iForest Biogeosciences and Forestry

Research Article
doi: 10.3832/ifor1661-008
vol. 9, pp. 370-374

Effect of family, crown position, number of winter buds, fresh weight and the length of needle on rooting ability of *Pinus thunbergii* Parl. cuttings

Tetsuji Hakamata [(1)], Yuichiro Hiraoka [(2)], Shigehiro Yamamoto [(3)], Kimihiko Kato [(4)]

As a measure for contrasting pine wilt disease, which caused serious damage in Japanese black pine (*Pinus thunbergii* Parl.) by the pine wood nematode (*Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle), resistant trees have been widely planted in Japan. The propagation of resistant trees using cuttings obtained from healthy stock plants and inoculated with pine wood nematode is expected to further increase in the next future. To improve the cutting propagation of Japanese black pine trees resistant to pine wilt disease, the factors associated with rooting and root volume were investigated. The type of cutting and the crown position of stock plants from which cuttings were taken, were markedly associated with rooting. The crown position did not show significant interactions with any other investigated factor, while fresh weight of cuttings and their number of winter buds did not affect rooting. The rooting percentage was markedly higher for cuttings taken from the lower crown than for those from the upper crown, as already reported for other coniferous tree species. The length of the longest needle was significantly correlated with the root volume of cuttings, and showed significantly interactions with crown position and fresh weight of cuttings. Such correlation suggests that the growth of needles can be considered a useful predictor in the assessment of the root volume of cuttings during the propagation period, allowing growers to transplant rooted cuttings at the appropriate time without excavating or uprooting. These findings may contribute to the improvement of cutting propagation of Japanese black pine.

Keywords: Cutting Propagation, Crown Position, Rooting, Root Volume, *Pinus thunbergii* Parl.

Introduction

As one of the countermeasures for pine wilt disease caused by the pine wood nematode [PWN, *Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle], which provoked serious damage in Japanese black pine (*Pinus thunbergii* Parl.), resistant trees have been widely planted in Japan. Seedlings originating from seed production orchards of the resistant mother trees are inoculated with PWN, and the healthy seedlings are available for commercial sale. However, an intensive summer work is required for producing these seedlings, and the mortality rate of approximately 50% after inoculation makes them expensive. Further, the percentages of symptomless seedlings in inoculation tests is not stable, fluctuating over the years because of varying environmental conditions (Toda 2004).

To avoid these problems, resistant trees are propagated by cuttings obtained from healthy young seedlings that have been inoculated with PWN. Although rooting rates are generally low in cuttings of Japanese black pine (Morishita & Ooyama 1972), the cuttings obtained from certain stock plants have exhibited high rooting rates (Mori et al. 2004). Moreover, rooted cuttings taken from resistant seedlings have shown a high survival percentage after inoculation with PWN (Mori et al. 2006b), suggesting that re-inoculation to confirm the resistance of the rooted cuttings is not required. The production of resistant rooted cuttings has been estimated by Ohira et al. (2009) to be less expensive than other methods of propagation, and its application for Japanese black pine propagation is expected to increase.

External conditions, such as the bed media type, treatment with a rooting-promoting agent, temperature, humidity, and lighting intensity, are associated with rooting responses in cuttings of black pine. Physiological features of cuttings are also important. The phenomenon wherein differences in the physiological status of cut-

(1) Shizuoka Prefectural Research Institute of Agriculture and Forestry, Forestry and Forest Products Research Center, 2542-8 Negata, Hamakita, Hamamatsu, Shizuoka, 434-0016 (Japan); (2) Forest Tree Breeding Center, Forestry and Forest Products Research Institute, 3809-1 Ishi, Juo, Hitachi, Ibaraki, 319-1301 (Japan); (3) Shizuoka Prefectural Agriculture and Forestry College, 4034-5 Oro, Hamakita, Hamamatsu, Shizuoka, 434-0015 (Japan); (4) Shizuoka Prefectural Research Institute of Agriculture and Forestry, 678-1 Tomioka, Iwata, Shizuoka, 438-0803 (Japan)

@ Tetsuji Hakamata (tetsuji1_hakamata@pref.shizuoka.lg.jp)

Received: Apr 06, 2015 - Accepted: Dec 10, 2015

Citation: Hakamata T, Hiraoka Y, Yamamoto S, Kato K (2016). Effect of family, crown position, number of winter buds, fresh weight and the length of needle on rooting ability of *Pinus thunbergii* Parl. cuttings. iForest 9: 370-374. – doi: 10.3832/ifor1661-008 [online 2016-01-11]

