Growth Stimulatory Substances for Mycorrhizal Fungi in Composted Organic Matter

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Abstract

Non-gaseous growth stimulatory substances for vesicular-arbuscular mycorrhizal (VAM) fungi in 45 day-fermented bahiagrass (*Paspalum notatum* Flügge.) shoots and leaves were isolated and identified. The VAM growth stimulants were eupalitin and 2, 3-dihydroeupalitin. Eupalitin at concentrations high enough to stimulate the hyphal growth of VAM fungi was detected in this composted bahiagrass shoots and leaves. The results suggest that some flavonoids such as eupalitin and 2, 3-dihydroeupalitin remain even in the composted organic matter or its water extract. We indicate that the effectiveness of composted organic matter application that stimulates the growth of mycorrhizal fungi will result from not only the evolution of gaseous mycorrhizal growth stimulants from the composted organic matter but also the presence of non-gaseous ones such as flavonoids.

INTRODUCTION

Organic matter and humic substances affect the formation of endomycorrizae (Hepper and Warner, 1983; Linderman and Davis, 2001; Soedarjo and Habte, 1993; Sreenivasa, 1994; Vallini et al., 1993; Yang et al., 2001b) and ectomycorrhizae (Schisler and Linderman, 1989; Tan and Nopamonbordi, 1979). In particular, we reported that although the application of raw organic matter to the soil severely inhibited VAM development, fermented organic matter stimulated VAM development and growth of citrus seedlings (Ishii and Kadoya, 1996). When the liquid obtained from the fermented organic matter, such as the shoots and leaves of *Artemisia princeps* Pampan., bahiagrass, *Cayratia japonica* Gagn., *Stellaria media* Villars and *Trifolium repens* L. and rice straw, was applied to the trifoliate orange-planted soils, every liquid treatment promoted root colonization and growth of trifoliate orange seedlings (Ishii et al., 2001).

When we fractionated the 80% methyl alcohol (MeOH) extracts of fermented and raw bahiagrass shoots and leaves by using a flash chromatograph, both growth stimulatory and inhibitory substances for mycorrhizal fungi existed in the raw shoots and leaves. After fermentation, however, the inhibitory effects on the 100% MeOH fraction containing a great amount of growth inhibitory substances disappeared and the stimulatory effects on the 25% MeOH fraction containing a great amount of growth stimulatory substances remained (Yang et al., 2001a). These results indicate that the growth stimulatory substances for mycorrhizal fungi in the 25% MeOH fraction are stable during the period of fermentation.

In this study, we isolated and identified the growth stimulatory substances for mycorrhizal fungi in composted organic matter.

MATERIALS AND METHODS

The shoots and leaves of bahiagrass were fermented during 45 days with the added nitrogen (40g N / Kg plant materials). After fermentation, the growth stimulatory substances for mycorrhizal fungi were extracted by 80% MeOH solution. As shown in Fig. 1, the crude extracts were fractionated by flash chromatography (Fuji Silysia

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Chemical Ltd., Nagoya). And then the 25% MeOH eluates, which stimulates the hyphal growth of VAM fungi, were evaporated at 45ºC, and purified by using a Rotofor (Bio-Rad Laboratories). After the fractions which have the VAM activity were sampled and evaporated, VAM growth stimulants in the concentrate were isolated by high pressure liquid chromatography (HPLC)(Hitachi L-7000, Tokyo). The ODS column (10 mm in diameter x 250 mm in length) was eluted with 10% MeOH at 1.2 ml/min. A detector (Hitachi L-4000, Tokyo) monitored the eluates at 254nm (Ishii et al., 1997). The concentration of eupalitin and 2, 3-dihydroeupalitin in the eluates was determined using their standard solutions already isolated and identified by Ishii et al. (1997).

RESULTS AND DISCUSSION

As shown in Fig. 2, eupalitin and 2, 3-dihydroeupalitin were detected in the 25% MeOH eluates of fermented bahiagrass shoots and leaves. These compounds are classified as flavonoids, and their stimulatory effects on hyphal growth of *Gigaspora margarita* were observed. Particularly, 3 and 10 ppm of eupalitin remarkably stimulated the hyphal growth (Ishii et al., 1997). In this study, the concentration of eupalitin was 177.09µg/gDW of the fermented organic matter, and this concentration was high enough to stimulate the hyphal growth of VAM fungi. These results indicate that some flavonoids which stimulate the growth of mycorrhizal fungi remain in composted organic matter.

Currently, flavonoids (Carr et al., 1985; Gianinazzi-Pearson et al., 1989; Nair et al., 1991), polyamines (El Ghachtouli et al., 1996; Ishii et al., 2000), alginate oligosaccharide (Ishii et al., 2000) and ethylene (Ishii et al., 1996; Geil and Guinel, 2002) have been known to influence markedly the growth of mycorrhizal fungi. Alginate oligo-saccharide is generally contained in some algae. Although polyamines and flavonoids exist in raw organic matter, polyamines will easily decompose during fermentation, but some flavonoids will remain in fermented organic matter. Also, no polyamines such as putrescine, spermidine and spermine were detected after fermentation (unpublished data). We reported that although hyphal growth of VAM fungi was severely inhibited at 0.2 ppm ethylene and above, spore germination and hyphal growth were stimulated at concentrations ranging from 0.01 to 0.1 ppm (Ishii et al., 1996), and that soils incorporated with fermented organic matter produced sufficient ethylene to stimulate the growth of VAM fungi (Ishii and Kadoya, 1984). The application of composted organic matter that stimulates VAM development and growth of citrus seedlings (Ishii and Kadoya, 1996) would result in the evolution of gaseous mycorrhizal growth stimulants from the composted organic matter and/or the existence of non-gaseous ones in it.

