Survival rate and shoot growth of grafted Dahurian larch (*Larix gmelinii* var. *japonica*): a comparison between Japanese larch (*L. kaempferi*) and F_1 hybrid larch (*L. gmelinii* var. *japonica* × *L. kaempferi*) rootstocks

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Abstract

We grafted scions of Dahurian larch (*Larix gmelinii* var. *japonica*) onto Japanese larch (*L. kaempferi*) and F_1 hybrid larch (*L. gmelinii* var. *japonica* × *L. kaempferi*) rootstocks and examined rootstock–scion compatibility by assessing the survival rate (SR) in two independent experiments. Scion overgrowth on the rootstock was not observed. SR was not significantly different among rootstocks due to large interquartile ranges (IQR) among clones within a rootstock type. Results suggested that the SR was more dependent on the clonal characteristics of the scion than on the growth vigor of the rootstock. Shoot elongation of grafts on F_1 hybrid rootstock was superior to that of grafts on Japanese larch rootstock. Selection of an appropriate combination of scion and rootstock may improve the SR of grafted Dahurian larch and shorten the cultivation period.

Key words: *Grafting, scion, rootstock, compatibility, terminal leader, Dahurian larch, hybrid larch, Japanese larch*

Introduction

Cuttings and grafting are two common asexual propagation methods in trees (Copes, 1999; Goldschmidt, 2014; van Nocker and Gardiner, 2014). However, some tree species cannot be rooted from cuttings, and for those that can, rooting capacity gradually decreases with aging of the mother tree (e.g. Pallardy, 2008). Grafting is often more successful and, with an appropriate rootstock, can produce a tree that exhibits the best qualities of both the rootstock and the scion trees (Copes, 1980; 1999; Kita et al., 2013). Therefore, grafting is used for propagating difficult-to-root species (Fujisawa et al., 2012; Pallardy, 2008), and grafting methods are useful for preserving clonal elite trees and establishing clone banks (Goldschmidt, 2014).

In northern Japan, Japanese larch (*Larix kaempferi*) has been widely planted for use as pit props in coal mines since the early decades of the 20th century (Kita, 2013; Kita et al., 2009). It is not a native species in northern Japan and suffers from several types of abiotic and biotic stress (Ryu et al., 2009; Koike, 2009). Moreover, its timber is of low quality due to strong twisting in the trunk and high concentration of resin (Kita et al., 2009). Through the efforts of the forest tree breeding and timber engineering programs at the Forest Products Research Institute, Hokkaido Research Organization (HRO), new timber technologies and processes have been successfully developed, and a new F_1 hybrid larch (*L. gmelinii* var. *japonica* × *L. kaempferi*) with high bulk density in the trunk has been produced (Kita et al., 2009; Kita et al., 2018). After public feedback, Kita et al. (2009) named this new F_1 hybrid larch as the "Clean larch" because it absorbs CO_2 quickly and stores it in larger amounts (approximately 120%) per unit volume than other larches. The hybrid was produced by crossing Dahurian larch (*L. gmelinii* var. *japonica*) with Japanese larch. This hybrid is highly resistant to biological stresses, such as grazing by red-back vole and

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shoot blight disease (Ryu et al., 2009; Kita et al., 2018), and displays heterosis, making it promising under abiotic stress, such as air pollution (Agathokleous et al., 2017; Wang et al., 2018).

Forestry practices require large amounts of planting stocks of F_1 hybrid larch for reforestation, and the demand for planting stocks is rapidly increasing (Kita et al., 2013; Hokkaido Statistics, 2018); however, demand is currently exceeding supply due to the limited availability of scions. To improve the production of planting stock of the hybrid larch, new seed orchards and scion gardens should be maintained (Moriguchi et al., 2008). However, grafting success strongly depends on rootstock–scion interaction and compatibility (Goldschmidt, 2014).

Good compatibility may be achieved using the same or a different species of rootstock and scion within same genus (Copes, 1980; Kita et al., 2014a). However, we often fail in keeping good grafting materials in scion gardens and seed orchards of tree species. The site where scion and rootstock meet, the graft union, is usually fragile and susceptible to mechanical damage caused by strong wind and/or heavy snowfall (Kita et al., 2013). Failure is mainly due to mismatch of the grafting materials, and graft imbalances are often observed in seed orchards, for example, the scion overgrowing the rootstock (Fig. 1) or vice versa (Webber, 1948; Goldschmidt, 2014).