Communicated by: Gianfranco Minotta

tings taken from the same tree influence the rooting ability is called "topophysis" (Dodd & Power 1988). As an example of topophysis, the rooting ability of cuttings from the lower crown of stock plants is higher than those taken from the upper crown in some coniferous trees (Foster et al. 1984, Morgenstern 1987, Maeda et al. 1997, Peer & Greenwood 2001). Furthermore, the rooting percentage varies in different morphological cuttings from the same tree (Henry et al. 1992). In black pine, it has been reported that the length and diameter of cuttings influence the rooting ability (Ohira et al. 2006, Watanabe 2006) and that the removal of winter buds from cuttings improves rooting (Sasaki et al. 2004). However, Miyazaki (2003) reported that the removal of winter buds was not related with the rooting percentage in black pine. Thus, the effect of the removal of winter buds remains poorly elucidated. Other cutting factors, such as the original number of winter buds, the fresh weight, and the position on the crown of stock plants from which cuttings are obtained, have not been fully explored. Clarifying the association of the rooting ability with crown positions and morphological characteristics of cuttings can increase the practical success rate of the cuttings.

Most previous studies on cutting propagation in black pine have focused on rooting percentages; however, this parameter is insufficient for the assessment of rooting success. The root volume is also important because it affects the growth of rooted cuttings (Struve et al. 1984).

Mori & Miyahara (2002) suggested a practical though effective method to assess rooting in Japanese black pine: cuttings not easily uprooted from the bed medium have to be considered as rooted. However, this practice has the disadvantage of being stressful for rooted cuttings. In pine tree cuttings, needles from new shoots do not grow before rooting, even if the shoots grow (Morishita & Ooyama 1972). The relation between rooting and growth of needles remains unclear. However, this could represent a quick method to infer rooting without excavating cuttings from the bed media. Indeed, the assessment of the root volume of cuttings based on the observation of needles during the propagation period would allow growers to transplant rooted cuttings at the appropriate time.

In the present study, the impact of the crown position of stock plants on the success of cuttings was analyzed in order to improve the propagation of cuttings from Japanese black pine. In addition, two morphological characteristics of cuttings, i.e., the original number of winter buds and the fresh weight, were included in the analysis. Finally, the relation between the root volume and growth of needles was investigated in rooted cuttings.

Material and methods

Plant materials

Half-sib seeds were collected from mother trees in seed production orchards of Japanese black pine of the following six pine wilt-resistant clones: Namikata-37, Namikata-73, Shima-64, Tanabe-54, Tosashimizu-63, and Tsuyasaki-50 (Tab. 1). These open-pollinated seeds were sown at the end of March 2010, and the seedlings were transplanted by the middle of March 2011 in a nursery field at the Shizuoka Prefectural Research Institute of Agriculture and Forestry, Forestry and Forest Products Research Center (Hamamatsu, Shizuoka prefecture, Japan). Some 17-19 seedlings per half-sib family were used as stock plants.

Preparation, planting and culture conditions

Cutting experiments were conducted in the middle of February 2013, which is an appropriate season for obtaining cuttings of Japanese black pine (Ohira et al. 2007).

Fifty cuttings were taken from each half-sib family for a total of 300 cuttings. Twenty-five cuttings were randomly obtained from branches in the upper crown of 17 to 19 stock plants without stumping, while the remaining half of the cuttings were taken from branches in the lower crown (Tab. 1). The height of stock plants was approximately 50-100 cm.

The cuttings were 5-cm long, and the needles were removed from all but the most upper part (2 cm) of the cuttings. A standard two-way slanting cut was made across the base. The original number of winter buds and the fresh weight of each cutting were recorded. The average fresh weight and number of winter buds of cuttings are shown in Tab. 1. Because of the better growth of branches in the upper crown than in the lower crown, both the average fresh weight and number of winter buds of cuttings from the upper crown were higher than those from the lower crown.

The cuttings were then dipped in the rooting hormone solution (indole butyric acid, 4000 ppm) for 5 s (Sasaki et al. 2004). They were then vertically inserted into the bed container (47×23×9 cm – L×W×H) filled with moist Kanuma soil medium (which contains low levels of organic nutrients suitable for cuttings) and planted within 1 day after their removal from stock plants.

