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# DEGRADATION CHARACTERISTICS OF GLOMALIN-RELATED SOIL PROTEIN IN CITRUS RHIZOSPHERE

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**ABSTRACT**

*Glomalin-relate soil protein (GRSP), participating in various biochemical functions in soil, is a kind of soil glycoprotein, originated from arbuscular mycorrhizal fungi (AMF). In the present work, we collected the soil from a citrus orchard and tested the changes in GRSP fractions viz., easily-extractable GRSP (EE-GRSP) and difficultly-extractable GRSP (DE-GRSP) at different temperature of storage time and form of storage of samples. There were significant increase in both EE-GRSP and DE-GRSP level upto 120 days of strorage; Low temperature of 5°C markedly restricted the degradation rate of EE-GRSP and DE-GRSP. The extent of degradation of EE-GRSP and DE-GRSP was observed as 52.3–80.1% and 50.8–70.1%, respectively, in temperature range of 5–35°C. However, in response to temperature sensitivity EE-GRSP was more vulnerable to degradation than DE-GRSP.*

**Key words:** Glomalin, Mycorrhiza, Temperature, Citrus, Degradation

## INTRODUCTION

Growth of citrus and rhizosphere properties are inter-related (Ngullie *et al.*, 2015) Glomalin is a kind of glycoproteins containing metal ions, which originated from spores and hyphal walls of arbuscular mycorrhizal fungi (AMF) (Wright and Upadhyaya 1996). After the spores and hyphae are decomposed, glomalin is released and deposited into the soil, called as glomalin-related soil protein (GRSP) (Rillig 2004). GRSP is not only an important source of C and N in soil but also resists against wind erosion and water erosion (Purin and Rillig 2007, Zou *et al.* 2015) by acting as glue material for cementing the soil particles. Studies have shown that GRSP is able to improve soil structure, stabilize aggregates, regulate soil water relations, and contribute towards enriching the soil organic carbon pool (Wu *et al.* 2008; Wu *et al.* 2014b; Zou *et al.* 2014). Such GRSP-mediated changes in soil properties are expected to improve crop performance. GRSP is divided into two fractions, namely, easily-extractable glomalin-related soil protein (EE-GRSP) and difficultly-extractable glomalin-related soil protein (DE-GRSP) (Koide and Peoples 2013; Wu *et al.* 2014a). The fraction EE-GRSP is a newly synthesized glomalin and relatively more labile while DE-GRSP, an older glomalin originated from EE-GRSP turnover, is difficult to extract and recalcitrant in soils. Total glomalin-related soil protein (T-GRSP) is the sum of EE-GRSP and DE-GRSP. Using the $^{14}C$ tracer technique, GRSP turnover in tropical forest soil was analysed by Rillig *et al.* (2001) which revealed GRSP turnover time as 6~42 years. Treseder and Turner (2007) reported that GRSP decomposition rate could be considerably lower in field than in the laboratory. Understanding the shelf life of GRSP will provide an additional knowledge about GRSP functioning, the current state of information regarding the degradation traits of the two GRSP fractions is very limited. In this background, the purpose of the present study was to assess the changes in EE-GRSP and DE-GRSP degradation under different storage temperatures and forms of storage.

## MATERIALS AND METHODS

### Collection and preparation

We collected soil samples from a citrus orchard (27-year-old *Citrus unshiu* no. 1 grafted on *Poncirus trifoliata*) of Yangtze University, Jingzhou, China. The soil taxonomically belonged to Xanthi-Udic Ferralsols. The soil had a pH of 6.0, 12.10 mg/kg $KMnO_4$-N, 15.7 mg/kg Bray-P, and 223.0 mg/kg neutral $NH_4OAc$-K.Covering four directions of the citrus trees; ~2 kg soil was collected underneath the tree canopy. The subsamples collected from tree, were mixed well using four trees, air-dried, ground, and then sieved (2 mm) for the analysis of GRSPs.

### Experimental setup

The experiment consisted of $3^3$ factorial randomized design, with four replicates having three storage temperatures (5, 20 and 35°C) and three storage periods(0, 60 and 120 days) tested under air-dried soil, supernatant of EE-GRSP and non-separated solution of EE-GRSP including supernatant and precipitation. Under these treatment conditions, the EE-GRSP and DE-GRSP level were determined at an interval of two months, with a total of three determinations.

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**GRSP analysis**

EE-GRSP was determined following the protocol as suggested by Wu *et al.* (2015). One gram air-dried soil was incubated in 8 mL citrate buffer (20 mM, pH 7.0), autoclaved for 0.5 h at 0.11 Mpa, and centrifugated at 10,000×*g* for 3 min. The supernatant was collected and stored at three different temperatures viz., 5, 20, and 35°C. The supernatant was used to analyze EE-GRSP level. For the treatment regarding non-separated solution of EE-GRSP, the autoclaved solution was centrifuged without collecting the supernatant. The supernatant and the precipited were stored as per designed temperatures. Similarly, DE-GRSP was extracted using the protocol of Wu *et al.* (2015). The precipitate of EE-GRSP centrifugation was incubated with 8 ml 50 mm citrate buffer (pH 7.0), autoclaved at 0.11 Mpa for 1 h, and centrifuged at 10,000×*g* for 3 min. The supernatant was analyzed as per the protocol of Bradford (1976) using bovine serum albumin solution as the standard. After storing the supernatent for 2 and 4 months, all the samples were centrifuged again for the EE-GRSP and DE-GRSP determination.