Fig. 1
An example showing overgrowth of scion against rootstock. The rootstock species is *Abies sachalinensis*. The scion is a variety of *Abies sachalinensis* called "Oni hada" ("rugged bark" in English) distributed in a small area of Central Hokkaido, Japan. The graft is approximately 50 years old.

In the case of the hybrid larch, elite trees of Dahurian larch are used as mother trees for propagation by grafting (Kita et al., 2013; Ishizuka, 2017). Japanese larch is used as the rootstock to avoid overgrowth of the scion as Dahurian larch has a slower growth rate than Japanese larch (Koike, 2009; Ryu et al., 2009). However, some scions of Dahurian larch clones produce an imbalance when grafted to rootstocks of Japanese larch, which may be attributed to scion–rootstock incompatibility (Kita et al., 2014a). To overcome this problem and to increase the production rate of planting stock, efforts have been made to employ F_1 hybrid larch (*L. gmelinii* var. *japonica* × *L. kaempferi*) as the rootstock for scions from elite Dahurian larch trees. This is because the growth rate of the F_1 hybrid is similar to that of Japanese larch (and higher than that of Dahurian larch) and high compatibility could be expected when the mother tree of rootstock and the scion are of the same species.

It could be expected that F_1 hybrid larch would provide suitable rootstock for receiving scions from Dahurian larch, and in this study, we examined that hypothesis. Our criteria for grafting compatibility were survival rate (SR) and elongation length of the scion, as both serve as indices for the agglutination of the scion and rootstock, and water and nutrition conductivity between the scion and rootstock. They are also the primary factors considered when transplanting from nursery to seed orchard or, in particular, after transplantation due to snow damage.

Material and Methods

Plant Materials

Scions were obtained from the progeny test site for Dahurian larch in Mikasa City, Hokkaido (43.3°N, 141.9°E; 100 m a.s.l.). This progeny test site was established in 1993, and tree density was 1600/ha.

The progeny test site comprised two different breeding populations. The first population was derived from the F_1 progeny of intraspecies crosses of Dahurian larch trees selected in Hokkaido between 1966 and 1970. The artificial crossing was performed in 1985 at the clonal bank of the Forestry Research Institute, HRO in Bibai, Hokkaido. Seeds produced by the artificial crossing were sown in the nursery beside the clonal bank in 1990, and 3-year-old seedlings were planted at the progeny test site. The second population was obtained from Dahurian larch seeds collected in southern Sakhalin, Russia, in August 1988. Seeds collected in southern Sakhalin were sown in 1991 in the same nursery as the seeds arising from artificial crossing, and 2-year-old seedlings were planted at the progeny test site.

Grafting was performed twice (once in 2013 and 2014) with scions sampled from the progeny from each population to propagate and preserve the genetic resources of superior individuals selected from each population. The individuals selected for grafting were chosen based on having high growth and/or good wood properties (Kita, 2013; Ishizuka, 2017). Scions (from 17 trees; Table 1) for the first grafting experiment in 2013 were selected in the first population and harvested in February, 2013 (23 years old). Genetic gains based on breeding value of individuals selected for grafting in 2013 were modulus of elasticity of green logs (5.5 %), trunk straightness (17.2 %), tree height (0.1 %), and diameter at breast height at 18 years old (0 %) (Kita, 2013). Scions (from 19 trees; Table 1) for the second grafting experiment in 2014 were selected in the second population and harvested in February 2014 (23 years old). Because Japanese larch was planted in the area from where the seed was collected and open pollination would have occurred, chloroplast-DNA analyses were performed to ensure that only pure Dahurian larch individuals were selected as the source for the scions (Kita et al., 2014b). Genetic gains from individuals selected for grafting in 2014 were tree height (20.4

%), diameter at breast height (38.9 %), trunk straightness (5.0 %), and modulus of elasticity of green logs at 20 years old (−4.50 %) (Tamura et al., 2015).

