Original Article

Rooting ability of cuttings from 1000-year-old Yaku-sugi (*Cryptomeria japonica*)

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【Abstract】

Yakushima in Japan was registered as a World Natural Heritage Site in 1993 for its ancient natural environment. On this island, extraordinarily long-lived Japanese cedars (*Cryptomeria japonica* D. Don), known as Yaku-sugi, can be found living to more than 1000 years old. It is estimated that there are now only 1000 specimens of these cedar trees because they have been felled during past 500 years for their irreplaceable value.

In this study, clonal propagation of those long-lived Yaku-sugi was tried by cutting. Cuttings were obtained from four naturally occurring Yaku-sugi estimated at 880, 1150, 1340, 1480 years old, and one young planted 85-year-old Yaku-sugi. NAA treatments were carried out at 0, 100, 200, and 400ppm for 24 hrs prior to the planting of cuttings and the rooting rate, root number, and root length were examined after 5 months.

Surprisingly, cuttings from the four long-lived Yaku-sugi rooted without NAA, though with low rooting rates below 30%, showing that they did have rooting-ability. Meanwhile, the young Yaku-sugi did not produce any roots, suggesting that there might be a genetic difference among the cedars examined. Variations in rooting rate, root number, and root length depended on the Yaku-sugi examined. The five cedars were classified into two groups, an easy rooting and a difficult rooting. The easy rooting group was highly sensitive to NAA concentration, that is, nearly 100% of the cedars examined rooted at 100ppm NAA. Whereas the difficult rooting group was less sensitive to the concentration, that is, their rooting rate increased with an increase in concentration. Root number and root length increased with NAA concentration. All the cedars examined showed high rooting rates from 70% to 100% when treated with 400ppm NAA, enough for practical use. The sensitivity of the young 85-year-old Yaku-sugi to NAA was similar to that of long-lived difficult rooting ones. This result suggests that the 85-year-old cedar may be applicable as a substitute material for the long-lived cedars in order to develop a clonal propagation technique.

Key Words: *Cryptomeria japonica*, cutting, long-lived, NAA, rooting ability, Yaku-sugi
キーワード：ヤクスギ、挿し木、スギ、高齢、NAA、発根能力

Introduction

Yakushima, locating in the southern part of the Japanese archipelago (Fig. 1), was registered as a World Nature Heritage Site in 1993 for its ancient natural environment. This island is the southern limit of Japanese cedar, *Cryptomeria japonica* D. Don (Taxodiaceae). Japanese cedar is one of the most important trees for Japanese forestry and there are more than 20 natural local races (Miyajima, 1989). The cedar on Yakushima is one such local race and it is called Yaku-sugi (Fig. 2). Among Japanese cedars, the Yaku-sugi have special characteristics, in particular they have an extraordinarily long lifespan. The average for Japanese cedars is about 500 years, but in the case of Yaku-sugi, trees more than 2000-year-old can be seen (Fig. 3). On Yakushima, cedars over 1000 years old are called Yaku-sugi, and those under 1000 years Ko-sugi, which means young or small cedar. The long-lived Yaku-sugi have irreplaceable value and are very expensive as decorative material.

The felling of Yaku-sugi started about 500 years ago and over time this has gradually reduced the huge cedars in number. It is estimated that are now only 1000 long-lived Yaku-sugi left. Because of this threatening situation, the development of a clonal propagation technique for the Yaku-sugi is necessary not only for their gene pool preservation but also for forestry on Yakushima.

