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In Vitro Plantlet Regeneration from Axillary Buds and the Growth of Acclimated Plantlet of *Kunugi* (*Quercus acutissima*)

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Summary

Plantlets of *Kunugi* were regenerated from axillary buds of juvenile seedlings. The basic media used in this study were BTM and WSM. The extent of the shoot elongation from the explant was affected by the medium type, the plant hormone and other factors. The axillary shoots were rooted on the 1/2 BTM with IBA (0.2 ppm) and NAA (0.05ppm). Acclimatization of the regenerated plantlet was successfully done under conditions of controlled humidity condition.

The growth of acclimated plantlets was compared with that of seedlings. The tree height and the basal diameter were not significantly different between the regenerated plantlets and the seedlings.

1 Introduction

Kunugi is one of the most important tree species providing bed logs for the culture of *Shiitake* mushrooms. The breeding program for this species has already been started by selecting plus trees. It is difficult to propagate *Kunugi* by conventional methods of cutting and grafting. Therefore, new propagating techniques should be established for this species[5].

There have been some reports on the tissue culture of *Kunugi*. The plantlet regeneration has been also reported[1, 2, 6, 7]. However, no studies have been reported on the plantlet growth after acclimatization.

This paper therefore reports on the *in vitro* plantlet regeneration from axil lary buds, and compares the growth of acclimated plantlets with that of seedlings.

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2　Materials and Methods

2．1 *In vitro* plantlet regeneration

Acorns were collected in 1989 from the *Kunugi* experimental forest for bed logs of *Shiitake* mushrooms at the Kansai Regional Breeding Office, National Forest Tree Breeding Center, in western Honshu, Japan. The *Kunugi* experimental forest was established in 1980 with the half-sib families of Kunugi candidate trees selected from Okayama prefecture. The acorns were collected from the candidate trees Katsuyama2, Katsuyama4, Yamakuse-oku3 and Yamakuse-oku12. The collected acorns were stored at 5℃ until spring 1990, and were planted in containers of vermiculite in a laboratory.

The acorns germinated immediately, and the seedlings were grown to about 15cm in height. Nodal segments of 1.5-2cm were cut from the seedlings and used as explants for tissue culture. After removing the leaves, the explants with petioles were sterilized with a 70% ethanol solution for 3 minutes, and then with a 3% hydrogen peroxide solution for 15 minutes. The basal ends of the segments damaged by the surface sterilization were removed. To reduce the exudation of tannin, the newly cut ends of the explants were soaked in a 0.1% sterilized silver nitrate solution for 3 seconds, and placed on a sterilized filter paper for 30 minutes to permeate of the silver nitrate solution.

The sterilized explants were inoculated on solid nutrient media prepared for axillary shoot elongation in test tubes. The composition of the media is described below. After the axillary shoot elongation, the shoots were transplanted to a medium for root formation described below.

The regenerated plantlets were planted in containers of vermiculite. Each plantlet was capped with an empty test tube, and the container was covered with saran-wrap®. After 20-30 days the plantlets were planted, the test tubes were taken away and 2-3 mm diameter holes were made incrementally in the saran-wrap® covering the container to acclimate the plantlets to the outside air.

Throughout the culturing and acclimating period, the cultures were kept at 25℃ with a 16 h／day period of constant illumination by fluorescent lighting of about 4000 lux.

Broad leaved tree medium (BTM) and Wolter and Skoog medium (WSM) were used in this culture as the basic media. To elongate the axillary shoots, 0.8% agar, 0.1% gelrite, 0.5 mg／ℓ or 1.0 mg／ℓ 6-benzylaminopurine (BAP), 1.0% active carbon and 3% sucrose were added to the basic medium separately. The components of the media are shown in Table 1. The composition of the medium prepared for rooting was 0.2mg／ℓ β-indolebutylic acid (IBA), 0.05mg／ℓ α-naphthylacetic acid (NAA), 3% sucrose, 0.8% agar and 1／2 BTM. The pH of all the media was adjusted to 5.8 before adding the agar.