Table 1
Clones used for scions and number of grafts per clone in the experiments of 2013 and 2014

2013				2014			
A/A [1]	Scions of Dahurian larch clone[2]	Number of grafts: Japanese larch rootstock	Number of grafts: F_1 hybrid larch rootstock	A/A [1]	Scions of Dahurian larch clone[2]	Number of grafts: Japanese larch rootstock	Number of grafts: F_1 hybrid larch rootstock
1	10A-1-4	4	4	21	So9-1-8	8	6
2	10A-15-6	7	4	22	So-1-4	6	5
3	10B-4-4	15	24	23	So3-1-6	5	6
4	10B-6-8	7	4	24	So27-2-7	6	7
5	10D-2-5	6	4	25	So1-2-6	6	6
6	17C-1-6	4	4	26	So14-2-4	6	6
7	18B-9-7	4	4	27	So14-3-9	6	6
8	18C-2-9	7	4	28	So5-4-7	7	6
9	7A-4-8	4	4	29	So17-4-5	10	14
10	7A-6-6	7	4	30	So10-4-6	8	10
11	10E-1-6	15	27	31	So2-2-4	10	9
12	18C-8-4	8	7	32	So5-3-9	6	6
13	18D-2-8	4	7	33	So5-4-9	6	18
14	21A-0-7	4	4	34	So8-2-5	6	6
15	7C-8-5	6	4	35	So14-2-9	7	8
16	7C-8-9	12	28	36	So9-2-8	6	6
17	7E-1-7	6	4	37	So5-4-6	5	9
18				38	So27-2-6	8	8
19				39	So1-2-4	7	9
Total		120	141			129	151

[1]A/A indicates the clone in Fig. 3.
[2]Clone abbreviations: the three parts of the abbreviation from left to right indicate the symbol of progeny, replication number, and number of individuals within a plot.

Scion shoots of approximately 20 cm in length were obtained from the sunny crown of Dahurian larch in February 2013 and 2014. They were immediately stored in a low temperature underground stockroom under natural snow cover at the Forestry Research Institute, HRO, Bibai, Hokkaido.

Three-year-old rootstocks were used for the grafting of Japanese larch and F_1 hybrid larch in the nursery at Bibai, which has brown forest soil (Dystric Cambisol). Rootstock of Japanese larch for 2013 was obtained from bulk seeds collected in Ebeotsu near Bibai and Shibetsu located at northern Hokkaido and at Shibetsu for 2014. Rootstocks of F_1 hybrid larch were offered by Kunnnepu seed orchard located in eastern Hokkaido for both years. The mean (min.–max.) number of grafts per clone in each rootstock was 7.1 (4–15), 8.3 (4–28), 6.8 (5–10) and 7.9 (5–18) for Japanese larch rootstock in 2013, F_1 hybrid larch rootstock in 2013, Japanese larch rootstock in 2014 and F_1 hybrid larch rootstock in 2014, respectively.

Grafting

Grafting was performed within a wooden shading frame in the nursery during the latter part of April to early May in 2013 and 2014. On the upper surface of the shading frame, a double layer of 2-mm shading mesh was attached to achieve suitably shady conditions (relative light intensity: 3 %–5 %).

A scion approximately 4 cm long was cut from a stored shoot and grafted to the rootstock using the cleft method, with the lower half of the scion inserted into the rootstock at the grafting point. The grafting point was located at a height of 25 cm measured from the base of 1- or 2-year-old rootstock stems (26.1 ± 9.7 cm for Japanese larch and 27.6 ± 7.3 cm for hybrid larch), a position chosen to allow the retention of some lower branches to maintain rootstock activity. Grafted materials were sealed using white vegetable wax mixed with resin and lard, fixed with rubber band, and wrapped in a plastic bag to prevent excess transpiration (Sunagawa, 1994; Kita et al., 2014a).

Cultivation

The top part of the plastic bag was cut to allow aeration once the dormant buds of the scion started unfolding. Once the new shoot started elongating, the plastic bag was removed for acclimation to the environment. After new leaves started appearing on the graft, the shading material was gradually removed over a 3-month period (Sunagawa, 1994; Kita, 2013). All grafted materials were kept in the nursery under snow shelters during periods of snow. All branches emerging from the rootstocks, except the scion, were removed over 2 years.