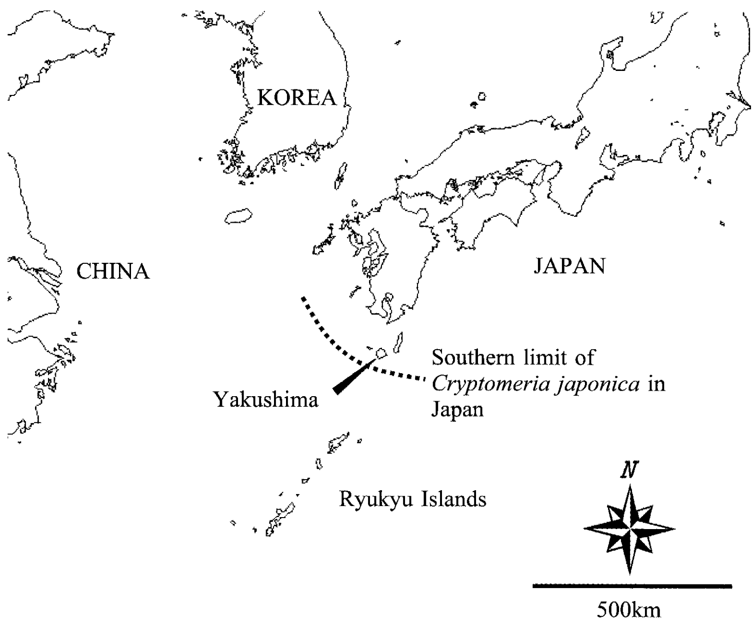

Fig.1 Location of Yakushima. Yakushima is known as the southern limit of *Cryptomeria japonica* distribution.

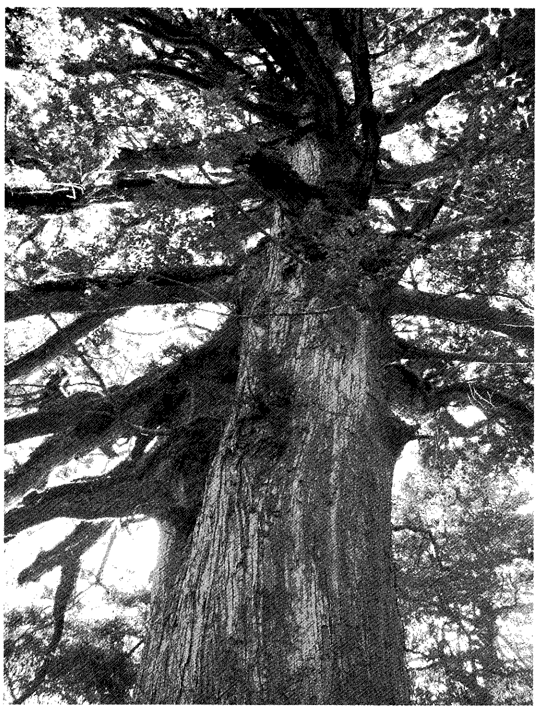

Fig. 2 A naturally occurring Yaku-sugi cedar, a local race of *Cryptomeria japonica*, estimated at 1340 years old (source S-1 in this study).

The advantage of clonal propagation is to obtain genetically identical clones from the selected mature trees that are proved superior in the natural forest, and propagation of Japanese cedar by cuttings has been carried out for at least 500 years (Ritchie, 1997). However, no clonal propagation has been attempted through cuttings in Yaku-sugi, though propagation by grafting was recently carried out (Kumamoto National Forest Agency, 2004; Umata et. al., unpublished).

Concerning propagation by cuttings, in general, two factors, namely, genotype and age of source tree have been thought to influence rooting significantly (Miyajima, 1989). Yaku-sugi has been thought as one of the most difficult-to-root genotypes in Japanese cedars (Hatusima, 1991), and established Yaku-sugi cultivars were either unknown (Miyajima, 1989) or few were known (Ishikawa & Tanaka, 1970). Probably due to this reason, the artificial Yaku-sugi forests were established by seedlings and not by cuttings. In addition, this cedar's extraordinarily long life of over 1000 years may make the rooting of cuttings difficult.

