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Site characteristics of *Betula ermanii* range-wide provenance trials [revised version]

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ダケカンバ広域産地試験地のサイト特性［修正版］

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Introduction

Provenance trials have been established with the aim of identifying the most suitable source populations of seeds for the targeted planting of forestry tree species (Morgenstern, 1996). Recently, attempts have been made to predict the response of trees to global warming by analyzing data from range-wide provenance trials (Refeldt *et al*., 2002). The climate of Japan differs markedly between the Sea of Japan and Pacific Ocean regions of the country, which are characterized by large differences in meteorological

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conditions such as temperature and precipitation. Consequently, it might be anticipated that plant genotypes distributed in these different regions are genetically adapted to the local climates. Range-wide provenance trials enable us to evaluate the effects of differing climates on the growth and survival of tree species, and in Japan, such trials have been conducted for major forestry coniferous species such as *Pinus densiflora* (Nagamitsu *et al*., 2015), *Larix kaempferi* (Nagamitsu *et al*., 2018), and *Abies sachalinensis* (Tsuyama *et al*., 2020). In contrast, with the exception of *Fagus crenata* (Hiura, 1998; Osada *et al*., 2018), very few provenance trials have been conducted for broadleaved tree species in Japan. As a consequence, it is currently difficult to predict the potential effects of global warming on the status of cool–temperate and/or alpine forest ecosystems in Japan.

In 2012, during the Japanese Association of University Forests conference held at Niigata University, an agreement was reached regarding the establishment of range-wide provenance trials at the national scale for evaluating the impact of global warming on *Betula ermanii*. According to this agreement, 11 provenance test sites for *B. ermanii* were to be established. The selection of these provenance test sites, selection of seeds, and cultivation of seedlings have recently been described by Paing *et al*. (2021). In the present paper, we describe the characteristics of the 11 sites used for these range-wide *B. ermanii* provenance trials.

Materials and Methods

Cultivation and labeling of containerized saplings

In 2016 and 2017, seeds of *B. ermanii* were collected from 11 natural populations growing throughout Japan (Fig.1), which were subsequently sown in a nursery in April 2018. In June of the same year, the freshly germinated seedlings were transplanted to 150 cm^3 JFA containers (Paing *et al*., 2021). The containerized saplings were thereafter cultivated in a greenhouse through two successive growing seasons in 2018 and 2019. The saplings derived from seeds collected from the different populations were labeled with different colored vinyl tapes and assigned individual IDs (Table S1). With the exception of the populations GYS, AKS, and CKS, we selected 20 saplings per population from the remaining eight populations (Table 1). The total number of saplings per site was 183 (10 saplings from GYS+nine from AKS+four saplings from CKS+20 saplings per populations from eight populations), and overall, we planted 2,013 saplings for this study (11 sites × 183 saplings).

Delivery of containerized seedlings

In August 2019, the height of saplings was measured and in October of the same year, saplings from each population were partially packed using CTM cardboard, such that the IDs tags would not become detached, and were subsequently transported by refrigerated shipping for autumn planting. For the spring planting sites, the containers were placed directly on the floor of the greenhouse until thawed, and then