The bed containers were placed on shelves in a greenhouse and misted for 25 to 30 s every 25-30 min all day long. During the experiment period, the temperature in the greenhouse ranged from -2.0 °C to 40.7°C. Bed containers were rotated once a week in order to avoid differences in irrigation and light conditions.

Measuring needle length, rooting and root volume

Winter buds of the cuttings revealed a gradual growth into new shoots after being planted. The length of the longest new needle from a new shoot in each cutting was measured at the beginning of October 2013 (approximately 8 months after planting).

Cuttings were excavated from the bed containers and their rooting was examined. Roots were washed with water to rinse off soil residuals, and then gently wiped with a towel. The root volume was estimated by measuring the increase in the water volume after the roots were dipped into a 100-mL graduated cylinder filled with 80 mL water, according to Kathiravan et al. (2009).

Data analysis

To identify the factors associated with rooting, a logistic regression analysis was performed using the R statistical software (version 2.15.1) on data from 300 cuttings of six half-sib families. The rooting was de-

Tab. 1 - Summary statistics of Japanese black pine cuttings. Planting occurred during the 3rd week of February 2013, and the excavation was conducted 33-34 weeks later. Numbers in brackets (last row) represent mean values. (Nsp): number of stock plants; (Npl): number of planted cuttings; (FW): fresh weight; (Nwb): number of winter buds; (RP): rooting percentage; (MRV): mean root volume.

Half-sib family from stock plant	Nsp	Upper crown					Lower crown					Overall average	
		Npl	FW (g)	Nwb	RP (%)	MRV (cm^3)	Npl	FW (g)	Nwb	RP (%)	MRV (cm^3)	RP (%)	MRV (cm^3)
Namikata-37	18	25	8.5 ± 2.3	4.7 ± 1.5	60.0	2.20	25	6.7 ± 1.8	3.1 ± 1.4	92.0	4.19	76.0	3.41
Namikata-73	18	25	7.2 ± 2.7	4.6 ± 1.4	36.0	3.05	25	4.8 ± 1.6	3.1 ± 1.5	80.0	3.34	58.0	3.25
Shima-64	19	25	6.7 ± 2.9	4.1 ± 1.6	40.0	3.52	25	5.5 ± 1.6	3.6 ± 1.5	88.0	3.04	64.0	3.19
Tanabe-54	17	25	7.9 ± 2.0	5.3 ± 1.4	16.0	1.95	25	5.1 ± 1.7	3.2 ± 1.8	84.0	2.73	50.0	2.60
Tosashimizu-63	18	25	4.7 ± 1.6	4.0 ± 1.6	24.0	2.67	25	4.7 ± 1.4	3.0 ± 1.5	44.0	3.40	34.0	3.14
Tsuyasaki-50	18	25	7.6 ± 2.8	4.6 ± 1.6	32.0	2.91	25	6.3 ± 1.9	4.6 ± 1.7	60.0	3.52	46.0	3.31
Total (Average)	*108*	*150*	*(7.1 ± 2.7)*	*(4.6 ± 1.6)*	*(34.7)*	*(2.75)*	*150*	*(5.5 ± 1.8)*	*(3.4 ± 1.6)*	*(74.7)*	*(3.37)*	*(54.7)*	*(3.17)*

signated as the response variable, and the family of the stock plant, the crown position of the cutting (location on the stock plant), the fresh weight of cuttings, and their original number of winter buds were considered as explanatory variables. The additive models, including all four explanatory variables and an intercept, were constructed using the logit link function as rooting is binomial. The model used is represented by the following equation (eqn. 1):

$$logit(rooting) = \beta_0 + \beta_1 \cdot family_i + \beta_2 \cdot position_i + \beta_3 \cdot weight_i + \beta_4 \cdot bud_i$$

where β_0 is the intercept, and β_1, β_2, β_3 and β_4 are the effects of the family, crown position, fresh weight, and number of winter buds, respectively.

The analysis of the root volume was conducted on data obtained from 164 rooted cuttings. For the additive model of the root volume designated as the response variable, a generalized linear model (GLM) was used with a log function because the root volume data exhibited a gamma distribution. The family, crown position, fresh weight, number of winter buds, and length of the longest needle were designated as explanatory variables, such that (eqn. 2):

$$\log(root\ volume) = \beta_0 + \beta_1 \cdot family_i + \beta_2 \cdot position_i + \beta_3 \cdot weight_i + \beta_4 \cdot bud_i + \beta_5 \cdot needle_i$$

where β_0,is the intercept, while β_1, β_2, β_3, β_4 and β_5 are the effects of family, crown position, fresh weight, number of winter buds, and length of the longest needle, respectively.