It was reported that indole-3-butyric acid (IBA) was effective for the rooting of Japanese cedar cuttings (Ooyama & Kaminaga, 1970). According to Ishikawa & Tanaka (1970), cuttings from four cultivated clones of plus Yaku-sugi cedars did not root by 0ppm and 200ppm of IBA solution except one clone rooted with 1% IBA-talc powder. However, only one cutting per each IBA concentration was used in their study, the appropriate concentration had not been cleared, and their cultivated mother clones were young (personal communication). Moreover, alpha-naphthaleneacetic acid (NAA) was not tested.

In this study, NAA was used as auxin and its effect for the rooting of cuttings from naturally occurring long-lived Yaku-sugi over 1000 years old was examined. In addition, an 85-year-old planted Yaku-sugi was used for comparison of rooting ability between young Yaku-sugi and the extraordinarily old ones.

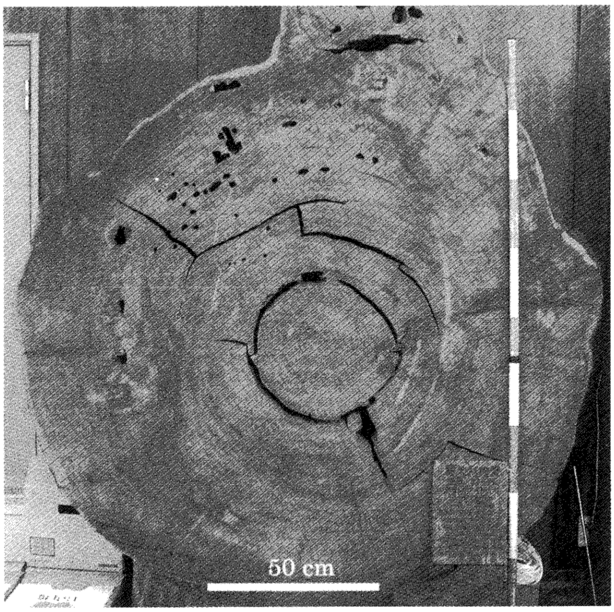

Fig. 3 A stem disk at the base height of a 1650 year old naturally occurring Yaku-sugi cedar, 220 cm in the maximum diameter and 164 cm in the minimum diameter, felled in 1968, belonging to Yakushima Forest Environment Conservation Center of Kumamoto National Forest Agency. Scale bar = 50 cm.

Materials and methods

Materials

The naturally occurring Yaku-sugi were growing at altitudes above 500m. Cuttings from the cedars were collected with the permission of the Yakushima Forest District of Kumamoto National Forest Agency. Four mother giant cedars were chosen in the forest of compartment 228 and the ages of the cedars were estimated based on the report of Sako et. al (1985). The data concerning the cedar sources are shown in Table 1.

In addition to the four long-lived cedars, one young Yaku-sugi was used from an 85-year-old seedling plantation of Kagoshima University Forest. The source seeds of the plantation were obtained from natural Yaku-sugi forests on Yakushima. Ten individual trees were chosen randomly from the 85-year-old plantation to examine their rooting ability preliminarily, and one cedar named KG-7 for convenience in this study was used. It was the most difficult-to-root among the 10 cedars examined.

Branches 50cm in length were obtained from the outer layer in the middle of individual cedar crown, and they were kept by burring their base to about 30cm in depth in a field of Kagoshima University Forest until the study started. Collection dates were 18-19 March 2003 on Yakushima and 21 April 2003 in Takakuma Forest, respectively. The study was carried out between 22 April and 25 September 2003.

Methods

First, 15cm-long cuttings were prepared from the branches, and ages and diameters of cuttings were measured at the bases with a magnifier and a vernier. Secondly, small lateral twigs were removed from the 5cm-basal end of the cuttings, and then, the bases of the cuttings were immersed 2 cm in depth in the solution of 0, 100, 200, and 400 ppm of NAA for 24 hrs, respectively. Next, the treated cuttings were planted into the pumice medium in containers (50 × 60 × 15cm) 5cm in depth. Finally, the containers were buried in soil up to their rims, and then they were covered with black cheesecloth for shading.

Fifteen S-1, 10 S-2, 20 S-3, 10 S-4, and 20 KG-7 cuttings were used for each NAA concentration, respectively.