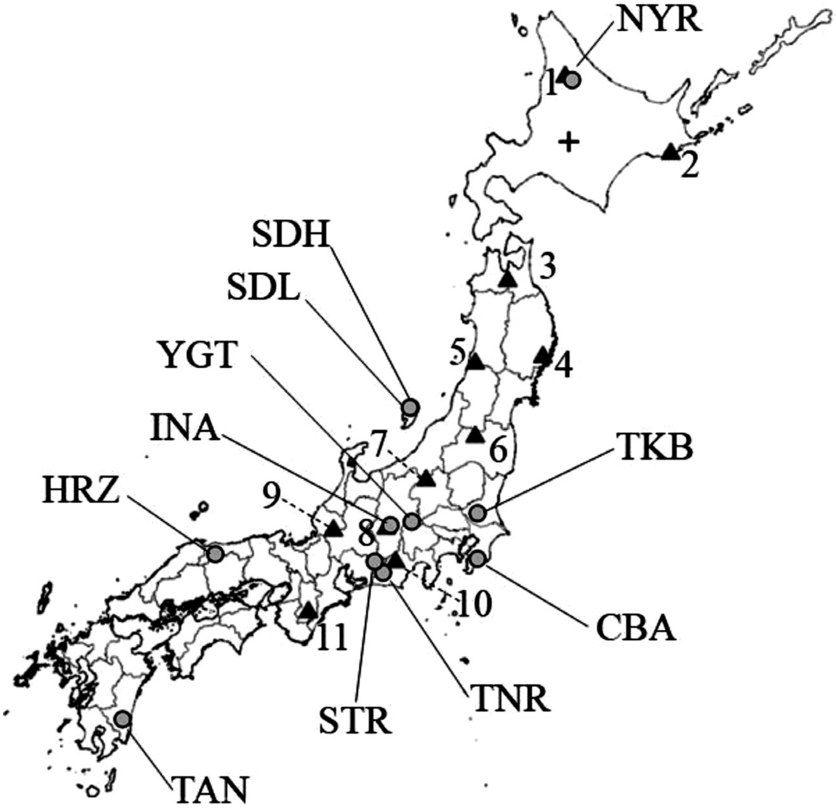

Fig. 1. Location of the sites used for range-wide provenance trials (gray circle). Black triangles represent seed source population. Population number is consistent with Table 1. Cross represents the place that grew containerized saplings.

Table 1. Number of samplings per population.

Pop. No	Pop. Name	Pop Abb.*	Lat (°)	Long (°)	Alt (m)	Tree line§	Num. saplings	Color of tape†
1	Uryu	URU	44.386	142.280	487		20	white
2	Akkeshi	AKS	42.990	144.923	105		9	black
3	Hakkoda	HKD	40.648	140.852	898		20	red
4	Goyo-San	GYS	39.182	141.738	842		20	light blue
5	Cyokai-san	CKS	39.070	140.044	1256	x	4	lime
6	Bandai-san	BDS	37.629	140.051	1076		20	gray
7	Mikuni-Touge	MKT	36.765	138.823	1293		20	orange
8	Alps-West	APW	35.811	137.837	2457	x	20	blue
9	Nougouhaku-san	NGH	35.771	136.513	1495	x	20	yellow
10	Alps-South	APS	35.137	138.053	1523		20	pink
11	Shakaga-Take	SHK	34.114	135.903	1780	x	20	green

Pop Abb.*: Population abbreviations follow those used by Paing *et al.* (2021). Tree line§: tree-line populations are represented by x. Color of tape†: the color of vinyl tape used to distinguish populations.

packed and transported using same procedure in 2020. Unfortunately, prior to packing and after thawing, approximately half of saplings were damaged by rabbits that had invaded the greenhouse. However, the height was recorded at the time of feeding damage and all seedlings were thereafter delivered for planting

(Table S1).

Establishment of range-wide provenance trials

The following 11 planting sites were established at locations distributed from the north to south of Japan (Fig. 1): Nayoro (NYR), Sado low altitude (SDL), Sado high altitude (SDH), Tsukuba (TKB), Yatsugatake (YGT), Ina (INA), Hiruzen (HRZ), Chiba (CBA), Shitara (STR), Tenryu (TNR), and Tano (TAN). The universities responsible for these sites are indicated in Table 2. With the exception of the TNR site, for both autumn 2019 and spring 2020 plantings, the containerized saplings were planted immediately after delivery to their respective destinations. In the case of TNR, saplings were delivered in the autumn of 2019, maintained in containers at the planting site during the winter, and then planted out in the spring of 2020.

Saplings were planted at 1.6-m intervals based on a random planting design to take into account within-site microtopographical and environmental differences (Fig. S1). Following planting, the height and diameter at 10 cm above ground level (two directions) were measured (Table S1). At sites in which damage caused by deer and rabbit was anticipated, deer or guard fences were installed to prevent feeding damage. Weeding and prevention of damage to other animals in addition to deer and rabbit were carried out depending on the planting sites.

Climate variables

To evaluate the effects of climate on *B. ermanii* growth and survival, for each planting site, we obtained meteorological data for the period 2011 to 2022 from those recorded by the Agricultural Research Organization (Japan Meteorological Agency 2002; https://amu.rd.naro.go.jp) with a spatial resolution of 1 × 1 km. Specifically, we obtained the following data: temperature variables: mean annual temperature (MAT), mean temperature of the coldest month (TMC), and Warmth Index (℃ · month: WI); precipitation variables: summer precipitation (May–Sept: PRS), winter precipitation (Dec–March: PRW), and total precipitation (PRT); solar radiation variables: SRS: solar radiation in summer (May–Sept: SRS), solar radiation in winter (Dec–March: SRW), and total solar radiation (SRT). The altitude of NARO data base was used the average altitude of 1km x 1km mesh. Therefore, temperature was adjusted for a reduction rate of 0.55℃ per 100 m, comparing altitude from NARO data base with actual altitude.