Table 1 Medium composition of the initial culture

Medium number	1	2	3	4	5
Basic medium	BTM	BTM	BTM	WSM	WSM
Agar	0.8%	0.8%	0.8%	0.8%	-
Gelrite	-	-	-	-	0.1%
Sucrose	3%	3%	3%	3%	3%
BAP	1mg／ℓ	0.5mg／ℓ	0.5mg／ℓ	1mg／ℓ	1mg／ℓ
Active carbon	-	-	1%	-	-

About 10mℓ of the medium was distributed to 25mm diameter × 120mm height test tubes. The test tubes were capped with alminium foil and autoclaved.

2. 2 Regenerated plantlet growth

The acclimated plantlets and same-family seedlings were planted in the nursery of the Kansai Regional Breeding Office in May 1991. The acorns had been stored since 1989 at 5 C, and were directly sown in the nursery. The surviving plantlets and germinated seedlings were transplanted in the nursery spacing of 50 × 50cm in July 1991. The transplanted families were Katsuyama2, Katsuyama4, Yamakuse-oku3 and Yamakuse-oku12.

The tree height and basal diameter of the regenerated plantlets and seedlings were measured in May 1993 and March 1994. The measured number of regenerated plantlets were 24 individuals and the measured number of seedlings were 52 individuals.

3 Results and Discussion

3. 1 *In vitro* plantlet regeneration

The contamination percentage was satisfactorily low level in the initial culture (Table 2). The surface sterilization method used in this experiment was effective in lowering the contamination level. Another reason for the low level of contamination might be that the explants were taken from seedlings which were germinated in the laboratory.

The axillary buds on the nodal segments began to sprout regardless of the medium composition, but the axillary shoot growth varied among medium compositions. Figure 1 shows the dependence of the axillary shoot length on the medium composition. The axillary shoot of the explants in the BTM elongated longer than that in the WSM. Moreover, the axillary shoot elongation in the agar medium was better than that in the gelrite medium. The media containing 0.5mg／ℓ BAP or 1.0mg／ℓ BAP stimulated axillary bud breaking. A comparison of these two concentrations of BAP shows that

Table 2　Contamination percentage in the initial culture

Medium	Family name	Number of explants	Contamination percentage
1	Katsuyama2	50	6%
	Yamakuse-oku3	50	18%
	Yamakuse-oku12	37	0%
2	Katsuyama4	35	3%
3	Katsuyama4	35	3%
4	Yamakuse-oku12	35	6%
5	Katsuyama2	40	3%
	Yamakuse-oku3	35	25%

the axillary shoot in the medium containing 0.5mg／ℓ BAP elongated longer than that in the medium containing 1.0mg／ℓ BAP. However, multiple shoot formation was observed in the medium containing 1.0mg／ℓ BAP. These results suggest the possibility of mass production of plantlets from a nodal segment. Active carbon was added to absorb the growth inhibitor exuded from the explants[3], but the active carbon seems to have inhibitory effect on the axillary shoot elongation. This suggests that the active carbon absorbed BAP which stimulates bud breaking[3].

