and/or *Frankia* bacteria CcB. After growing in the greenhouse, plants were harvested and parameters like shoot and root biomass, and arbuscular mycorrhizal colonization were assessed. In parallel, we analysed the expression of marker genes encoding a mycorrhizal phosphate transporter, an hemoglobin gene, a marker gene involved in *Frankia* infection and a marker gene encoding the nitrogenase. We evaluated the activity of mycorrhiza using histochemical techniques. Our results showed that Senegalese strains have a positive effect on *C. equisetifolia* growth and this correlates with an increased expression of symbiotic marker genes and with increased metabolic

activity. Our work will help to select the most efficient fungal strains to be used for the rehabilitation of Senegalese soils.

## 1 Introduction

In West Africa, ecosystem fertility decreases every year. The degradation of the ecosystem is generally due to natural and/or anthropological effects. To restore soil fertility in these areas, applications of chemical fertilizers are often used. However, these chemical products are expensive and not ecofriendly. An alternative strategy to increase soil fertility is the use of symbiotic fungi and bacteria. These microorganisms improve the plant growth by enhancing the uptake of nutrients and water. Several Australian trees have been introduced to West Africa. They are characterized by early rapid growth even on poor and disturbed soils. It has been demonstrated that Australian species could promote the rehabilitation of degraded ecosystems. In Senegal, *C. equisetifolia* was established in the Niayes region through a reforestation programme that began in 1948. This plantation is localized in the North littoral of Senegal between Dakar (west of Senegal) and St Louis (north of Senegal). It plays an important role in stabilizing coastal dunes, helping to protect adjacent agricultural areas by acting as a windbreak. The Niayes region production contributes to about 65% of the national vegetable production (Ba, 1991). The rehabilitation programme of Sahelian zone was performed using tree species able to achieve a symbiotic relationship with mycorrhizal fungi and nitrogen-fixing bacteria. *C. equisetifolia* forms a symbiosis with ectomycorrhizal (EM) and arbuscular mycorrhizal fungi (AMF). Our study was carried out with arbuscular mycorrhizal fungi, which are obligate biotrophs being unculturable in the absence of plants host (Declerck *et al.*, 2005). AMF symbiosis is a symbiotic relationship between the plant and the fungus of the Glomeromycota phylum (Schüssler *et al.*, 2001). This symbiosis is the most widespread and the oldest association dates back to the Devonian (Smith and Read, 2008).

AMF are associated with the roots of about 80% plant species. As a result of this symbiosis, the fungi deliver inorganic phosphate (Pi), nitrogen and other micronutrients, in turn they receive carbohydrates from plant (Smith and Read, 2008). AMF may consume up to 20% of total net plant photosynthetic carbon (Jacobson and Rosendahl, 1990). In addition to their biological fertilizer role, AMF increase plants production and also confer resistance against abiotic and biotic stresses (Evelin *et al.*, 2009; Li *et al.*, 2010). Duponnois *et al.* (2003) demonstrated that the inoculation of *C. glauca* and *Allocasuarina verticillata* with *Glomus intraradices* increased plant shoot biomass 1.8 and 2.6 times respectively. *Casuarina* also forms a symbiotic relationship with the soil actinomycete *Frankia*. With their nitrogen-fixing ability *Casuarina* trees increase the nitrogen content in soil. Forrester *et al.* (2006) demonstrated that *Casuarina* contributes to N uptake and increases indirectly the amount of nitrogen available to non-fixing trees planted in mixed plantations. Similar results were obtained by Dommergues and Subba-Rao (2000), who have shown that the total biomass of non nitrogen-fixing *Eucalyptus robusta* was higher when planted in mixture with *C. equisetifolia* than when planted as pure species. In an attempt to restore degraded ecosystems in Senegal, *C. equisetifolia* was introduced in the Niayes region.

The aim of this work is to study the effects of Australian and Senegalese fungal inoculation on *C. equisetifolia* growth and characterize the basis of differences obtained by using molecular and histochemical markers.