In conclusion, this study strongly indicates that composted organic matter and its water extract should be used as agricultural organic fertilizers in order to modifythe biological character of soils when soil physical and chemical characters have been amended.

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Figures

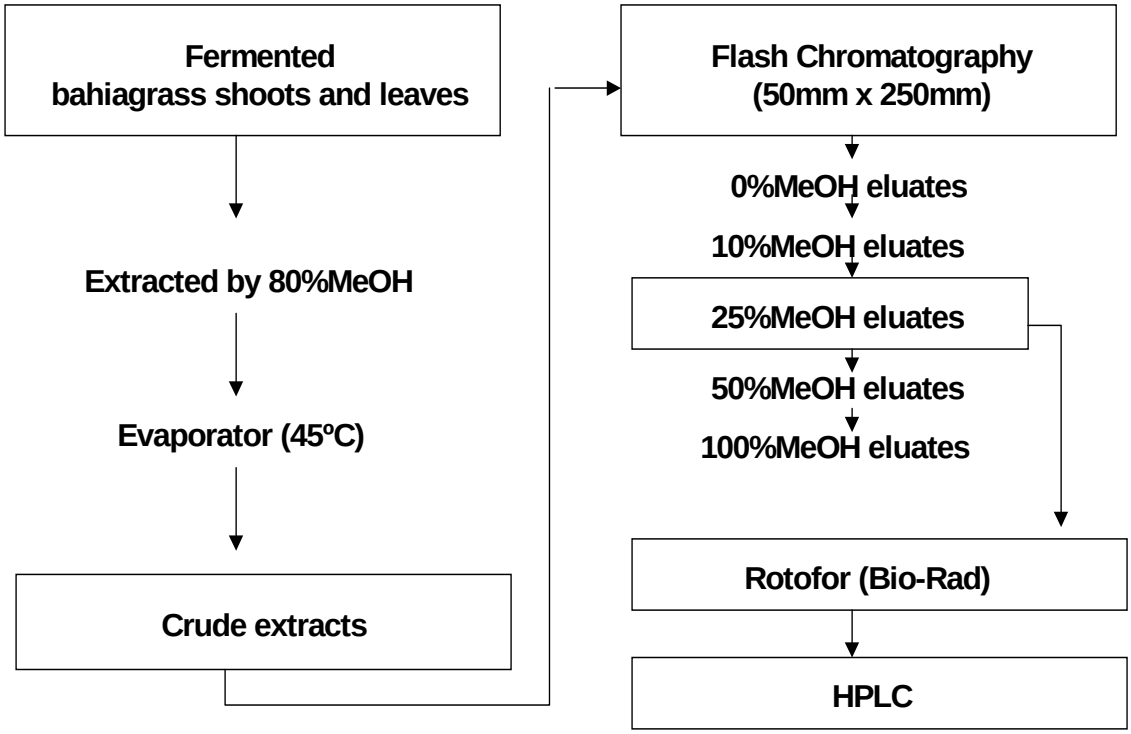

Fig. 1. Flow sheet for separation of VAM stimulatory substances in fermented bahiagrass shoots and leaves.

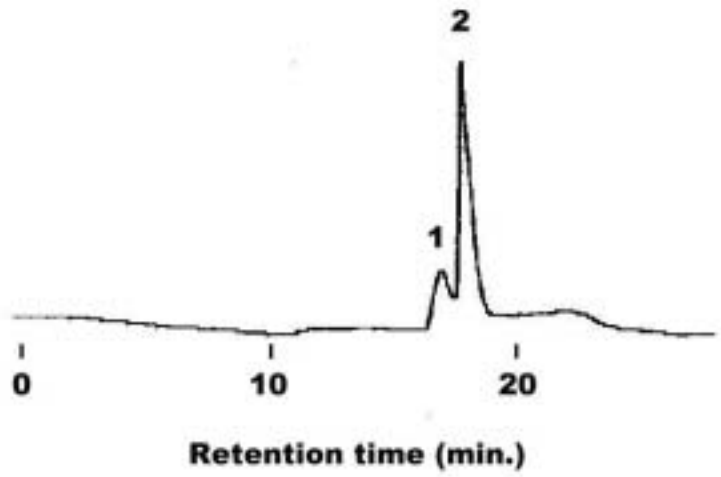

Fig. 2. The chromatograph of VAM stimulatory substances in fermented bahiagrass shoots and leaves by using a HPLC at 254 nm. 1: eupalitin, 2: 2, 3-dihydroeupalitin.