After conducting the logistic regression and GLM analysis, only explanatory variables which markedly affected response variables were considered for further analysis. Comparison of the rooting percentages between cuttings obtained from the lower and the upper part of the crown (crown position) was carried out by χ^2 test. In addition, the correlation between root volume and the length of the longest needle of cuttings (both from upper and lower crowns) was calculated using a permutational Spearman's rank test. Furthermore, all cuttings were divided into three groups based on their fresh weight (light, medium and heavy) with a nearly equal number of cuttings (55, 55, and 54, respectively), and the correlation coefficients between the root volume and length of the longest needle were also calculated for each group.

Tab. 2 - Results of the logistic regression and GLM analysis of the effects of the investigated factors on rooting or root volume of the rooted cuttings (Type II model ANOVA summary table). (*): $p < 0.05$; (**): $p < 0.01$; (***): $p < 0.001$.

Response variable	Explanatory variable	LR	χ^2 df	Pr(>χ^2)
Rooting	Family (F) ***	34.533	5	1.865×10^{-6}
	Crown position of cutting (C) ***	40.292	1	2.187×10^{-10}
	Fresh weight of cutting (W)	1.715	1	0.190
	Number of winter buds (B)	1.729	1	0.188
	F × C	5.599	5	0.347
	F × W ***	20.978	5	0.001
	F × B ***	24.188	5	1.998×10^{-4}
	C × W	0.010	1	0.921
	C × B	0.936	1	0.333
	W × B	0.058	1	0.810
Root volume	Family (F)	2.269	5	0.811
	Crown position of cutting (C)	2.035	1	0.154
	Fresh weight of cutting (W)	0.039	1	0.843
	Number of winter buds (B)	0.224	1	0.636
	Length of longest needle (N) ***	48.221	1	3.809×10^{-12}
	F × C	4.884	5	0.430
	F × W	3.125	5	0.681
	F × B	4.585	5	0.469
	F × N	6.220	5	0.285
	C × W	0.569	1	0.451
	C × B	0.634	1	0.426
	C × N*	4.890	1	0.027
	W × B**	8.160	1	0.004
	W × N*	6.093	1	0.014
	B × N	1.572	1	0.210

Results

Factors influencing rooting

The results of the logistic regression analysis indicated that family ($p < 0.001$) and crown position ($p < 0.001$) significantly associated with the rooting of cuttings (Tab. 2). The crown position did not interact with any other variable; however, family interacted with the fresh weight and winter bud number of the cuttings.

Rooting percentages of the six families ranged from 34.0% to 76.0%, with a mean of 54.7% for all the 300 cuttings (Tab. 1). The rooting percentage was considerably higher for cuttings taken from the lower crown (74.7%) than from the upper crown (34.7%). These rooting percentages significantly differed according to the χ^2 test ($p < 0.001$).

Factors influencing root volume

GLM analysis revealed that the length of the longest needle significantly correlated with the root volume ($p < 0.001$); however, family, crown position, fresh weight, and number of winter buds did not show any significant effect on the same parameter (Tab. 2). However, given the significant interaction ($p < 0.05$) of the length of the longest needle with two factors, *i.e.*, crown position and fresh weight of cutting, were significant, further analyses were conducted.

The mean root volume ranged from 2.60 to 3.41 cm^3 among families, and the mean volume across all families was 3.17 cm^3 (Tab. 1). The correlation between the length of the longest needle just before excavation and the root volume was significant in cuttings taken from both the upper ($r_s = 0.723$, $p < 0.001$) and lower ($r_s = 0.402$, $p < 0.001$) crowns (Fig. 1a, Fig. 1b). The correlation coefficients between the