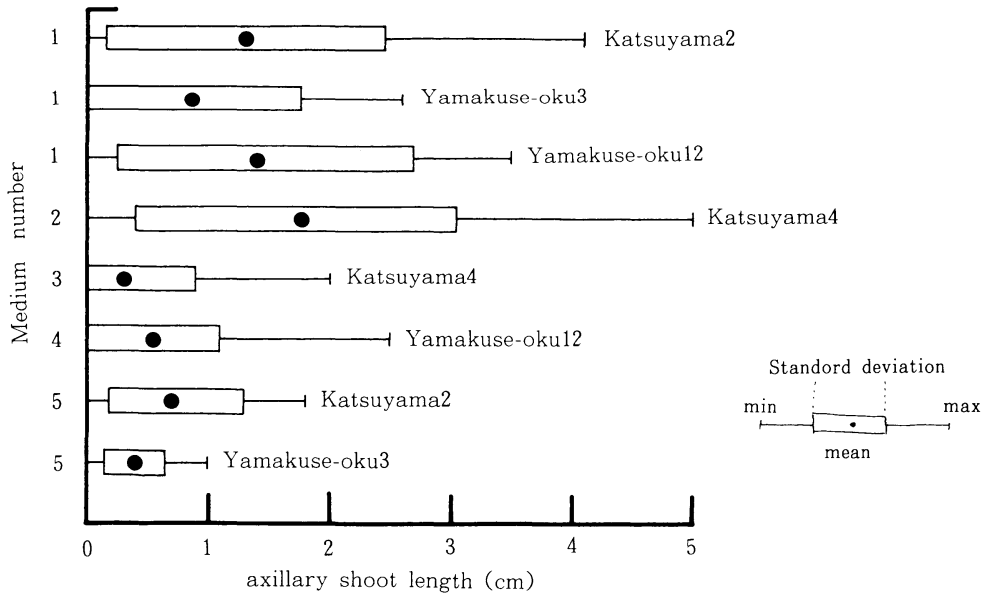

Fig. 1　Dependence of the axillary shoot length on the medium composition

We studied only a small number of families and media compositions. Further examination should be necessary to establish a standard method for tissue culture of *Kunugi*.

The axillary shoots elongated from the explants were transplanted to a rooting medium. Multiple shoots sprouted from an axillary bud were cut at the base of each shoot and each of the shoots was transplanted to the rooting medium. At forty days after transplanting to the rooting medium, rooting was observed in almost all of the plantlets. Rooting percentages were 69% for Katsuyama2, 80-90% for Katsuyama4, 88% for Yamakuse-oku3 and 75-81% for Yamakuse-oku12 (Table 3). The reason for high rooting percentage was that the explants used juvenile *Kunugi* seedlings.

Table 3 Rooting percentage and percentage of plantlets surviving the acclimatization process

Initial culture medium	Family name	Number of transplanted plantlet	Rooting percentage	Acclimatization percentage
1	Katsuyama2	35	69%	33%
	Yamakuse-oku3	55	88%	21%
	Yamakuse-oku12	77	81%	37%
2	Katsuyama4	67	90%	-
3	Katsuyama4	10	80%	-
4	Yamakuse-oku12	40	75%	35%

The regenerated plantlets were acclimated under conditions of controlled humidity condition. The percentage of rooted plantlets surviving the acclimatization process was 21-37% (Table 3).

3. 2 Regenerated plantlet growth

Many acclimating plantlets planted in the nursery died presumably because they were vulnerable to environmental stresses, such as desiccation, strong light conditions and variation in air temperature. The height of the regenerated plantlets when they were planted in the nursery was only 1.0cm - 6.0cm. The techniques for acclimatization to nursery conditions should be examined further.

Figure 2 shows the growth of regenerated plantlets and seedlings in the nursery from May 1993 to March 1994. The growth of regenerated plantlets and seedlings was analyzed four whole families. The tree height measured in May 1993 and March 1994 and basal diameter measured in May 1993 were not significantly different between the

regenerated plantlets and the seedlings ($P>0.05$ analysis of variance) and the basal diameter measured in March 1994 was different between the regenerated plantlets and seedlings ($P<0.05$ analysis of variance).