**Statistical analysis**

The data were analyzed by the ANOVA of SAS (8.1), and the significant differences between the treatments were compared with Duncan's multiple range tests at 0.05 level.

**RESULTS AND DISCUSSION**

**EE-GRSP degradation:** Significant change in concentration of EE-GRSP was observed in response to different treatments. EE-GRSP level was considerably decreased with increase in storage periods when analysed across three different sampling methods air-dried soil, supernatant and supernatant + precipitate. This decrease was rated as supernatant + Precipitate > supernatant > air-dried soil. After 120 days of storage, the degradation rate was 52.3-59.0% at 5°C, 65.6 -80.1% at 20°C and 62.6-68.9%, at 35°C. The rate of degradation of EE-GRSP at $5^0$C was much slower compared to either $20^0$ or $35^0$C. Possibly, at 5°C treatment, inactivation of the relevant degrading enzyme, such as soil proteas took place, resulting in the relatively slow degradation rate of EE-GRSP. Under similar storage temperatures, the EE-GRSP level registered the highest concentration in air-dried soil followed by supernatant and supernatant+ Precipitate. These results suggested that different sample forms strongly affected EE-GRSP degradation rate. EE-GRSP degradation is, hence, so much dependent on soil temperature and forms of the samples subjected to analysis.

Table 1: Changes of EE-GRSP level under different treatments conditions

| Storage temperature (°C) | Storage period (Days) | Air-dried soil | Supernatant | Supernatant+Precipitate |
|---|---|---|---|---|
| 5 | 0 | 0.663a | 0.663a | 0.663a |
| | 60 | 0.496b | 0.457c | 0.399cd |
| | 120 | 0.316de | 0.296efg | 0.272fg |
| 20 | 0 | 0.663a | 0.663a | 0.663a |
| | 60 | 0.376cd | 0.323de | 0.308ef |
| | 120 | 0.228fg | 0.208g | 0.132h |
| 35 | 0 | 0.663a | 0.663a | 0.663a |
| | 60 | 0.388cd | 0.360de | 0.316de |
| | 120 | 0.248fg | 0.212g | 0.206g |

*Different letters mean significant difference amongst different treatments ($p<0.05$)*

**DE-GRSP degradation:** DE-GRSP concentration markedly decreased with increase in length of the time storage, irrespective of temperature range, the samples were stored including the forms of storage. After 120 days of storage, the degradation rate of DE-GRSP was 50.8- 57.7% at 5°C, 61.8-70.1 at 20°C and 52.1-59.5% at 35°C. These degradation rate of DE-GRSP were much lower than degradation rate of EE-GRSP, indicating EE-GRSP as comparatively more labile and DE-GRSP as most recalcitrant fraction of GRSP in soil (Wu *et al.* 2015). A notably higher DE-GRSP was observed at $5^0$C followed by $35^0$C and $20^0$C, irrespective of time of storage and type of stored sample. We concluded from these observations that too low or high temperature restricted the activities of the relevant soil enzymes in the process of DE-GRSP degradation. Under similar temperature of storage and conditions of sample storage, the DE-GRSP level exhibited its maximum concentration in air-dried soil followed by supernatant and supernatant plus precipitation of storage. The observations are suggested of the fact that DE-GRSP degradation depended on soil temperature, form, and length of the period of storage. As stated by Treseder and Turner (2007), the GRSP decomposition rate was considerably lower in the field than in the laboratory.

We guess that different properties of soil (soil water content, soil clay content, soil enzymes, soil microbial activity, etc.) and type of vegetation could further influence the decomposition of GRSP in soil.

Table 2: Changes of DE-GRSP level under different treatments conditions

| Storage temperature (°C) | Storage Period (Days) | Air-dried soil | Supernatant | Supernatant+Precipitate |
|---|---|---|---|---|
| 5 | 0 | 1.039a | 1.039a | 1.039a |
| | 60 | 0.781b | 0.704cb | 0.773b |
| | 120 | 0.511d | 0.498de | 0.439efg |
| 20 | 0 | 1.039a | 1.039a | 1.039a |
| | 60 | 0.531cd | 0.515d | 0.490de |
| | 120 | 0.397fg | 0.355gh | 0.311h |
| 35 | 0 | 1.039a | 1.039a | 1.039a |
| | 60 | 0.735c | 0.702cd | 0.651cde |
| | 120 | 0.498de | 0.449def | 0.421ef |

Different letters mean significant difference amongst different treatments ($p<0.05$)

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