## 2 Material and Methods

### 2.1 Plant, fungi and bacteria

Seeds of *C. equisetifolia* were collected in Notto Gouye Diama (north of Thies, Senegal). Australian fungi were collected in Queensland under casuarina stand and Senegalese fungi were collected under Bel-Air casuarina plantation (Dakar). Fungal inoculum was prepared by extracting spores from a maize trap over 3-month period. A sandy sterile soil was inoculated with the trap soil containing propagules. For the actinorhizal symbiosis, plants were inoculated with the *Frankia CcB3*. The experiment was carried out in greenhouse. After 6 months period, 8 plants for each treatment were harvested and parameters like shoot and root biomass and mycorrhizal colonization were assessed. Histochemical activities and molecular analyses were carried out with plant growing in greenhouse during 4 months using 3 pooled plants.

### 2.2 Histochemical activities

After harvested, viable mycorrhiza roots were gently washed with tap water. Three plants were pooled to evaluate the metabolic activity in mycorrhizal roots using histochemical techniques. All metabolically active AMF structures (Hyphae, vesicle, spores and arbuscules) were evaluated in roots by staining with Nitro Blue Tetrazolium (NBT) as described by Vierheilig *et al.* (2001; 2005). In order to evaluate the senescence of mycorrhizal structures, roots were stained with the Diaminobenzidine (DAB) as described by Vierheilig *et al.* (2001). The intensities of metabolically active AMF structures and clumped structures were assessed using Trouvelot method (Trouvelot *et al.*, 1986).

### 2.3 Expression of marker genes

Expression of molecular markers was used to compare the effect of Australian and Senegalese fungi using the half part of the 3 pooled plants and 3 replicates were carried out to analyse the expression of marker genes.

For each symbiosis, a marker gene was isolated. For the mycorrhizal symbiosis, we isolated the *CePT4* gene encoded a phosphate transporter specifically expressed in mycorrhizal roots. As markers of the actinorhizal symbiosis we isolated *Ce12*, an ortholog of *Cg12*, a marker gene expressed specifically during *Frankia* infection (Svistoonoff *et al.*, 2003) and *CeHB*, an ortholog of *CgHb*, a symbiotic hemoglobin gene expressed in nodules (Jacobsen-Lyon *et al.*, 1995). Finally, we used the *Frankia* gene *NifH* encoding the nitrogenase (Normand *et al.*, 2007).

As an internal control gene, we isolated a gene coding an ubiquitin which is expressed constitutively. After harvesting, plant material was immediately frozen in liquid nitrogen and stored at - 80°C. The total RNA of mycorrhizal and nodulated roots was extracted using the ultracentrifugation method based on sedimentation of RNA through the cesium chloride as described by Chirgwin *et al.* (1979). RNA was purified using the Turbo DNase-free™ to remove contaminating genomic DNA (Udvardi *et al.*, 2008). The reverse transcriptase and the Real-Time PCR were carried out as described by Gherbi *et al.* (2008). The results were standardized with *CeUbi* expression level (Hoche *et al.*, 2006).

## 3 Results

Our results showed that inoculation with Australian and Senegalese fungal strains significantly

stimulated *C. equisetifolia* growth and mycorrhizal colonization in greenhouse conditions. The histochemical studies carried out with the mycorrhizal roots to evaluate the metabolic activity using enzyme such as succinate dehydrogenase (SDH) showed metabolically AMF structures active within roots. These observations are only possible when the roots were stained with the Nitro Blue Tetrazolium which reacts with the succinate dehydrogenase enzyme. Stronger metabolic activity in AMF was found within mycorrhizal plants roots inoculated with Senegalese fungi. Staining with the Diaminobenzidine allowed the visualization of clumped structures. A higher DAB activity was observed in roots which contained the lowest SDH metabolic activity AMF. In parallel, qPCR analysis showed a higher expression level of *CePT4* in plants inoculated with Senegalese fungi compared to those inoculated with Australian fungi. The expression of marker gene involved in actinorhizal symbiosis showed that expression of *Ce12*, *CeHB* and *Nif* marker genes involved in the actinorhizal symbiosis was up regulated in mycorrhizal roots inoculated with Australian fungus. Co-inoculation with fungi and bacteria increased *Ce12* and *Nif* expression in roots.