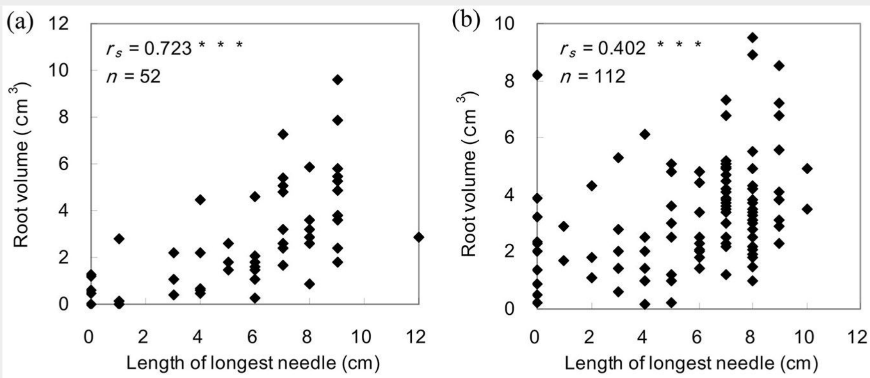

Fig. 1 - Correlation between the length of the longest needle and the root volume of rooted cuttings excised at two different positions on the stock plants: (a) cuttings taken from the upper crown; (b) cuttings taken from the lower crown. (rs): correlation coefficient based on a permutational Spearman's rank test; (n): sample size; (***): p < 0.001.

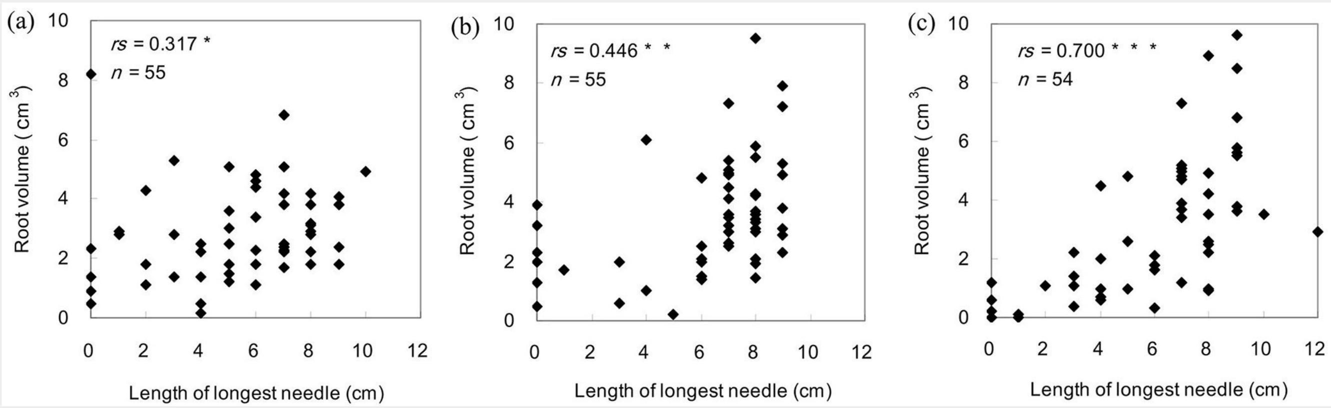

Fig. 2 - Correlation between the length of the longest needle and the root volume among groups of rooted cuttings. All cuttings were divided into three groups with nearly a equal number of cuttings, based on their fresh weight: (a) light, (b) medium, and (c) heavy. (rs): correlation coefficient based on a permutational Spearman's rank test; (n): sample size; (*): p <0.05, (**): p < 0.01, (***): p < 0.001.

length of the longest needle and the root volume were 0.317 ($p < 0.05$), 0.446 ($p < 0.01$), and 0.700 ($p < 0.001$) for cuttings in the light, medium, and heavy groups, respectively, and they showed an increasing trend with the fresh weight of the cuttings (Fig. 2a, Fig. 2b, Fig. 2c). Such analyses confirmed the significant correlations between the length of the longest needle and the root volume.

Discussion

The preparation of cuttings from stock plants with a high rooting ability is critical in order to achieve a high propagation rate. It has been universally recognized that the rooting ability differs among families not only in Japanese black pine (Mori et al. 2006a) but also in other pine species (Baltunis et al. 2005). In the present study, the rooting percentage varied among families of Japanese black pine, thus confirming that the genetic potential is an important factor to be considered for the successful establishment of cuttings.

