Mutants of the ectomycorrhizal mushroom *Tricholoma matsutake* that exert detrimental effects on its symbiotic partner *Pinus densiflora*†

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Tricholoma matsutake is an ectomycorrhizal fungus that produces the prized mushrooms "matsutake" in symbiotic association with conifers.[1)] In our attempts to induce mutants of *T. matsutake* that could be useful in artificial cultivation for fruiting by γ-ray irradiation at 500 Gy, we isolated a mutant, designated as G1, that grew better in substrates than the wild-type NBRC 33136.[1)] The phenotype of G1 on agar plates somewhat resembled that of Ar 59, which had been isolated by argon-ion-beam irradiation at 500 Gy; both mutants overproduced amylase and cellulase activities and exhibited a mycelial morphology different from the wild-type, forming a fuzzy cotton-ball-like colony, rather than a mycelial mat.[1,2)] In the present study, we examined how G1 and Ar 59 influence the plant growth of *Pinus densiflora* seedlings in vitro.

The inoculation was performed with two different methods. The first involved sterile 1-week-old *P. densiflora* seedlings, in which the seedlings were planted in a pumiceous soil substrate filled with *T. matsutake* mycelia and axenically co-cultured at 23°C for 120 days. The second involved sterile 1-month-old *P. densiflora* seedlings already grown in the pumiceous soil, in which *T. matsutake* mycelia were placed on the surface of the substrate where the shoot base emerged; they were axenically co-cultured at 23°C for 150 days.

In the first experiment, G1 exhibited a significant detrimental effect on both above- and below-ground organs; root systems were severely underdeveloped or collapsed, leading in turn to aboveground wilting (Fig. 1).

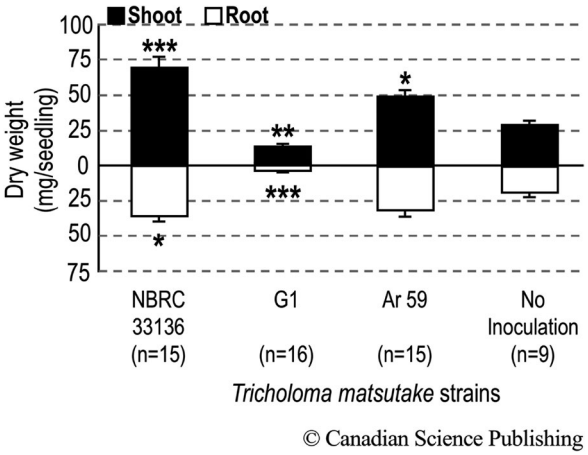

Fig. 1. Biomass measurements of 1-week-old *P. densiflora* seedlings co-cultured with *T. matsutake* strains for 120 days.

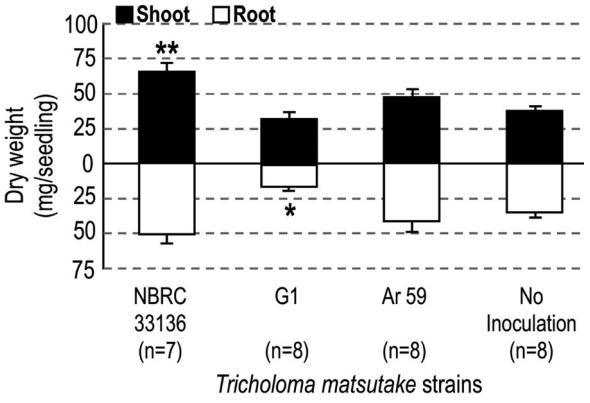

Fig. 2. Biomass measurements of 1-month-old *P. densiflora* seedlings co-cultured with *T. matsutake* strains for 150 days.

This result contrasts with the plant-growth-promoting effect of Ar 59, as well as NBRC 33136; the former allowed the seedlings to gain significantly higher aboveground and total biomasses than the control, while the latter allowed them to gain significantly higher aboveground, belowground, and total biomasses (Fig. 1).

In the second experiment, seedlings associated with G1 exhibited significant decreases in their belowground biomasses, and many lateral roots degenerated significantly, reducing their rhizosphere biomasses (Fig. 2). Seedlings associated with Ar 59, in contrast, exhibited neither plant-growth-promoting nor plant-growth-inhibiting effects (Fig. 2). Unlike these mutants, NBRC 33136 promoted plant growth; the aboveground and total biomasses significantly increased, but the belowground biomass did not (Fig. 2).

Data suggest that there could be a regulatory region in the genome of *T. matsutake* that determines plant associating behaviors, depending upon where the mutations occur and what environment the fungus inhabits based on its endophytic association with non-host plants.[3)] The overproduction of degrading enzymatic activities is not the cause of G1's trait of harming its symbiotic partner, because Ar 59 overproduced the same enzymatic activities without harming *P. densiflora* seedlings. Genomic and transcriptomic analyses of these mutants will allow us to unearth the unknown regulatory mechanisms involved in the ectomycorrhizal symbiosis of *T. matsutake*.

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