## 4 Discussion

Our study showed that inoculation with mycorrhizal fungi increased *C. equisetifolia* growth. This effect could be attributed to the fungus strains which improve nutrient uptake and directly increase *Casuarina* growth (Seddas *et al.*, 2009). Our results showed that the fungi inoculation increased the shoot biomass. Similar results were obtained by Rajendran and Devaraj (2004) who demonstrated that the inoculation with AMF increased the biomass and the growth of *C. equisetifolia*. This positive effect is probably due to the enhancement of shoot branching. The important branching could be attributed to an effectiveness of the mycorrhizal symbiosis which enhances the amount of nutrient uptakes by the fungus. It is well known that AMF with their phosphatase enzyme activities can enhance Pi availability for roots. Soluble Pi is directly absorbed and transported to the periarbuscular membrane by fungi before being released to the plant through a plant specific phosphate transporter.

The positive effect of fungal strains on *C. equisetifolia* development is also correlated with an increased metabolic activity in mycorrhizal roots staining with SDH. SDH is a tricarboxylic acid cycle enzyme present in AMF cycle and it reacts with the Nitro Blue Tetrazolium resulting in insoluble dark blue-purple formazan clearly distinguished in roots (Vierheilig *et al.*, 2005). Inoculation with Senegalese fungi showed the highest metabolic activity in roots; due to the presence of many viable arbuscules. It has been demonstrated that the amount of living arbuscules could reflect a surface area for nutrient exchange between living symbionts and symbiotic efficiency (Smith and Dickson, 1991). The efficiency of mycorrhizal symbiosis in plants inoculated with Senegalese fungi is also probably linked with the low number of senescent structures within the roots. In opposite, a higher DAB activity was observed in plants inoculated with Australian fungi and Vierheilig *et al.* (2001) demonstrated that their clumped structures are collapsed arbuscules detected through the presence of H<sub>2</sub>O<sub>2</sub>. Alexander *et al.* (1988) and Blee and Anderson (1996) have demonstrated that H<sub>2</sub>O<sub>2</sub> induction occurred in the later stages of arbuscule life cycle when they degenerate. These results could explain the basis of differences obtained between plants inoculated either with Senegalese or Australian strains. Our results also suggest that the inoculation with Australian fungi and *Cc13* results in an earlier arbuscule development which would cause their rapid degeneration compared to plants inoculated only with Australian fungi.

Since *CePT4* encodes a symbiotic phosphate transporter, the higher *CePT4* expression in mycorrhizal roots inoculated with Senegalese fungi would results in a higher level of Pi which is linked to the

effectiveness of mycorrhizal symbiosis. In *Medicago truncatula*, this phosphate transporter is localized in the periarbuscular membrane and its expression is probably coordinated to arbuscular development and degeneration (Harrison *et al.*, 2002; Pumplin and Harrison, 2009). It has been demonstrated that optimal Pi absorption occurs in mature arbuscules (Pumplin and Harrison, 2009). In *C. equisetifolia*, *CePT4* is also probably a marker of functional symbiosis and allows a continuous arbuscule development. Javot *et al.* (2007) showed that without this mycorrhiza specific phosphate transporter, arbuscules died prematurely. Recent studies showed that, the arbuscule lifetime is influenced by their ability to deliver phosphate (Javot *et al.*, 2007). The mutation of *MtPT4* results in a premature degradation of arbuscules (Javot *et al.*, 2007) demonstrating a correlation between *MtPT4* expression and the metabolic activity in roots. The post regulation of *CePT4* expression obtained in mycorrhizal roots with Australian fungi, corresponding to a lower metabolic activity and a higher DAB activity within these roots may be related to a weaker effectiveness of the mycorrhizal symbiosis.

The expression levels of the molecular markers involved in actinorhizal symbiosis is higher in mycorrhizal roots inoculated with Australian fungus compared with roots inoculated with Senegalese fungi. Since the fungal inoculum was collected under *Casuarina* natural stand, inoculum from Australia (the native region) may be associated with more efficient bacteria. The efficiency of these *Frankia* induce a better actinorhizal symbiosis development and hence increase the expression of molecular markers involved in this symbiosis like *Ce12*, *CeHb* and *NifH*.