The crown position on the stock plant the cutting are taken from is one of the decisive factors in producing successful cuttings of economically important tree species. Cuttings taken from the lower part of the crown have shown a higher rooting percentage in western hemlock (*Tsuga heterophylla* (Raf.) Sang. – Foster et al. 1984), dawn redwood (*Metasequoia glyptostroboides* – Ogasawara & Shidei 1966), black spruce (*Picea mariana* (Mill.) B. S. P – Tousignant et al. 1995), fraser fir (*Abies fraseri* (Pursh) Poir – Rosier et al. 2006), larch (*Larix* spp. – Peer & Greenwood 2001), and Japanese cypress (*Chamaecyparis obtusa* – Maeda et al. 1997). The 3-year-old stock plants used in this experiment were slightly younger and smaller than those used in the aforementioned examples. However, as already observed in other coniferous tree species, the crown position of cuttings was substantially associated with their rooting in Japanese black pine. Indeed, the rooting percentage of cuttings from the lower crown was significantly higher than that from the upper crown.

In other coniferous species, the different rooting potential of cuttings from different parts of the tree is believed to be related to their varying amounts of growth-promoting and rooting-inhibiting substances. In dawn redwood (*M. glyptostroboides*), cuttings with high rooting ability taken from the lower crown contained high levels of hormones (Ogasawara & Shidei 1966). Tannins, which seemed to inhibit rooting, were present at low levels in the lower branches of Japanese cypress (Hashizume & Taniguchi 1981). Browne et al. (1996) found that the nutrient status of cuttings differed with the cutting position in jack pine (*Pinus banksiana* Lamb.). Moreover, Tousignant et al. (1995) pointed out differences in the physiological maturation of plant tissues from different crown positions. The cuttings from the upper crown revealed advanced maturation, while those from the lower crown remained at a juvenile stage. The rooting percentage was higher in cuttings from shade-grown stock plants in Japanese umbrella pine (*Sciadopitys verticillata* – Yates et al. 2006), suggesting that sunlight may negatively affect the rooting ability of tissues to be excised. Indeed, the cuttings from the lower crown are subject to shading.

In the present study, the reason underlying the differences in the rooting percentage observed between the two crown positions of cuttings was not investigated. Japanese black pine may possess different quantities of growth-promoting and rooting-inhibiting substances or exhibit different levels of physiological maturation in different parts of the tree. Both shade and nutrient effects may contribute to the high rooting ability of cuttings taken from the lower crown of Japanese black pine. The association of these factors with the rooting ability of cuttings calls for future research on this species. In any case, we can recommend that cuttings should be taken from the lower crown, particularly in the case of 3-year-old Japanese black pine stock plants. Nonetheless, Browne et al. (1996) reported that the lower branches of jack pine did not always exhibit higher rooting percentages than the upper branches among stock plants of various ages. Thus, further experiments are required for supporting our findings in older stock plants of Japanese black pine.

Cuttings of appropriate sizes should be used for effective propagation, as mentioned by Kathiravan et al. (2009). Ohira et al. (2006) reported that small cuttings (mean length 3.9 cm; mean diameter 3.8 mm) of Japanese black pine exhibited a higher rooting percentage than both medium (mean length 7.0 cm; mean diameter 4.5 mm) and large (mean length 12.3 cm; mean diameter 7.6 mm) cuttings. In order to further explore the effect of the size of cuttings on rooting ability, we investigated the influence of fresh weight of 5-cm-long cuttings on rooting. However, based on our results, the fresh weight of cuttings did not affect the rooting ability. Because fresh weight of cuttings is not reported by Ohira et al. (2006), an exact comparison of the fresh weight of cuttings in their study with that observed in this investigation is not feasible. However, based on the estimated volume of cuttings reported by Ohira et al. (2006), they seems to use cuttings of a wider range of fresh weights than those used in the present study. This difference in the cutting size may likely explain possible differences in their rooting.

Statistical analysis revealed that the number of winter buds did not affect rooting. Although the influence of winter bud removal on rooting was previously reported only twice (Miyazaki 2003, Sasaki et al. 2004), the present study can support the conclusion of Miyazaki (2003) that the winter bud removal did not influence the rooting percentage in Japanese black pine.

Rosier et al. (2006) found that the primary needle length of cuttings just before their insertion into the bed correlated with the rooting percentage and root length of cuttings in Virginia pine (*Pinus virginiana* Mill.). According to Morishita & Ooyama (1972), the needles of non-rooted pine cuttings do not grow, regardless of the elongation of the new shoots. In addition to these associations, we report a new finding about Japanese black pine – the length of the longest needle just before transplanting correlated with the root volume in the case of young stock plants. This result suggests that the growth of needles may successfully predict the root volume without excavation of cuttings. Based on this prediction, growers can transplant rooted cuttings at the appropriate time, resulting in well-grown rooted cuttings.