Numbers used for each NAA concentration were 15, 10, 20, 10, 20 cuttings in S-1, S-2, S-3, S-4, and Kg-7, respectively.

The experiments were carried out in a field of Kagoshima

Table 1. Source name, gbh, height, estimated age, and habit of the tested Yaku-sugi cedars, a local race of *Cryptomeria japonica*

Source name	Gbh m	Height m	Estimated age*1 year	Habitat	Location
S-1	7.85	20.5	1340	Mountain ridge, 1350m above sea level	Compartment 228
S-2	5.71	21.7	880	Edge of ridge, 1350m above sea level	Compartment 228
S-3	8.38	18.4	1480	Edge of ridge, 1350m above sea level	Compartment 228
S-4	7.07	9.1*2	1150	Mountain ridge, 1350m above sea level	Compartment 228
KG-7*3			85	Mountainside, 480m above sea level	Compartment 7

*1; Estimated ages of long-lived Yaku-sugi cedars were obtained based on the report by Sako et al (1985).

*2; The upper part of stem might be broken by heart rot and lastly a typhoon.

*3; KG-7 was planted in 1921 in the Kagoshima University Forest.

University Forest. The cuttings were watered properly over the cheesecloth. The root number and root length of the cuttings were examined 5 months after planting. Structures, which did not show clearly as root with the naked eye, were categorized as calluses.

Results and discussion

Rooting rates of cutting

Fig. 4 showed that over 80% of the cuttings were alive in each source, indicating that Yaku-sugi keep an exuberant vitality even at 1000 years old. Among the living cuttings from the long-lived cedars, many formed calluses on their bases, suggesting that this might ensure that cuttings live. Surprisingly, over 10% of the cuttings from long-lived cedars rooted, which showed that long-lived cedars have the potential to root.

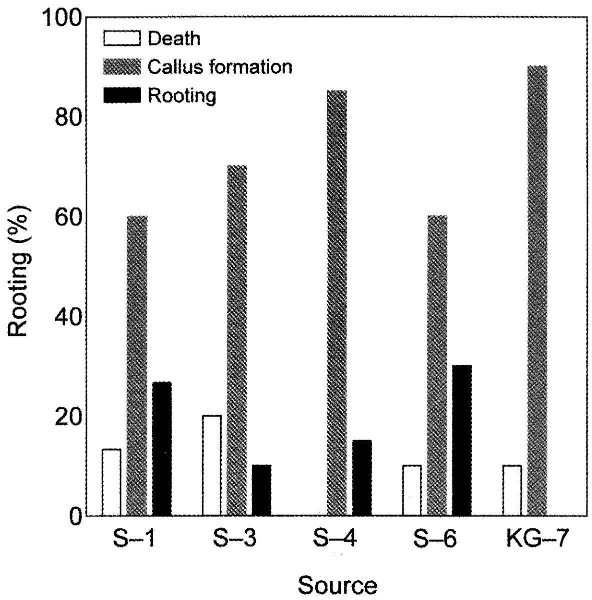

Fig. 4 Rooting rate of cuttings from the long-lived Yaku-sugi cedars without NAA treatment after 5 months of planting.

This may be the first report of the rooting of cuttings from trees more than 1000 years old in a tree species. The rooting performance of cuttings from such long-lived Yaku-sugi had been believed to be unsuccessful, but such a belief proved unfounded. Successful cuttings from some long-lived trees have been reported though rarely, and clonal plants were obtained, for example, from a cultivated 250-year-old Japanese cypress, *Chamaecyparis obtusa* Endl. (Miyajima, 1962) and a naturally occurring 500-year-old Japanese cypress (Umata et al., unpublished) through cuttings, moreover, from 500-year-old *Sequoia sempervirens* (Lamb. ex D. Don) Endl. (Fouret et al., 1988) and 100-year-old *Sequoiadendron giganteum* (Lindley) Buchholz (Monteuuis, 1991) through in vitro culture of the meristem. These results indicated that many long-lived trees might have great potential for propagation by means of cutting or in vitro culture.