Despite these positive effects, the best *C. equisetifolia* development occurred in plants inoculated with Senegalese fungi. This result suggests that under the conditions tested the enhancement of the mycorrhizal symbiosis has a stronger effect on plant growth than the enhancement of the actinorhizal symbiosis.

## 5 Conclusion

Our results show that Senegalese fungi have higher positive effect on *C. equisetifolia* growth probably because these strains are already adapted to the local conditions. The marker genes isolated allow study on the molecular basis of growth enhancement by AMF or *Frankia*.

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## References

- Alexander, T., Meier, R., Toth, R. and Weber, H.C. 1988. Dynamics of arbuscule development and degeneration in mycorrhizas of *Triticum aestivum* L. and *Avena sativa* L. with reference to *Zea mays* L. *New Phytologist*, 110: 363-370.
- Ba, M. 1991. Les systèmes d'élevage dans la zone des Niayes. ISRA Etudes et Documents, Vol 4: 14.
- Blee, K. A. and Anderson, A. J. 1996. Defense-related transcript accumulation in *Phaseolus vulgaris* L. colonized by the arbuscular mycorrhizal fungus *Glomus intraradices* Schenk & Smith. *Plant Physiologist*, 110: 675-688.
- Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. and Rutter, W. J. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry*, 18: 5294-5299.
- Declercq, S., Strullu, D. G. and Fortin, J. A. 2005. *In vitro* culture of mycorrhizas. In: Varma A (ed) *Soil*

- biology, Springer Berlin, vol 4, pp388-400.
- Dommergues, Y. R. and Subba-Rao, N. S. 2000. Introduction of N<sub>2</sub>-fixing trees in non-N<sub>2</sub>-fixing tropical plantations. *In*: Subba-Rao, N. S. and Dommergues, Y. R. (eds), *Microbial Interactions in Agriculture and Forestry*. Science Publishers, Enfield, N. H, USA, pp131-154.
- Duponnois, R., Diedhiou, S., Chotte, J. L. and Sy, M. O. 2003. Relative importance of the endomycorrhizal and/or ectomycorrhizal associations in *Allocasuarina* and *Casuarina* genera. *Canadian Journal of Microbiology*, 50: 691-696.
- Evelin, H., Kapoori, R. and Giri, B. 2009. Arbuscular mycorrhizal fungi in alleviation of salt stress; a review. *Annals of Botany*, 104: 1263-1280.
- Forrester, D. I., Bauhus, J., Cowie, A. L. and Vanclay, J. K. 2006. Mixed-species plantations of *Eucalyptus* with nitrogen-fixing trees: a review. *Forest Ecology and Management*, 233: 211-230.
- Gherbi, H., Markmann, K., Svistoonoff, S., Estevan, J., Autran, D., Giczey, G., Auguy, F., Peret, B., Laplaze, L., Franche, C., Parniske, M. and Bogusz, D. 2008. *SymRK* defines a common genetic basis for plant root endosymbioses with AM fungi, *rhizobia* and *Frankia* bacteria. *Proceedings of National Academy of Sciences of the United States of America*, 105: 4928-4932.
- Harrison, M. J., Dewbre, G. R. and Liu, J. 2002. A phosphate transporter from *Medicago truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. *The Plant Cell*, 14: 2413-2429.
- Hoher, V., Auguy, F., Argout, X., Laplaze, L., Franche, C. and Bogusz, D. 2006. Expressed sequence-tag analysis in *Casuarina glauca* actinorhizal nodule and root. *New Phytologist*, 169: 681-688.
- Jacobsen, I., Rosendahl, L. 1990. Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. *New Phytologist*, 115: 77-83.
- Jacobsen-Lyon, K., Jensen, E. O., Jørgensen, J., Marcker, K. A., Peacock, W. J. and Dennis, E. S. 1995. Symbiotic and nonsymbiotic hemoglobin genes of *Casuarina glauca*. *The Plant cell*, 7: 213-223.
- Javot, H., Penmetza, R. V., Terzaghi, N., Cook, D. R. and Harrison, M. J. 2007. A *Medicago truncatula* phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. *Proceedings of National Academy of Sciences of the United States of America*, 104: 1720-1725.
- Li, R., Jiang, Y., Xu, J., Zhou, B., Ma, C., Liu, C., Yang, C., Xiao, Y., Xu, Q. and Hao, L. 2010. Synergistic action of exogenous salicylic acid and arbuscular mycorrhizal fungus colonization in *Avena nuda* seedlings in response to NO<sub>2</sub> exposure. *Bulletin of Environmental Contamination and Toxicology*, 84: 96-100.
- Normand, P., Lapiere, P., Tisa, L. S., Gogarten, J. P., Alloisio, N., Bagnarol, E., Bassi, C. A., Berry, A. M., Bickhart, D. M., Choisine, N., Couloux, A., Cournoyer, B., Cruveiller, S., Daubin, V., Demange, N., Francino, M. P., Goltsman, E., Huang, Y., Kopp, O. R., Labarre, L., Lapidus, A., Lavire, C., Marechal, J., Martinez, M., Mastrorunzio, J. E., Mullin, B. C., Niemann, J., Pujic, P., Rawnsley, T., Rouy, Z., Schenowitz, C., Sellstedt, A., Tavares, F., Tomkins, J. P., Vallenet, D., Valverde, C., Wall, L. G., Wang, Y., Medigue, C. and Benson, D. R. 2007. Genome characteristics of facultatively symbiotic *Frankia* sp. strains reflect host range and host plant biogeography. *Genome Research*, 17: 7-15.
- Pumplin, N. and Harrison, M. J. 2009. Live-cell imaging reveals periarbuscular membrane domains and organelle location in *Medicago truncatula* roots during arbuscular mycorrhizal symbiosis. *Plant Physiology*, 151: 809-819.
- Rajendran, K. and Devaraj, P. 2004. Biomass and nutrient distribution and their return of *Casuarina equisetifolia* inoculated with bio-fertilizers in farm land. *Biomass and Bioenergy*, 26: 235-249.
- Schüßler, A., Schwarzott, D. and Walker, C. 2001. A new phylum, the Glomeromycota phylogeny and evolution. *Mycological Research*, 105: 1414-1421.
- Seddas, P. M. A., Gianinazzi-Pearson, V., Schoefs, B., Küster, H. and Wipf, D. 2009. Communication and signaling in the plant-fungus symbiosis: the mycorrhiza. *In*: Baluska, F. (ed), *Plant-Environment Interactions from Behavioral Perspective*. Springer Verlag, Berlin, Heidelberg, New York, pp47-71.
- Smith, S. E. and Dickson, S. 1991. Quantification of active vesicular-arbuscular mycorrhizal infection using image analysis and other techniques. *Australian Journal of Plant Physiology*, 18: 637-648.
- Smith, S. E. and Read, D. J. 2008. *Mycorrhizal Symbiosis*. Academic Press, London.

