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Production of Kunugi (*Quercus acutissima* CARRUTH) Plants for the Bed Log of Shiitake (*Cortinellus shiitake* P. HENN.) by *in vitro* Tissue Culture

—Propagation from the Mature Tree of Kunugi—

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A Kunugi (*Quercus acutissima* CARRUTH) tree is the most useful species for the bed log (Hodagi) of Shiitake (*Cortinellus shiitake* P. HENN.) cultivation, and the production of these bed logs forms an important industry in the districts of Shiitake cultivation adjacent to the mountains all over the country.

Generally, a Kunugi tree is propagated by use of the seeds. But, there are some problems such as the fluctuation of the yield of seeds in each year and in each district, and the difficulty in storing seeds. Moreover, the propagation by cutting is recognized to be difficult because of the difficulty in rooting.

Therefore, this paper deals with the mass propagation of Kunugi trees by using *in vitro* tissue culture of stem sections obtained from the mature tree of kunugi for the purpose of stable supply of the clonally propagated elite-trees as the bed log with good quality for shiitake[1].

Materials and Methods

A wild 8-year old kunugi tree was dug up wholly in March of 1987 and transported into the green-house after removing the top of the stem. The shoots sprouting from the hidden buds on the stump were used for materials of the experiment.

The shoots (current green sprouts) of about 30 cm long with about 2 mm diameter were cut into about 5 cm long and the leaves were removed. Then, they were sterilized with 70% ethyl alcohol for a few seconds and with 2% of anthiformin (contained 8.5-13.5% NaClO) on the stirrer for 15 min, successively. After sterilization, they were rinsed with sterile water over 3 times and divided into 1 to 2 cm long by use of the sterilized scissors, and the cuttings were planted on the culture medium.

The basal medium of MS (Murashige and Skoog, 1962) used for the first culture was added with 0.5 mg/l 6-BA (6-benzylamino-purine). Next, the basal medium of WPM (Lloyd and Mccown, 1981) added with 0.5 mg/l 6-BA was used for the subculture. The WPM medium added with 1.0 mg/l IAA (β-indoleacetic acid) was used for rooting.

For the culture, the phytotron was used in which the upper ends of all test-tubes were illuminated by the fluorescent lamp of 4,000 lx with the day-length of 16 hours and the temperature was maintained at 25 ± 2℃.

Results and Discussion

Although almost all materials were contaminated by microorganisms when the materials were provided from the mature tree in open air, there was comparatively few contamination in case of using the sprout in the green-house. Consequently, all of the experimental materials were collected from the mature tree

grown in the green-house. From the fact that many shoots elongate from the hidden buds on the cut part of stump under green-house conditions, the culture materials can be obtained easily and also in large numbers.

After initiation of the first culture, sprouting was found on the node part of cut stem after about one month, and more than ten shoots were obtained per explant (**Fig. 1**).

When the part of this sprouting shoot was transplanted on the subculture medium for multiplication, some more sprouts were found after one month (**Fig. 2**).

After the shoots attained 1 to 2 cm long, they were transplanted on the culture medium for rooting. The differentiation of root was found after one to two weeks (**Fig. 3**) and the acclimatizable plantlet could be obtained after one month.

After acclimatization, the plantlets presented the feature of rosette at the initial stage, but afterwards

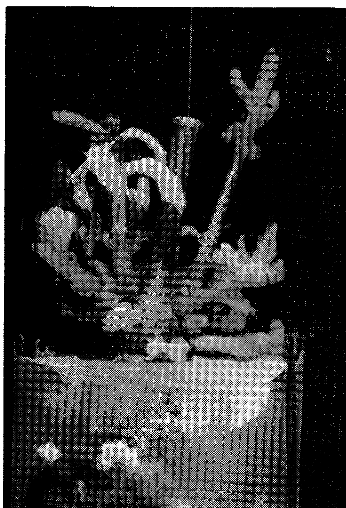

Fig. 1. Shoots after one month of the first culture.

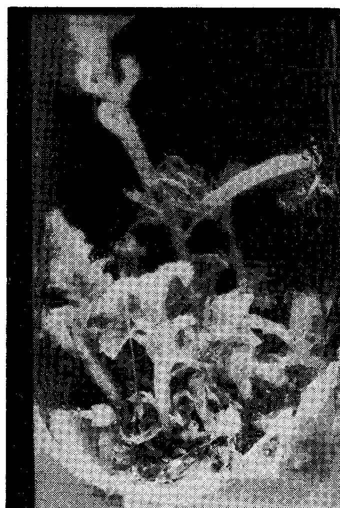

Fig. 2. Shoots after one month of the subculture for multiplication.

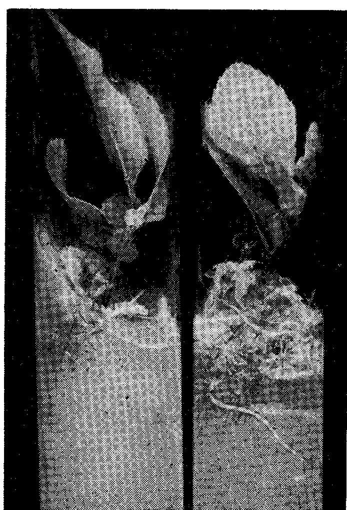

Fig. 3. Plants after two weeks on the rooting medium.

Fig. 4. Acclimatized plant.

showed favourable growth (**Fig. 4**).

As to the study on the micro-propagation of kunugi tree, Sato *et al.*[2] cultured the parts of epicotyl, hypocotyl, radicle, and cotyledon in the seed and obtained many shoots. Ide *et al.*[3] also obtained the plantlet by the culture using the current shoot of mature tree, but it is recongnized that the multiplication is difficult by contamination in many cases. In contrast, Gyokusen[4] reported that many plantlets were obtained by sprouts from the sterilized seeds of Kunugi tree and Konara tree (*Quercus serrata* THUNB.).

As to the report on the tree species of *Quercus*, there are only papers of Chalupa[5] and Vieitez *et al.*[6] in which the plantlets were obtained by using the explant of the terminal bud and node (axillary bud) of 2 to 4 months or one year old seedling of *Quercus robur*. However, there are few examples in these papers on the success with multiplication from the mature tree of age 8 to 20 years.

From the results of this experiment, the following method could be used to obtain effectively many clonal plantlets from the mature tree of Kunugi. First, the hidden buds of bed log are sprouted and the cuttings of elongating current green shoots are planted on the *in vitro* medium added with cytokinin. Next, the sprouting shoots are multiplicated on the subculture, and then the plantlets are made on the medium of rooting. When the elite-tree is grafted on the stock and the sprout from its hidden bud is propagated by this method, mass multiplication from the elite-tree is possible.

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≪和文要約≫

組織培養を利用したシイタケ原木（クヌギ）の生産
—クヌギの成木からの増殖—

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クヌギ (*Quercus acutissima* CARRUTH) は，シイタケ (*Cortinellus shiitake* P. HENN.) の榾木として重要であり，成木からのクローン増殖が強く望まれている．筆者らは，8年生のクヌギを温室内に搬入し，育成して得た当年枝を材料として試験管内（挿し木）増殖を試みた．まず，MS 培地に 0.5 mg/*l* の 6-BA を添加した初代培養では1カ月後に外植体1本から 10 数本のシュートが得られ，WPM 培地に 0.5 mg/*l* の 6-BA を添加した継代培養でさらに多数のシュートが得られた．次に得られたシュートを発根培地である WPM 培地（1.0 mg/*l* の IAA 添加）に移植したところ 1〜2 週間後に発根が見られ，約1カ月後には馴化可能な個体が得られた．