The present study showed that long-lived Yaku-sugi over 1000 years old rooted while 85 year-old KG-7 did not produce any roots. Considering that there was a very large age difference among the cedars examined, rooting might be influenced little by the age but significantly by the source (i.e. genetic difference). As shown in Fig. 5, the difference in the rooting rates of 5 cedars related with difference in the source, when treated with NAA. Variability in rooting-ability of cuttings owing to genetic difference was known in cultivated clones of plus Japanese cedar (Sato, 1973) and in many tree species, such as *Acacia mangium* Willd. (Monteuuis et al., 1995), *Cupressus sempervirens* L. (Stankova et Panetsos, 1997; Capuana et al, 2000), and *Juniperus virginiata* L. (Henry et al, 1992).

The rooting rates in each Yaku-sugi were very low, namely, below 30% as shown in Fig. 4. It is well known that most conifers and a few hardwood genera are difficult-to-root when explants are taken from mature trees (Berhe and Negash, 1998; Henry et al., 1992; Monteuuis et al., 1995; Nautiyal et al., 1991; Teklehaimanot, 2000). In the case of Nangou-hi cypress, a cultivated clone of Japanese cypress, 18% of cuttings rooted from a 250-year-old cypress while 66% rooted from a 10-year-old one (Miyajima, 1962). A similar result was obtained in Japanese cedar (Higo et al., 1950). According to our unpublished data, around 70% of cuttings from some 15-year-old grafted long-lived Yaku-sugi, such as the Jomon-sugi, Okina-sugi, or Bandai-sugi cedars estimated above or around 2000 year-old (Yakusugi Museum, 1993), rooted without NAA treatment, though we could not attempt to study rooting of cuttings from those long-lived cedars directly.

In conclusion, the genetic difference of the Yaku-sugi may control rooting and its ageing may act as an important factor on the rooting rate.

NAA treatment

NAA markedly affected root formation of the cuttings in all the cedars examined (Fig. 5). The five cedars were divided into two groups, one is an easy rooting group for S-1 and S-4, and