Soil collection and analysis

To obtain basic information on surface soils, three samples of soil were collected from each planting site from July to October 2020, avoiding periods immediately after rainfall, using a 100 mL stainless steel sample cylinder (DIK-1801; Daiki Rika Kogyo). The cylinders containing the samples were sealed and taken to the University of Tsukuba, at which the following parameters were promptly analyzed.

Moisture content: The wet weight of a sample of the collected soil was initially measured, after which the sample was dried at 70℃ for 72 h for dry weight determinations. Soil moisture content was calculated from the difference between the wet and dry soil weights.

pH (H_2O): A 10 g of sample collected was suspended in 25 mL of distilled water, and after thorough mixing on a shaker for 30 min, the pH of the resulting turbid suspension was measured using an MM41-DP pH meter (TOA-DKK).

Total carbon and nitrogen contents: A sample of the collected soil was initially passed through a 2 mm mesh sieve, and thereafter dried at 70℃ for at least 48 h. The nitrogen and organic carbon contents were then subject to dry combustion using a SUMIGRAPH NC-22 nitrogen/carbon analyzer (Sumika Analysis Service), and thereafter analyzed by gas chromatography in conjunction with heat conductive detection (GC-8A, SHIMADZU) to determine the total carbon and nitrogen contents of each sample.

Characterization of site conditions

Using the data obtained for climate variables and soil conditions, we performed principal component analysis using the prcomp function of R software (R Core Team, 2020), and then characterized each site based on environmental conditions.

Results

Range-wide provenance trials

Details of the location (latitude, longitude, and altitude), slope, treatment (mulching, fencing, chemical, and weeding), and description of each site are summarized in Table 2. The 11 selected planting sites were distributed throughout the length of Japan from Hokkaido to Miyazaki prefectures (Fig. 1), 10 of which were characterized by a flat topography, and four and five of the sites were established in nurseries and cut-over forests, respectively (Table 2).

Climate variables

MAT ranged from 5.5℃ at NYR to 15.8℃ at TAN, whereas TMC ranged from -15.8℃ at the coldest site to 1.9℃ at the warmest site. The lowest and highest PRS values were 487.0 mm at NYR and 1728.7 mm at TAN, respectively, and a similar pattern was found for PRT. These data accordingly indicate that the climatic characteristics of the warmest sites are hot and humid rather than hot and dry. SRS ranged from 15.9 to 18.3 MJ/m^2, SRW ranged from 9.1 to 12.8 MJ/m^2, and SRT ranged from 11.6 to 14.4 MJ/m^2 (Table 3).

Soil conditions

Details of the soil conditions at each site are presented in Table 4. The mean gravimetric soil water content was found to range from 45.3% at HRZ to 76.8% at SDL, whereas soil pH ranged from 4.27 to 6.20. Mean soil carbon content ranged from 2.0% at SDL to 27.1% at HRZ, and mean soil nitrogen content ranges from 0.4% at SDL to 2.7% at HRZ. The highest and lowest mean CN ratios were 10.1 at HRZ and 5.07 at SDL, respectively. The YGT, INA, TNR, and STR sites were characterized by intermediate ranges

Table 2. Location, planting season, management, and description of selected trial sites.

Site No.	Site abb.	Address	Latitude	Longitude	Altitude	Slope	Planting	Mulching	Fence†	Chemical§	Weeding	Description	University※
1	NYR	Tokuda, Nayoro, Hokkaido	44.329	142.454	99	Flat	Oct, 2019	Oct, 2019	No	DC (2/yr)	several/yr	Past nursery	Hokkaido
2	SDH	Okura, Sado, Niigata	38.206	138.435	800	Flat	May, 2020	May, 2020	M (Rab)	No treat	several/yr	Cut-over forest	Niigata
3	SDL	Koda, Sado, Niigata	38.207	138.418	350	Flat	Oct, 2019	Oct, 2019	M (Rab)	No treat	several/yr	Cut-over forest	Niigata
4	TKB	Tennodai, Tsukuba, Ibaraki	36.120	140.100	30	Flat	Oct, 2019	Feb, 2022	M (Rab)	DC (1/yr)	2/yr	Nursery	Tsukuba
5	YGT	Nobeyama, Minamisakugun Minamimakimura, Nagano	35.940	138.470	1350	Flat	April, 2020	No	M (Rab)	DC (1/yr)	2/yr	Nursery	Tsukuba
6	INA	Minami-minowa-mura, Kami-ina-gun, Nagano	35.865	137.932	770	Flat	April, 2020	No	No	IC1	several/yr	Cut-over forest	Shinshu
7	TNR	Nishifujidaira, Hamamatsu Tenryu-ku, Shizuoka	34.908	137.746	415	30°N	Feb, 2020	No	M (Deer)	DC (1/yr, 2020)	2/yr	Cut-over forest	Shizuoka
8	STR	Higashinagura, Kitashitaragun Shitara-cho, Aichi	35.138	137.555	680	Flat	Oct, 2019	No	E (Deer)	IC2 (2/yr, 2021)	10/yr	Past pasture	Nagoya
9	CBA	Orikisawa, Kimitsu, Chiba	35.193	140.109	212	Flat	April, 2020	No	E (Deer)	DC (2/yr, 2020)	several/yr	Nursery	Tokyo
10	HRZ	Hiruzenkamitokuyama, Maniwa, Okayama	35.293	133.585	640	Flat	Oct, 2019	No	No	No treat	1/yr	Cut-over forest	Tottori
11	TAN	Otsu,Tano-cho, Miyazaki, Miyazaki	31.866	131.275	180	Flat	April, 2020	No	E (Deer), W (Indiv)	DC (2/yr, 2020)	2/yr	Cut-over forest	Miyazaki

