Notes Plant Biotechnology, **14**(2), 123-125(1997)

Tannins and Related Phenolics in Callus and Root Cultures of *Quercus glauca*

Kazutaka NISHIKAWA*, Norie TANAKA*, Fumi NAKANISHI**, Koichiro SHIMOMURA** and Kanji ISHIMARU*

Received 5 February 1997; accepted 8 May 1997

Quercus plants (Fagaceae) are regarded as a rich source of polyphenol constituents, *e.g.* hydrolyzable and/or procyanidin-type tennins. Several studies on the chemistry [1-5] and on *in vitro* culture techniques for the mass propagatiorn [6] and for the determination of secondary metabolites (tannins)[7-10] have been done on some species, *Q. mongolica*, *Q. acutissima*, *Q. robur etc.* In this study, callus and adventitious root cultures of *Q. glauca* Thunberg were established and the production of major phenolics [(+)-catechin (**1**), gallic acid (**2**), β-glucogallin (**3**)[11], 1, 2, 3, 6-tetra-*O*-galloyl-β-D-glucose (**4**)[12, 13] and 1, 2, 3, 4, 6-penta-*O*-galloyl-β-D-glucose (**5**)[12, 13]] in these cultures was investigated.

Q. glauca acorns were collected at the garden of Saga Prefectural Museum in November in 1994. After taking off the epicarps, the acorns were sterilized by an usual method (3% NaOCl, 10 min.), and placed on hormone-free one-tenth strength Murashige-Skoog (1/10 MS) solid medium [14], which was solidified with 5 g/*l* agar with 30 g/*l* sucrose, in the light (3,000 lux, 16 hr photoperiod per a day). The axenic seedlings obtained (germination rate was almost 100%) were transferred to hormone-free Woody Plant (WP) solid medium [15], which was solidified with 2.5 g/*l* gelrite with 20 g/*l* sucrose, and subcultured in the same condition as above at one month intervals to produce *in vitro* plantlets.

For the induction of the callus, the leaf segments cut from the axenic plantlets *in vitro* were placed on half-strength (1/2) MS solid medium supplemented with 2.0 mg/*l* naphthaleneacetic acid (NAA) and 0.1 mg/*l* benzyladenine (BA) in the dark. The calli derived from the segments were subcultured on the same medium at one month intervals in the dark for over a half year.

For the establishment of the adventitious root cultures, the root tissues of the *in vitro* plantlets were also cut off, transferred into 1/2 MS, WP or Gamborg B5 [16] (B5) liquid media supplemented with various combinations of indoleacetic acid (IAA), NAA and indolebutyric acid (IBA). They were subcultured on a rotary shaker (100 rpm) in the dark at one month intervals for over a half year. Among the media tested, B5 basal medium with 3.0 mg/*l* NAA was the best for the growth of the adventitious roots, and these roots were used for the following experiments.

Callus and adventitious root cultures established as above were used for the determination of time course of the growth and polyphenol produciton. Calli [inoculum: *ca.* 30 mg, dry weight (dw)] and adventitious roots (inoculum: *ca.* 100 mg, dw) were cultured in the light and dark for 12 and 8 weeks, respectively. The cultures were harvested periodically (2 weeks intervals) and the growth (dw) and the phenolic production were determined. HPLC for analysis of the phenolics was performed as follows. Lyophilized tissues (callus and root, *ca.* 20-30 mg) were mashed and extracted with MeOH (2 m*l*) for 16 hr at room temperature. Each extract, after filtration through a millipore filter (0.45 mm), was subjected to HPLC: column, TSK gel ODS 80Ts (4.6 mm × 250 mm); mobile phase, 1 mM tetra-butylammonium (adjusted to pH 2.9 with AcOH)-CH_3CN (9 : 1 → 1 : 4, in 30 min.); flow rate, 0.6 m*l*/min.; column temp., 40°C; detection, 280 nm (UV); R_t (min.): **3** (6.3), **2** (8.5), **1** (17.1), **4** (22.8), **5** (23.8). Culture temperature was 25°C and the data were the means of three replicates.