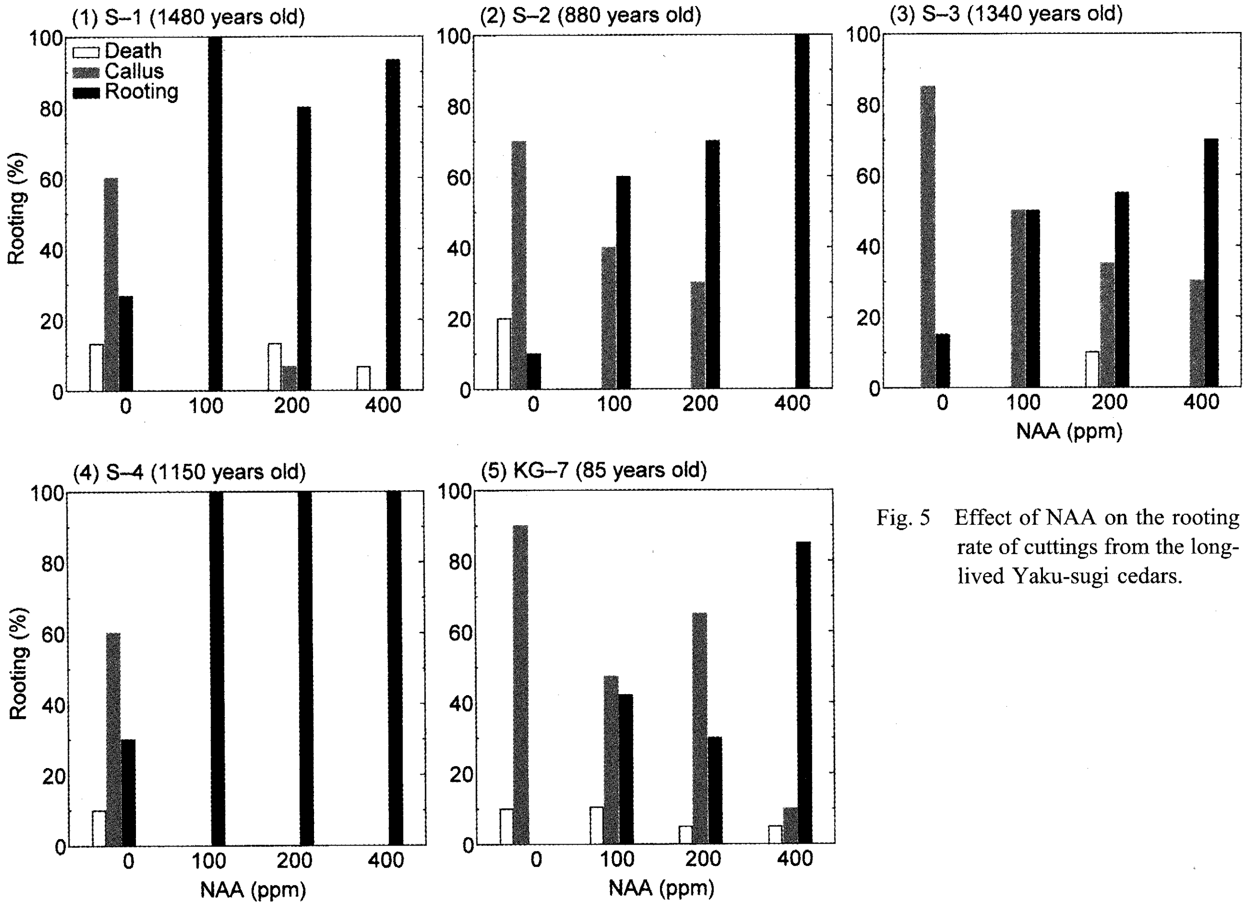

Fig. 5 Effect of NAA on the rooting rate of cuttings from the long-lived Yaku-sugi cedars.

the other is a difficult rooting group for S-2, S-3, and KG-7 (Fig.5). The former group was clearly distinguished from the latter group by its 100% rooting rate at 100ppm NAA, whereas the latter group showed less than 60 % at 100ppm NAA. The difficult rooting group increased its rooting rate as NAA concentration increased, and each cedar reached the highest rooting rate at 400ppm NAA. The number of roots and root length closely related NAA concentration, that is, both increased according to the increase in NAA concentration (Fig.6, 7). Only S-1 produced the greatest number of roots and the longest root at 100ppm NAA.

Auxins were known to induce rooting in the cuttings from *Cupressus sempervirens* L. (Capuana & Lambardi, 1995); and they are necessary for root formation in *Arbus andrachne* L. (Al Salem Mohammed & Karam Nabila, 2001). On the other hand, they had little or no effect when applied to difficult woody cuttings (Wilson, 1994) and *Irvingia gabonensis* Baill. (Shiembo et al., 1996). Yaku-sugi cuttings were known to be very difficult to root, but in the present study NAA application was found to enhance rooting markedly and to be necessary, in particular, for KG-7 cuttings that did not root without NAA.

Classification of the Yaku-sugi cedars examined into the easy rooting group and the difficult rooting one indicates that there are genetic differences in sensitivity to NAA concentration. Sources showing a high rooting rate without NAA (S-1, 4) were classified into the easy rooting group while those with low rate (S-2, 3) were into the difficult one. This suggests that sensitivity difference can be identified in the rooting rate without NAA treatment. Furthermore, genetic difference was seen concerning root number and root length, that is, the easy rooting group produced many and longer roots compared with the difficult rooting group. Among the difficult rooting group, only S-2 reached a 100% rooting rate at 400ppm NAA, suggesting that S-3 and KG-7 may require a higher NAA concentration for 100% rooting. On the other hand, S-1 exhibited a maximum rooting response at 100ppm NAA in rooting rate, root number, and root length, indicating that S-1 had the greatest sensitivity to NAA among the five specimens. Ishikawa & Tanaka (1970) reported that four cultivated clones of plus Yaku-sugi cedars did not root when treated with 0ppm and 200ppm IBA solution for 18 hrs although one rooted with 1% powder of IBA. This indicates that further studies will be needed concerning the auxin concentration, application type, or treatment time. In addition, the rooting of many other long-lived cedars must be examined.

