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¹⁵N tracing studies preference for different nitrogen forms of *Fusarium oxysporum* f. sp. cubense tropical race 4

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1. Introduction

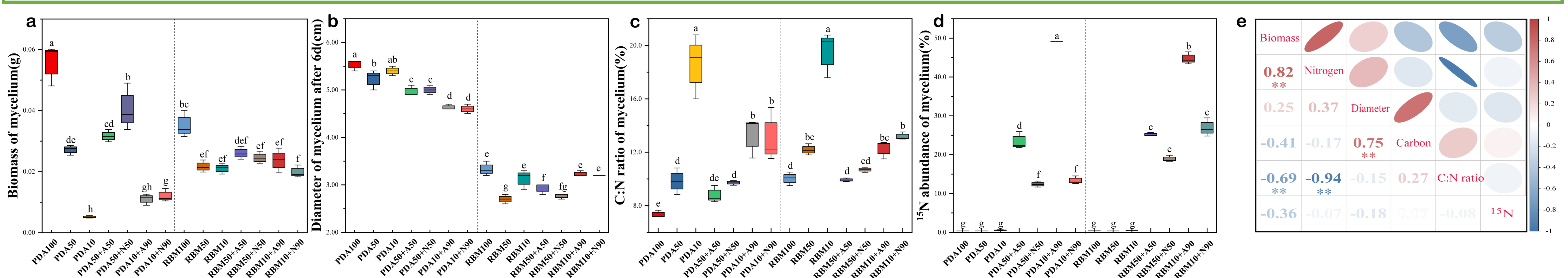
Fusarium wilt of banana (FWB), induced by the soil-borne fungus *Fusarium oxysporum* f. sp. cubense tropical race 4 (Foc TR4), is a serious disease affecting bananas that damages the vascular system and induces cell necrosis to reduce crop yield (Butler, 2013). Foc TR4 is highly pathogenic and can infect almost species of banana (Dita et al., 2018). The overuse of chemical nutrients, especially nitrogen (N), exacerbates disease resistance in Foc TR4. N is an important nutrient for plant growth and has long been known to play a key role in plant diseases (Fagard et al., 2014). Previous studies have focused mostly on the effect of different nitrogen forms on Foc TR4, but none have investigated which form of nitrogen is preferred by the pathogen. Therefore, it is important to investigate Foc TR4's response mechanism to nitrogen fertilization in terms of physiological characteristics.

2. Materials and methods

This experiment was conducted in 2021 at Hainan University (Haidian Campus). ¹⁵NH₄NO₃/NH₄¹⁵NO₃ was added to Potato Dextrose Agar (PDA) and Rose Bengal Medium (RBM) to prepare the new culture medium with three replicates for each of the following composition: PDA100 (100% organic nitrogen), PDA50 (50% organic nitrogen), PDA50+A50 (50% organic nitrogen+50% ¹⁵NH₄NO₃), PDA50+N50 (50% organic nitrogen+50% NH₄¹⁵NO₃), PDA10 (10% organic nitrogen), PDA10+A90 (10% organic nitrogen+90% ¹⁵NH₄NO₃), PDA10+N90 (10% organic nitrogen+90% NH₄¹⁵NO₃), RBM100 (100% organic nitrogen), RBM50 (50% organic nitrogen), RBM50+A50 (50% organic nitrogen+50% ¹⁵NH₄NO₃), RBM50+N50 (50% organic nitrogen+50% NH₄¹⁵NO₃), RBM10 (10% organic nitrogen), RBM10+A90 (10% organic nitrogen+90% ¹⁵NH₄NO₃), RBM10+N90 (10% organic nitrogen+90% NH₄¹⁵NO₃).

3. Results

In addition to being significantly affected by nitrogen form, mycelium biomass and diameter were closely related to nitrogen uptake and utilization. Compared with the control (PDA100), biomass decreased significantly on PDA and RBM (p<0.05). Biomass of the combined organic and inorganic nitrogen treatment was significantly lower than that of the pure organic nitrogen treatment (PDA100 or RBM100) on PDA and RBM. Foc TR4 preferentially utilized organic nitrogen rather than inorganic nitrogen (Figure 1a). In the combined organic and inorganic nitrogen treatment, mycelial diameter was higher on PDA compared to RBM (Figure 1b). The C:N ratios of mycelia in PDA10+A90 and PDA10+N90 treatments were 81.60 and 77.73% higher, respectively, than that of the control on PDA (p<0.05). In addition, the C:N ratio in mycelia grown on RBM exhibited a similar pattern (Figure 1c). In the ¹⁵N abundance experiment, tracer uptake was higher on PDA50+A50 than on PDA50+N50 and higher in PDA10+A90 than in PDA10+N90, with rates of uptake 88.63 and 267.53% higher, respectively, than growth on PDA (p>0.05). A similar pattern was observed in mycelia grown on RBM, suggesting that Foc TR4 prefers to use ammonium as a nitrogen source over nitrate when organic nitrogen is limited (Figure 1d). Pearson correlation analysis showed that the biomass of mycelium was significantly correlated with nitrogen content and C:N ratio (Figure 1e).



4. Discussion and Conclusion

In conclusion, the combination of organic and inorganic nitrogen treatments reduced mycelium biomass and nitrogen content significantly compared to an organic N source (PDA100 or RBM100); mycelium carbon content and C:N ratio increased significantly on PDA and RBM; and the mycelium biomass was negatively correlated with C:N ratio. A significant increase in ¹⁵N abundance was observed in mycelium grown on PDA50+A50, PDA10+A90, RBM50+A50, and RBM10+A90. These results indicate that mycelial biomass and diameter decreased as the C:N ratio increased, and that Foc TR4 preferentially utilized organic nitrogen over inorganic nitrogen. More importantly, Foc TR4 preferred to use ammonium nitrogen over nitrate to promote growth when organic N is limited. Therefore, we hypothesize that excessive application of ammonium nitrogen will aggravate the degree of banana wilt disease, while treating crops with a combination of organic and inorganic nitrogen fertilizers will reduce disease severity. This provides a new idea and reference for using nitrogen fertilization in banana cultivation management in the future.

5. References

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