Evaluation

The percentage SR in each autumn in 2013 and 2014 was evaluated as follows:

$$SR = (\text{grafts with surviving scions}/\text{total number of grafts}) \times 100.$$

The length of the new terminal leader emerging from the grafted scion was also measured at the end of growing season in 2013 and 2014.

Data handling

The cut-off for statistical significance was set at an alpha level of 0.05 for all tests. To eliminate the effects of genetic variability and allow the drawing of robust conclusions regarding the difference between the two rootstock types, the influence of the rootstock genetic base was standardized by averaging the determinations per clone. This resulted in one robust value per clone per rootstock type and hence a stabilized sample size (n = 17 in 2013 and n = 19 in 2014) for statistical comparisons between the rootstock types.

SR data did not conform to a Gaussian distribution even after a Box–Cox power transformation (high skewness). Therefore, SR data were subjected to a Kruskal–Wallis test by rank to test whether samples originated from the same or different distributions and to a Spearman rank correlation to test clone agreement between F_1 hybrid and Japanese larch rootstocks. Elongation data were transformed by a Box–Cox power transformation to approach a normal distribution, as described previously (Agathokleous et al., 2016a). Given the abovementioned factors, shoot elongation was tested using simple contrast analysis between Japanese larch and hybrid larch rootstocks upon which Dahurian larch scion was grafted.

When the simple contrast returned a statistically significant difference, the magnitude of the difference was calculated using Cohen's δ corrected for bias (Hedges and Olkin, 1985; Cohen, 1988), as described previously (Agathokleous et al., 2016a). To translate the differences into percentile gain, which appeared in the experimental condition, the Cohen's U_3 index was calculated (Cohen, 1977); δ was also converted to overlapping coefficient (OVL) (Reiser and Faraggi, 1999). The magnitude of difference was arbitrarily defined as neutral (*δ* = 0.00–0.50), small (*δ* = 0.50–1.50)], moderate (*δ* = 1.50–3.00) or large (*δ* = >3.00) (Cohen, 1988; Agathokleous et al., 2016b). Absolute *δ* values in the interval 0.50–1.50 suggest educational significance, and *δ* values >1.50 suggest practical significance (Wolf, 1986; Agathokleous et al., 2016b).

Data processing and statistical analyses were performed in MS EXCEL 2010 (Microsoft ©) and STATISTICA v.10 (StatSoft Inc. ©) software, except for the Spearman rank correlation for which we used R v.3.12 (https://www.R-project.org).

Results and Discussion

Graft SR

In the experiments started in 2013, in the first year, the median SR for Dahurian larch scion (Fig. 2) was 93.3 % when grafted on Japanese larch rootstock (E13Y1JL) and 92.6 % when grafted on hybrid larch rootstock (E13Y1HL), a difference that was not statistically significant. Results in the second year were similar; the median SR of Dahurian larch scion was 83.3 % when grafted on Japanese larch rootstock (E13Y2JL) and 75.0 % when grafted on hybrid larch rootstock (E13Y2HL), with the difference not being statistically significant.

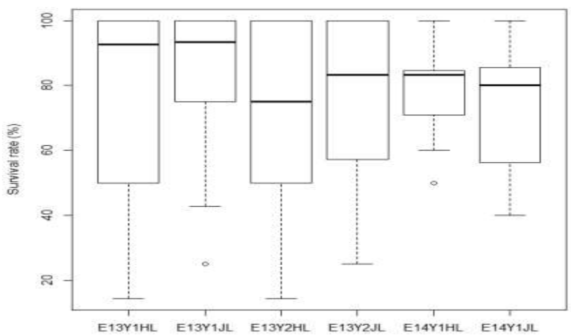

Fig. 2
Boxplot diagram of the survival rate of Dahurian larch scions grafted onto either Japanese larch or F_1 hybrid larch rootstocks in the first and second year of the experiment started in 2013 and 2014. Median values are indicated by the bold line in the boxes; interquartile range (IQR) is represented by the box; extreme values (within 1.5 times the IQR) are at the end of the vertical lines extending from the IQR. White circles indicate outliers. Abbreviations on the horizontal axis: E13, experiment in 2013; E14, experiment in 2014; Y1, 1st year; Y2, 2nd year; JL, Japanese larch rootstock; HL, F_1 hybrid larch rootstock.