According to previous reports, auxin effect varied among tree species, that is, auxin had effect on the root number but not on the rooting rate of *Acacia mangium* (Monteuuis et al., 1995) and *Chamaecyparis thyoides* (L.) B.S.P. (Hinesley & Snelling, 1997). On the other hand, it had effect on both the root number and rooting rate of *Maackia amurensis* Rupr. et Maxim (McNamara et al., 1994). In the case of Yaku-sugi cedars, NAA concentration strongly influenced root number and root length besides the rooting rate (Fig. 6-7). As shown typically in S-4 (Fig. 4-5), 100% rooting was achieved at 100, 200, and 400ppm NAA, but the root number and root length at 100ppm NAA were considerably smaller and shorter than those at 200 and 400ppm (Fig. 6-7). Among the examined concentrations of NAA, 400ppm was optimal in rooting rate (Fig. 5), root number (Fig. 6), and root length (Fig. 7) in all the cedars. Application of 400 ppm NAA induced 70% to 100% rooting rates (Fig. 5), which were thought to be enough for practical use.

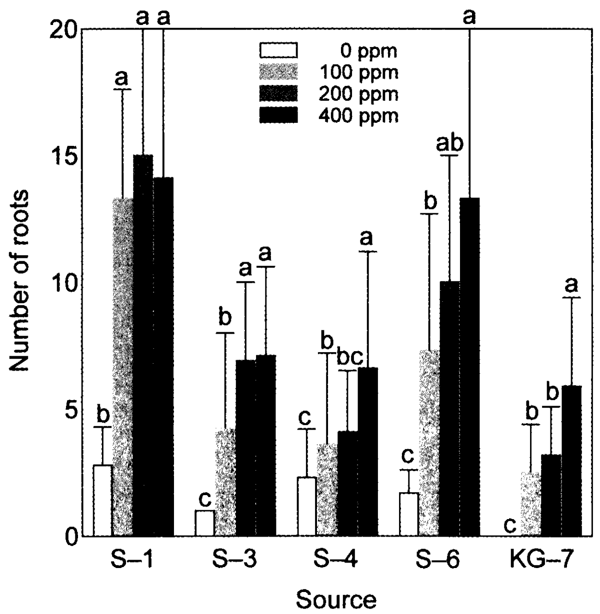

Fig. 6 Effect of NAA on the root number of rooted cuttings from the long-lived Yaku-sugi cedars. The different letters above each column indicate significant differences by Tukey's test (P<0.05). Error bars represent one standard deviation.

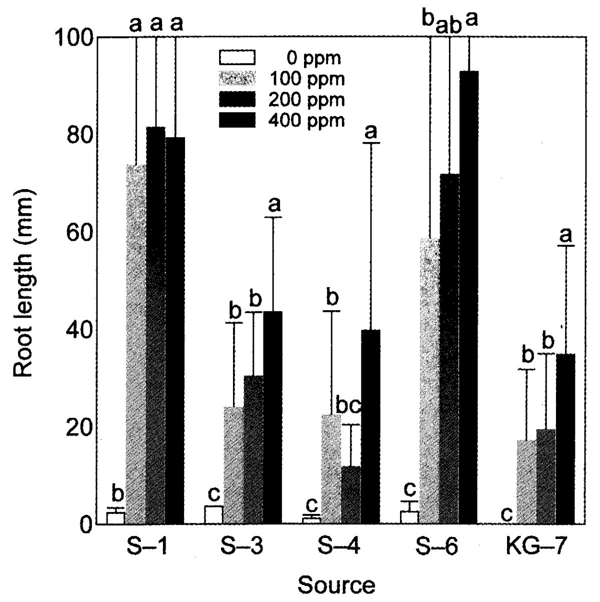

Fig. 7 Effect of NAA on the root length of rooted cuttings from the long-lived Yaku-sugi cedars.The different letters above each column indicate significant differences by Tukey's test (P<0.05). Error bars represent one standard deviation.