- Svistoonoff, S., Laplaze, L., Auguy, F., Runions, J., Duponnois, R., Haseloff, J., Franche, C. and Bogusz, D. 2003. *Cg12* expression is specifically linked to infection of root hairs and cortical cells during *Casuarina glauca* and *Allocasuarina verticillata* actinorhizal nodule development. *Molecular Plant-Microbe Interactions*, 16: 600-607.
- Trouvelot, A., Kough, J. and Gianinazzi-Pearson, V. 1986. Evaluation of VA infection levels in root systems. Research for estimation methods having a functional significance. *In*: Gianinazzi-Pearson, V. and Gianinazzi, S. (eds), *Physiological and Genetical Aspects of Mycorrhizae*. INRA-Press, Paris, pp217-221.
- Udvardi, M. K., Czechowski, T. and Scheible, W. 2008. Eleven golden rules of quantitative RT-PCR. *The Plant Cell*, 20: 1736-1737.
- Vierheilig, H., Knoblauch, M., Juergensen, K., van Bel, A., Grundler, M. W. and Piche, Y. 2001. Imaging arbuscular mycorrhizal structures in living roots of *Nicotiana tabacum* by light, epifluorescence and confocal laser scanning microscopy. *Canadian Journal of Botany*, 79: 231-237.
- Vierheilig, H., Schweigerb, P. and Brundrett, M. 2005. An overview of methods for the detection and observation of arbuscular mycorrhizal fungi in roots. *Physiologia Plantarum*, 125: 393-404.

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