In the experiments started in 2014, the median SR of Dahurian larch scion (Fig. 2) was 80.0 % when grafted on Japanese larch rootstock (E14Y1JL) and 83.3 % when grafted on hybrid larch rootstock (E14Y1HL), with the difference not being statistically significant (U = 151.5, P = 0.405).

No significant difference between the two rootstock types can be attributed to the high variance among clones. The interquartile range (IQR) of the SR was 25 % and 43 % for Japanese larch and 50 % and 50 % for hybrid larch in the first and second years, respectively, in the 2013 experiment. In 2014 experiment, IQR of SR was 29 % for Japanese larch and 14 % for hybrid larch. To evaluate the agreement in the SR between Japanese larch and hybrid larch, correlation analysis was performed. The agreement was significant only in the first year of the 2013 experiments (Fig. 3, ρ = 0.603, p = 0.010) but not significant in either the second year of the 2013 experiments or in the 2014 experiments. These results suggest that the SR varied among clones, depending on the genetics of the rootstock species. From these results, clones which can offer high SRs can be selected based on the rootstock type (Japanese or hybrid larch) for practical use.

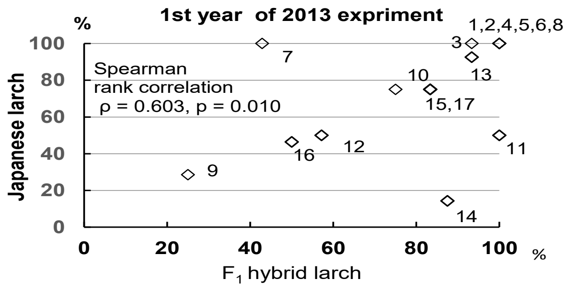

Fig. 3
Relationship between the survival rate of Dahurian larch (*Larix gmelinii* var. *japonica*) scions grafted onto Japanese larch (*L. kaempferi*) and F_1 hybrid larch rootstocks in the experiment started in 2013. The numbers 1–17 beside the diamond symbols indicate different clones of Dahurian larch scions (column "A/A" in Table 1). Correlation analysis was performed at *α* = 0.05.

Scion shoot elongation

In the experiments started in 2013, the terminal leader emerging from Dahurian larch scion was significantly longer when grafted on hybrid larch rootstock than that when grafted on Japanese larch rootstock in both the first (129 %, F = 6.3, P = 0.017) and second (120 %, F7, 4, P = 0.010) years (Fig. 4). The difference was small and of educational significance in both the first (*δ* = 0.79, U_3= 0.79, OVL = 0.69) and second (*δ* = 0.91, U_3= 0.82, OVL = 0.65) years. The educational significance is reflected by an approximately 80 % percentile gain of hybrid larch over Japanese larch, with the two group pairs overlapping by 65-69 %, in both years.

In the experiments started in 2014, the length of the terminal leader emerging from Dahurian larch scion was 115 % longer when grafted on hybrid larch rootstock than that when grafted on Japanese larch root stock ($F = 8.8$, $P < 0.010$) (Fig. 4). Similar to that in the experiment started in 2013, the difference was small and of educational significance, with an 83 % percentile gain of hybrid larch over Japanese larch and a 63 % overlap of the group pair ($\delta = 0.95$, $U_3 = 0.83$, OVL = 0.64).