Conclusions

This study identified the factors that affect the rooting ability of cuttings from young Japanese black pine stock plants, as well as the significant correlation between the root mass and needle growth. The crown position substantially and independently associated with the rooting. Furthermore, the rooting percentage of cuttings taken from the lower crown was markedly higher than that of cuttings excised from the upper crown. The length of the longest needle significantly correlated with the root volume of cuttings and also interacted with other factors. These findings constitute a useful contribution to the practical propagation of Japanese black pine cuttings resistant to pine wilt disease.

Acknowledgements

We thank Dr. I. Tamaki (Gifu Academy of Forest Science and Culture, Japan), Dr. T. Mitsunaga (NARO Agricultural Research Center, Japan) and Mr. T. Hoshikawa (Shizuoka Prefectural Research Institute of Agriculture and Forestry, Forestry and Forest Products Research Center, Japan) for providing us with appropriate suggestions.

TH and SY carried out experiments of cuttings. YH performed the statistical analysis. KK conceived the study and helped to draft the manuscript.

References

Baltunis BS, Huber DA, White TL, Goldfarb B, Stelzer HE (2005). Genetic effects of rooting loblolly pine stem cuttings from a partial diallel mating design. Canadian Journal of Forest Research 35: 1098-1108. - doi: 10.1139/x05-038

Browne RD, Davidson CG, Gobin SM (1996). Effects of crown position and plant age on rooting of jack pine long shoot cuttings. Tree Planters' Note 47 (3): 100-104. [online] URL: http://www.rngr.net/publications/tpn/47-3/

Dodd RS, Power AB (1988). Clarification of the term topophysis. Silvae Genetica 37 (1): 14-15. [online] URL: http://cat.inist.fr/?aModele=afficheN&cpsidt=7121470

Foster GS, Campbell RK, Adams WT (1984). Heritability, gain, and C effects in rooting western hemlock cuttings. Canadian Journal of Forest Research 14: 628-638. - doi: 10.1139/x84-114

Hashizume H, Taniguchi S (1981). Vegetative propagation of the family of Hinoki (*Chamaecyparis obtusa* Endl.) plus tree using physiologically immature cuttings from the scion stool of the low-cut style and some studies on the relationship between the physiological aging of cuttings and rooting ability. Bulletin of the Tottori University Forests 13: 1-17. [in Japanese]

Henry PH, Blazich FA, Hinesley LE (1992). Vegetative propagation of eastern redcedar by stem cuttings. HortScience 27 (12): 1272-1274. [online] URL: http://hortsci.ashspublications.org/content/27/12/1272.full.pdf

Kathiravan M, Ponnuswamy AS, Vanitha C (2009). Determination of suitable cutting size for vegetative propagation and comparison of propagules to evaluate the seed quality attributes in *Jatropha curcus* Linn. Natural Product Radiance 8 (2): 162-166. [online] URL: http://nopr.niscair.res.in/handle/123456789/4038

Maeda M, Yoshino Y, Maeda C (1997). Cyclophysis in Hinoki (*Chamaecyparis obtusa*) - Differences of rooting ability and growth of rooted cuttings between cuttings obtained at different height of trees. Applied Forest Science 6: 183-184. [in Japanese]

Miyazaki J (2003). Cutting propagation of Japanese black pine resistant to the pine wood nematode. III. Investigation of cuttings methods for holding down cost of raising seedling. Kyushu Journal of Forest Research 56: 188-189. [in Japanese] [online] URL: http://ffpsc.agr.kyushu-u.ac.jp/jfs-q/kyushu_forest_research/56/56sc017.pdf

Morgenstern EK (1987). Methods for rooting larch cuttings and applications in clonal selection. The Forestry Chronicle 63: 174-178. - doi: 10.5558/tfc63174-3

Mori Y, Miyahara F (2002). Examination of the rooting conditions in cutting of Japanese black pine plantlets. Kyushu Journal of Forest Research 55: 134-135. [in Japanese]

Mori Y, Miyahara F, Goto S (2004). The usefulness of rooted cuttings for producing nematode-resistant Japanese black pine plantlet. Journal of the Japanese Forest Society 86 (2): 98-104. [in Japanese with English abstract] [online] URL: http://www.jstage.jst.go.jp/article/jjfs1953/86/2/86_2_98/_pdf