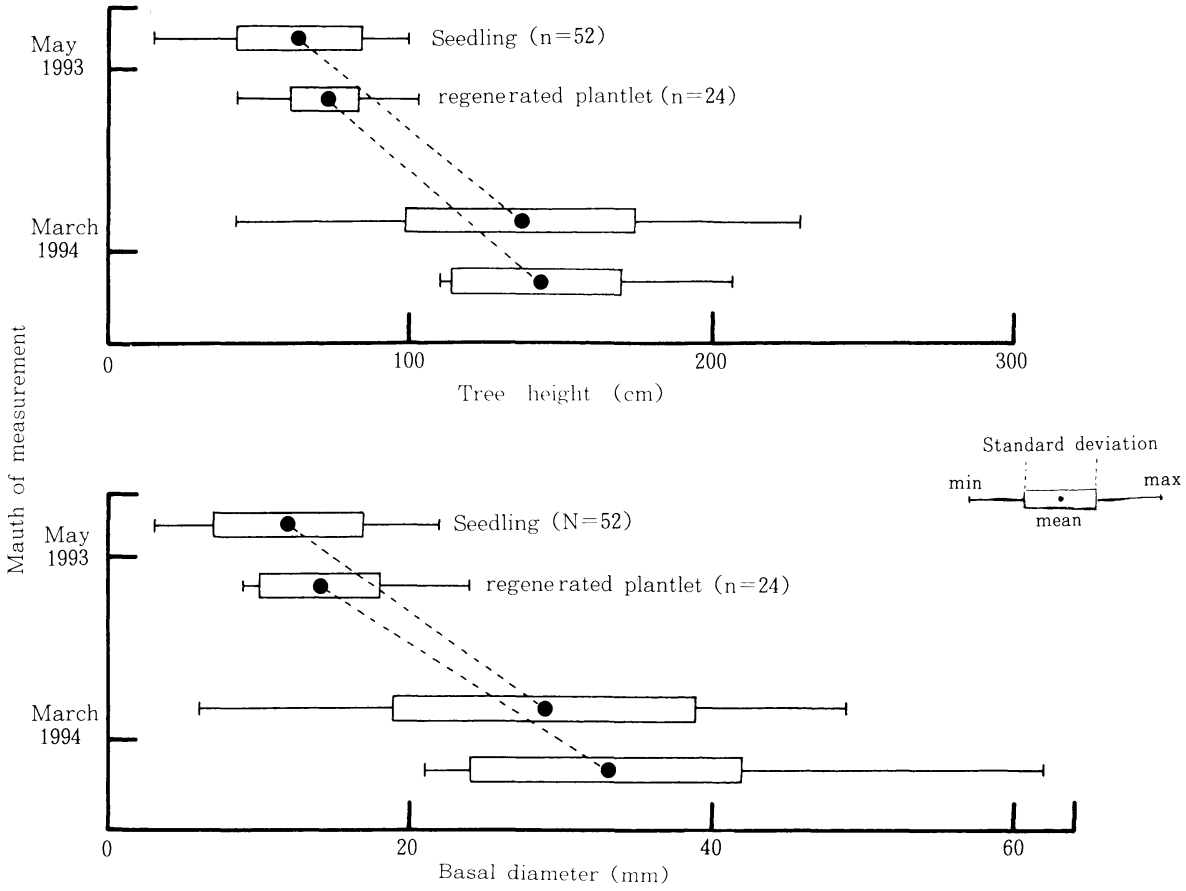

Fig. 2　Increase in the height and basal diameter of the regenerated plantlets and seedlings from May 1993 to March 1994

The standard deviation values of the seedlings were larger than those of the regenerated plantlets. Presumably, the growth of seedlings was dependent on acorn size. YAMADA and HANDA[8] reported that seedling growth of *Fagaceae* species was influenced by acorn characteristics, because *Fagaceae* tree species store food to foster the growth of seedlings in the acorn[4]. Therefore, standard deviation values of the regenerated plantlets that store little food were smaller than those of the seedlings.

However, it is possible that the genetic variation of regenerated plantlets were smaller than that of the seedlings, because the number of regenerated plantlets used to analyze were smaller than that of seedlings. The growth of regenerated plantlet should be examined further.

The growth of the regenerated plantlets was observed for 3 years. The experiment showed that there was no significant difference between the size of the regenerated plantlets and that of the seedlings. Mass production of nursery stocks for actual useshould become possible if the acclimatization techniques are improved.

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クヌギ葉腋からの幼植物体の再生と順化後の成長

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要旨：クヌギ実生の葉腋から幼植物体を再生した。基本培地としてＢＴＭ培地とＷＳＭ培地を使用した。葉腋からのシュートの伸長量は培地に加えた植物ホルモンなどの組成によって異なった。葉腋から伸長したシュートは1／2ＢＴＭ培地にＩＢＡとＮＡＡを加えた培地で発根した。発根した幼植物体は湿度を調節することにより順化することができた。順化した幼植物体と実生苗の成長を３年間比較した結果，両者の成長に違いは認められなかった。

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