Excessive concentrations of auxins were not effective for rooting in teak (Nautiyal et al., 1991), African Pencil cedar (Berhe & Negash, 1998), and *Prunus africana* (Hook.f.) Kalkman (Tchoundjeu et al., 2002), and they were inhibitory in Douglas fir (Copes & Mandel, 2000). Concerning Japanese cedar, about 100ppm auxin for 24 hrs was recommended as the optimal concentration (Machida, 1974). For example, application of 100ppm IBA for 24 hrs was very effective for rooting of plus cultivated clones, showing a 48% to 90% rooting rate, however they showed a 10% to 27% rooting rate without IBA (Ooyama and Kaminaga, 1970). This concentration suggests that there may be considerable difference in sensitivity to auxin between Yaku-sugi cedar and other Japanese cedars, and this low sensitivity of Yaku-sugi cedar to auxin may be one of the factors why it is difficult to root.

Young Yaku-sugi as a substitute material for long-lived ones

The reaction of the 85-year-old KG-7 to the NAA treatment was similar to that of the over 1000 year-old S-2 and S-3 that were classified into the difficult rooting group in this study. This indicates the possibility that KG-7 can be used in a simulation study on the propagation of long-lived Yaku-sugi. As the amount of very old Yaku-sugi is insufficient in number, it is not agreeable to take many cuttings from those long-lived cedars even for experiments on propagation. For this reason, only a small number of cuttings were prepared in this study. Substitute cedars for the long-lived cedar have been sought for further study on the propagation and protection of long-lived Yaku-sugi. The present result shows that KG-7 can be a suitable substitute for them. The method obtained by the substitute may be applicable to other long-lived tree species.

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* The titles are approximate translations of the original Japanese title by the authors of this paper.

要 約

屋久島は1993年に世界自然遺産に登録された。 この島には1,000年以上の齢を持つヤクスギが生育しているが，このような高齢のヤクスギはその高価さ故に過去500年間にも及ぶ伐採によって減少し，今ではわずか1,000本を数えるにすぎない。本研究ではこれら高齢のヤクスギのクローンを挿し木によって得ることを試みた。

挿し穂は推定樹齢880年，1150年，1340年，1480年の高齢天然ヤクスギ4個体と，85年生の若齢人工植栽ヤクスギ1個体から採取した。挿し穂を0，100，200，400 ppmのNAA液で24時間処理してから挿し木を行い，5ヶ月後に発根状況を調査した。高齢ヤクスギにおいては0 ppm処理において30%以下の発根率ではあったが，発根能力を維持していることが明らかになった。これに対して若齢ヤクスギでは発根が認められず，その原因として個体間の遺伝的な差異が影響しているものと考えられた。NAA処理においては発根率，発根数そして根長は供試木によって異なり，5個体のヤクスギは発根が容易な群と困難な群の2つに分かれた。すなわち，100 ppmで100%近い発根率を示す感受性の高い個体群と，NAA濃度の増加とともに発根率の高くなる感受性の低い個体群であった。根の本数と長さは濃度が増すごとに増加した。しかし，いずれの個体においても400 ppmのNAAで処理すると発根率は70～100%となり，充分実用に耐える得苗率が得られた。若齢のヤクスギのNAAに対する反応は感受性の低い高齢ヤクスギ個体の反応と類似した。このことから，今後の増殖試験において，85年生のヤクスギは発根性の低い高齢ヤクスギの代替品として利用可能と考えられた.