Fence†: M (Rab/Deer): Mesh fence for Rabbit/Deer, E (Deer): Electronic fence to prevent deer damage, W (indiv): Wire mesh fence for individual tree, Chemical§ : DC: Dithiocarbamates (Konifer-suiwa zai) (Daido Shouji Co. Ltd), IC: Insectcide, University ※ : University responsible for the site: Hokkaido: Hokkaido University, Niigata: Niigata University, Tsukuba: University of Tsukuba, Shinshu: Shinshu University, Shizuoka: Shizuoka University, Nagoya: Nagoya University, Tokyo: The University of Tokyo, Tottori: Tottori University, Miyazaki: University of Miyazaki

Table 3. Climate variables determined for the selected trial sites.

Site No	Site Abb.	Temperature* (°C)			Precipitation (mm)			Solar radiation (MJ/m^2)		
		MAT	TMC	WI	PRS	PRW	PRT	SRS	SRW	SRT
1	NYR	5.5	-15.8	58.0	487.0	513.5	1000.5	16.9	9.1	11.6
2	SDH	8.8	-4.9	106.8	946.4	1104.7	2051.1	18.3	9.5	12.7
3	SDL	11.4	-2.0	127.1	1306.2	1028.2	2334.4	17.9	9.3	12.5
4	TKB	14.0	-1.2	110.4	697.4	583.6	1281.0	16.0	11.8	13.0
5	YGT	7.1	-10.9	58.3	868.4	539.6	1408.0	18.1	12.8	14.4
6	INA	10.7	-6.6	86.6	869.0	664.2	1533.1	18.3	12.2	14.2
7	TNR	13.4	-1.3	104.9	1336.5	931.8	2268.3	16.5	12.3	13.6
8	STR	10.8	-4.2	83.4	1509.9	922.3	2432.2	16.5	12.4	13.7
9	CBA	13.6	-1.4	105.8	999.0	1074.7	2073.7	15.9	10.8	12.4
10	HRZ	10.1	-4.8	78.3	1028.2	1028.9	2057.1	16.7	9.5	12.2
11	TAN	15.8	1.9	129.7	1728.7	945.4	2674.2	17.2	12.3	13.9

Temperature*: Temperature was adjusted for a reduction rate of 0.55℃ per 100 m. MAT: mean annual temperature, TMC: mean temperature of the coldest month, WI: warmth index (℃・month). PRS: precipitation in summer (May–Sept), PRW: precipitation in winter (Dec–March), PRT: total precipitation. SRS: solar radiation in summer (May–Sept), SRW: solar radiation in winter (Dec–March), SRT: total solar radiation.

Table 4. Soil conditions at the selected trial sites.