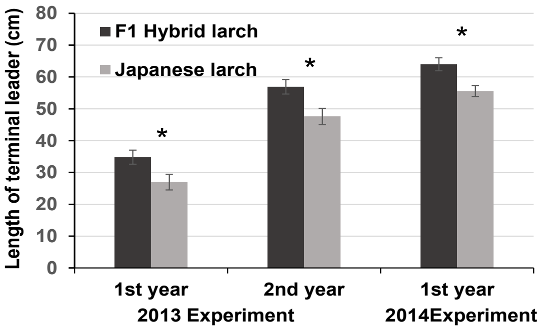

Fig. 4
Means ± SE of the terminal leader length of Dahurian larch (*Larix gmelinii* var. *japonica*) scions grafted onto either Japanese larch (*L. kaempferi*) or F_1 hybrid larch rootstocks in the first and second year in the experiments started in 2013 and 2014. Asterisk above SE bar indicates statistically significant difference between Japanese larch and hybrid larch rootstocks in a particular year according to simple contrast analysis at $\alpha = 0.05$. Each mean is the average of 17 and 19 values for 2013 and 2014 experiments, respectively, with each value representing the average performance of a unique clone.

The fact that SR did not differ significantly on the different rootstock types but elongation did suggests that elongation is not related to SR. The results of the 2013 and 2014 experiments also suggested that differences in SR among clones within a population were greater than SR among populations. Hence, if clones are utilized among those displaying high SR when grafted with hybrid larch rootstock (Fig. 3), grafting with hybrid larch would provide an opportunity for higher production. It should be mentioned that overgrowth of the scion on the rootstock (Webber, 1948) was not observed in this study, although overgrowth has been reported in *Pinus radiata* (Copes, 1980) and *Pseudotsuga menziesii* (Copes, 1999).

Intraspecific grafts (where rootstock and scion belong to the same botanical species) are nearly always compatible, whereas interspecific grafts (where rootstock and scion belong to different species in the same genus) are somewhat less compatible (Goldschmidt 2014). *L. gmelinii* var. *japonica* is a slow growing species. Therefore, we used *L. kaempferi* as rootstock for selected superior-growing clones in *L. gmelinii* var. *japonica* to avoid scion overgrowth. However, some of the selected clones showed incompatibility with *L. kaempferi* rootstock. The F_1 hybrid is expected to show higher compatibility with *L. gmelinii* var. *japonica* than with *L. kaempferi* because half of the F_1 hybrid genes are derived from *L. gmelinii* var. *japonica*. Moreover, the growth rate of the F_1 hybrid is high and similar to that of *L. kaempferi* (Kita et al. 2009). These characteristics suggest advantages in the use of F_1 hybrid larch as rootstock for the grafting of *L. gmelinii* var. *japonica*. In our study, this advantage was observed in shoot elongation. Although it would also be expected that future overgrowth of the scion on this rootstock would be avoided, were unable to confirm this beyond the two-year extent of this investigation into SR and shoot elongation.

In general, the cultivation period for grafting of Japanese larch has been proposed as >2 years (Kita, 2013). However, that for Dahurian larch is >3 years because the growth of Dahurian larch is slower than that of Japanese larch and hybrid larch (Ryu et al., 2009). In our experiments, new scion shoots were longer for Dahurian larch grafted on hybrid larch rootstock, implying that the cultivation period could be shortened to <3 years if the appropriate rootstock is used (Kita et al., 2014a). This would promote early seed production for progeny tests of trees in the second generation and beyond.

From the practical viewpoint, a variety of Dahurian larch ("Naka-shibetsu No 5") has been legally registered by the Japanese government as "super elite" mother trees for forestry (Kita, 2013; Ishizuka, 2017). We suggest that production of hybrid offspring of "Naka-shibetsu No. 5" × Japanese larch should be increased for silviculture. Results from this study showed that a shortened cultivation period for Dahurian larch is possible and that it has potential for the early establishment of hybrid seed orchards.

Conclusion

The SR of grafted Dahurian larch scions was related more to their clonal characteristics than to whether the grafting rootstock was from Japanese larch or hybrid larch. This outcome was somewhat unexpected, given that the Dahurian larch scion is more closely related to the hybrid larch rootstock than the Japanese larch rootstock. However, it is noteworthy that scions of some Dahurian larch clones grafted onto hybrid larch rootstock showed rapid growth of the new shoot after the graft union was established. If the most appropriate rootstock is chosen for each scion clone this would maximize graft SR for planting in a seed orchard, and shorten the cultivation time for establishing seed orchards.

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