The result of the time course experiment on the growth of calli and adventitious roots of *Q. glauca* is shown in **Fig. 1**. Both in the light and dark conditions, the calli showed maximum growth (dw) at week 6 (0.131 g in the light and 0.133 g in the dark). The growth in the dark was higher than that in the light during the entire culture period. The adventitious roots showed continuous increment of dw throughout the culture period (**Fig. 1**) showing the maximum level at week 8 (0.402 g).

The contents of tannins and related phenolics in the calli and adventitious roots are shown in **Fig. 2**. Among the five compounds examined, **1** and **5** were the major compounds in both callus and root cultures. Compound **5** showed maximum content in each culture (callus, 1.9% at week 6 in the light and 1.8% at week 8 in the dark; adventitious root, 1.6% at week 6) but the level was variable through the culture period compared to that of **1**.

*Department of Applied Biological Sciences, Faculty of Agriculture, Saga University, 1 Honjo, Saga 840, Japan
**Tsukuba Medicinal Plant Research Station, National Institute of Health Sciences, 1 Hachimandai, Tsukuba, Ibaraki 305, Japan

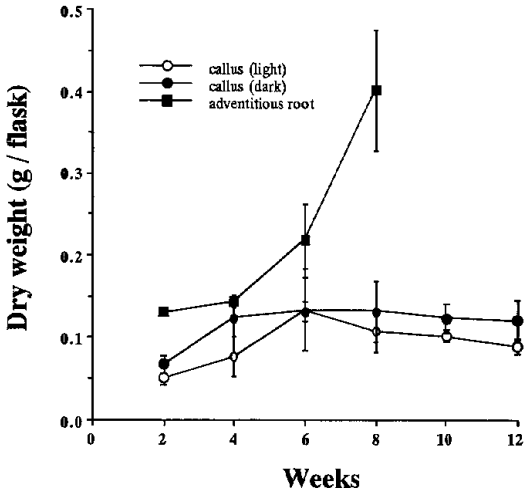

Fig. 1 Growth of callus and adventitious root cultures of *Quercus glauca*.

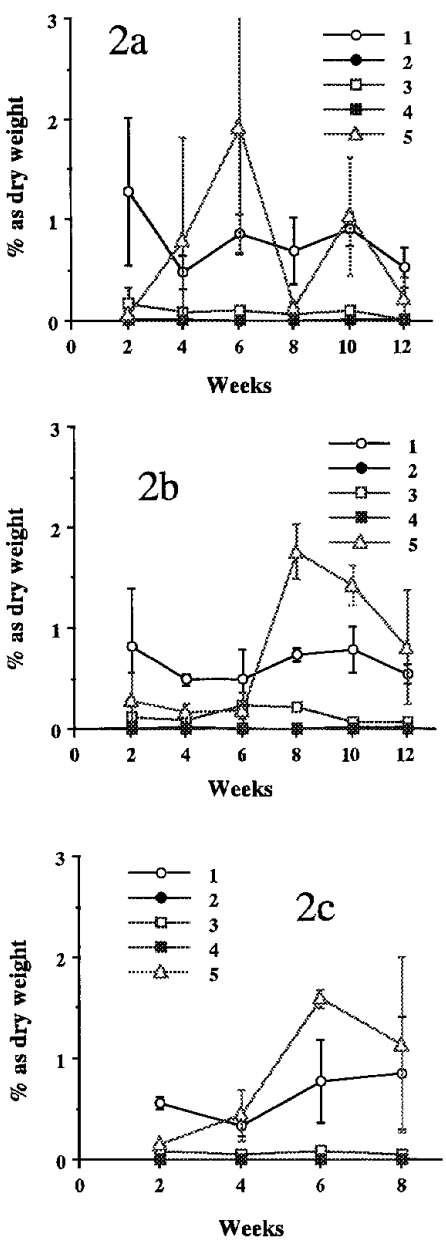

Fig. 2 Tannin and related phenolics content in callus and adventitious root cultures of *Quercus glauca*.
(**2a**: callus in the light, **2b**: callus in the dark, **2c**: adventitious root)