Mori Y, Miyahara F, Goto S (2006a). Clonal propagation of nematode-resistant Japanese black pine plantlet by cutting. Bulletin of the Fukuoka Prefectural Forest Research Center 7: 1-19. [in Japanese] [online] URL: http://agriknowledge.affrc.go.jp/RN/2010732962.pdf

Mori Y, Miyahara F, Goto S (2006b). Improving the pine wilt disease resistance of cutting-propagated Japanese black pine plantlet populations by inoculating ortets with pinewood nematodes. The Journal of Japanese Forest Society 88 (3): 197-201. [in Japanese with English abstract] - doi: 10.4005/jjfs.88.197

Morishita G, Ooyama N (1972). Practice of cuttings technique. In: "Theory and practice of cuttings". Chikyu Shuppann, Tokyo, Japan, pp. 169-276. [in Japanese]

Ogasawara K, Shidei T (1966). Studies on the cuttings of forest trees. Special relationship between the rooting responses of cuttings and the balance of phytohormones in cuttings. Bulletin of the Kyoto University Forests 35: 19-38. [in Japanese with English abstract]

Ohira M, Kuramoto N, Hiraoka Y, Okamura M, Taniguchi T, Fujisawa Y (2006). Effects of level of resistance to pine wilt disease, adjustment of cuttings and rooting environment on the rooting ability of *Pinus thunbergii* stem cuttings. Bulletin of the Forest Tree Breeding Center 22: 25-34. [in Japanese with English summary]

Ohira M, Mazaki S, Miyahara F, Mori Y, Yamada Y, Miyazaki J, Shiraish S (2007). Technological development of resistant seedlings to pine wilt disease by cuttings. Forest Tree Breeding (Special Issue): 29-32. [in Japanese]

Ohira M, Kuramoto N, Fujisawa Y, Shiraishi S (2009). Usefulness of the closed cutting system for the vegetative propagation of *Pinus thunbergii* resistant to pine wilt disease. Journal of the Japanese Forest Society 91 (4): 266-276. [in Japanese with English abstract] - doi: 10.4005/jjfs.91.266

Peer KR, Greenwood MS (2001). Maturation, topophysis and factors in relation to rooting in *Larix*. Tree Physiology 21: 267-272. - doi: 10.1093/treephys/21.4.267

Rosier CL, Frampton J, Goldfarb B, Blazich FA, Wise FC (2006). Improving the rooting capacity of stem cuttings of Virginia pine by severe stumping of parent trees. Southern Journal of Applied Forestry 30 (4): 172-181. [online] URL: http://www.ingentaconnect.com/content/saf/sjaf/2006/00000030/00000004/art00002

Sasaki M, Kuramoto N, Hiraoka Y, Okamura M, Fujisawa Y (2004). Effects of defoliation and disbudding on rooting ability of Japanese black pine cuttings. Journal of the Japanese Forest Society 86 (1): 37-40. [in Japanese with English abstract] [online] URL: http://ci.nii.ac.jp/naid/110006279023

Struve DK, Talbert JT, McKeand SE (1984). Growth of rooted cuttings and seedlings in a 40-year-old plantation of eastern white pine. Canadian Journal of Forest Research 14 (3): 462-464. - doi: 10.1139/x84-082

Toda T (2004). Studies on the breeding for resistance to the pine wilt disease in *Pinus densiflora* and *P. thunbergii*. Bulletin of the Forest Tree Breeding Center 20: 83-217. [in Japanese with English summary]

Tousignant D, Villeneuve M, Rioux M, Mercier S (1995). Effect of tree flowering and crown position on rooting success of cuttings from 9-year-old black spruce of seedling origin. Canadian Journal of Forest Research 25: 1058-1063. - doi: 10.1139/x95-116

Watanabe K (2006). The treatment of rooting promotion of Japanese black pine cuttings using young seedling. Bulletin of the Yamagata prefectural Forest Resesearch and Instruction Center 30: 29-33. [in Japanese]

Yates D, Earp B, Levy F (2006). Propagation of *Sciadopitys verticillata* (Thunb.) Sieb. & Zucc. by stem cuttings and properties of its latex-like sap. HortScience 41 (7): 1662-1666. [online] URL: http://hortsci.ashspublications.org/content/41/7/1662.full.pdf