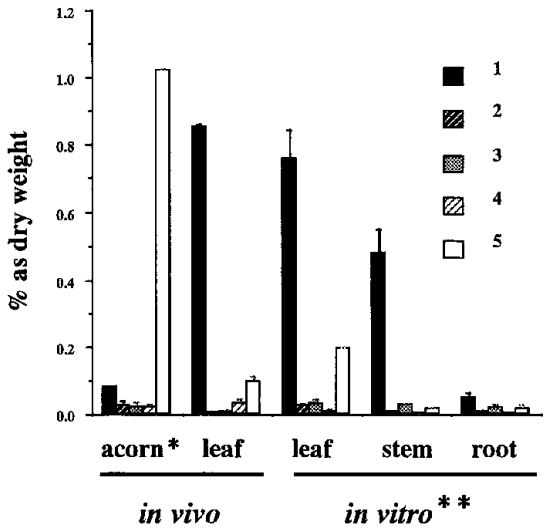

Fig. 3 Tannin and related phenolics content in the parent plant (*in vivo*) and *in vitro* plantlets of *Quercus glauca*.
acorn*: whole part of acorns was used for the extraciton
*in vitro***: plantlets cultured on hormone-free WP medium for 4 weeks in the light

The contents of tannins and related phenolics in acorns and leaves of the parent plant and in leaves, stems and roots of *in vivo* plantlets of *Q. glauca* are shown in **Fig. 3**. The leaves contained mainly compound **1** while the acorns contained a high level of **5**. The roots produced poor levels of these phenolics. The higher productivity of **1** and **5** in callus and adventitious root cultures than that in the parent plant and *in vitro* plantlets makes these cultures useful for the production and for biosynthetic studies of tannins and related phenolics in this species.

Acknowledgments

This work was supported in part by the subsidy from The San-Ei Gen Foundation for Food Chemical Research.

References

[1] Ishimaru, K., Nonaka, G., Nishioka, I., 1987. Phytochemistry, **26**: 1147-1152.
[2] Ishimaru, K., Nonaka, G., Nishioka, I., 1987. Chem. Pharm. Bull., **35**: 602-610.
[3] Nonaka, G., Ishimaru, K., Mihashi, K., Iwase, Y., Ageta, M., Nishioka, I., 1988. Chem. Pharm. Bull., **36**: 857-869
[4] Nonaka, G., Ishimaru, K., Azuma, R., Ishimatsu, M., Nishioka, I., 1989. Chem. Pharm. Bull., **37**: 2071-2077.
[5] Scalbert, A., Haslam, E., 1987. Phytochemistry, **26**: 3191-3195.
[6] Shoyama, Y., Sasaki, Y., Nishioka, I., Suzaki, T., 1992. Biotechnology in Agriculture and Forestry,

Vol. 18, High-Tech and Micropropagation II (ed. by Bajaj, Y. P. S.), p. 179-192, Springer-Verlag Berlin Heidelberg.

[7] Scalbert, A., Monties, B., Favre, J. M., 1988. Phytochemistry, **27**: 3483-3488.

[8] Favre, J. M., Scalbert, A., H-du Penhoat, C. L. M., 1993. Biotechnology in Agriculture and Forestry, Vol. 24, Medicinal and Aromatic Plants V (ed. by Bajaj, Y. P. S), p. 300-312, Springer-Verlag Berlin Heidelberg.

[9] Krajci, I., Gross, G. G., 1987. Phytochemistry, **26** : 141-143.

[10] Tanaka, N., Shimomura, K., Ishimaru, K., 1995. Phytochemistry, **40**: 1151-1154.

[11] Kashiwada, Y., Nonaka, G., Nishioka, I., 1984. Chem. Pharm. Bull., **32**: 3461-3470.

[12] Haddock, E. A., Gupta, R. K., Al-Shafi, S.M.K., Haslam, E., Magnolato, D., 1982. J. Chem. Soc. Perkin Trans. I: 2515-2524.

[13] Haddock, E. A., Gupta, R. K., Haslam, E., 1982. J. Chem. Soc. Perkin Trans. I: 2535-2545.

[14] Murashige, T., Skoog, F., 1962. Physiol. Plant., **15**: 473-497.

[15] Lloyd, G. B., McCown, B. H., 1980. Int. Plant. Prop. Soc., **30**: 421-427.

[16] Gamborg, O. L., Miller, R. A., Ojima, K., 1968. Exp. Cell Res., **50**: 151-158.