Site No	Site Abb.	Gravimetric Soil Water Content (%)			pH			C content (%)			N content (%)			CN ratio		
		mean	±	std	mean	±	std	mean	±	std	mean	±	std	mean	±	std
1	NYR	65.2	±	0.3	4.27	±	0.15	14.7	±	0.6	1.6	±	0.1	9.26	±	0.17
2	SDH	64.2	±	3.1	5.47	±	0.32	3.4	±	0.1	0.6	±	0.1	5.68	±	0.55
3	SDL	76.8	±	2.6	4.87	±	0.06	2.0	±	0.4	0.4	±	0.1	5.07	±	0.17
4	TKB	64.1	±	1.6	6.20	±	0.36	4.0	±	1.2	0.7	±	0.2	5.98	±	0.24
5	YGT	56.8	±	1.8	5.03	±	0.15	17.5	±	10.5	2.4	±	1.4	7.16	±	0.19
6	INA	52.4	±	3.9	4.43	±	0.51	18.7	±	4.7	2.0	±	0.5	9.28	±	0.12
7	TNR	70.5	±	3.4	6.13	±	0.15	10.1	±	10.5	1.3	±	1.3	7.61	±	0.29
8	STR	58.6	±	4.0	4.70	±	0.20	16.4	±	16.0	2.0	±	1.9	7.88	±	0.51
9	CBA	70.4	±	0.6	6.03	±	0.21	6.0	±	3.9	1.1	±	0.7	5.15	±	0.34
10	HRZ	45.3	±	4.9	4.93	±	0.21	27.1	±	23.1	2.7	±	2.2	10.1	±	0.43
11	TAN	71.1	±	6.8	5.77	±	0.15	8.2	±	1.0	1.0	±	0.2	8.58	±	0.49

pH: pH of soil, C: C content (%), N: N content (%), CN ratio: the ratio of the mass of carbon to the mass of nitrogen in soil.

of soil carbon and nitrogen contents. With the exception of NYR and HRZ which have high mean carbon and nitrogen contents, low mean values of carbon and nitrogen contents were observed at the rests of the northern and southern sites.

Site characteristics

The first (PC1) and second (PC2) axes of a principal component ordination plot obtained from an analysis based on climate variables and soil conditions explained 43.6% and 23.5% of the variances, respectively (data not shown). The plot indicated that YGT, HRZ and INA were clustered in a group in terms of soil nutrient contents (soil nitrogen content, carbon content, and CN ratio), whereas CBA and TKB were grouped in terms of soil water content. With respect to soil conditions and climatic variables, NYR and TAN were found to be distinct from the other sites (Fig. 2).

Conclusion

We successfully conducted range-wide provenance trials for *Betula ermanii* at selected sites distributed

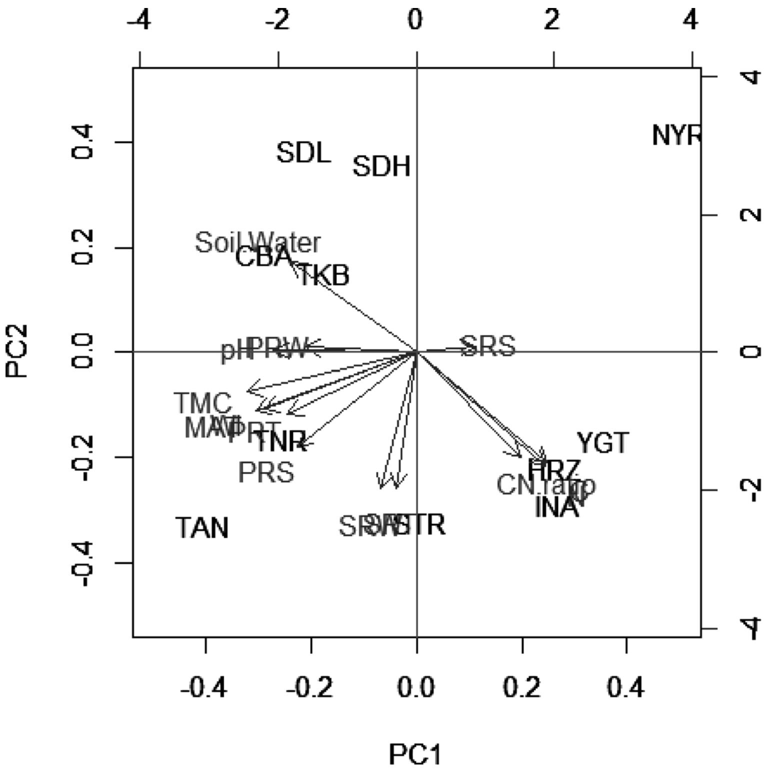

Fig. 2. Principal component analysis of site characteristics. Site abbreviation is consistent with Table 2. Environmental abbreviations are consistent with Table 3 and Table 4.

throughout Japan. The combinations of seed sources and planting sites examined in this study provide a representative assessment of the wide range of elevated temperatures. The data obtained from these range-wide provenance trials will provide a useful basis for evaluating the potential impacts of global warming on cold temperate and/or alpine forest ecosystems.

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Supplementary materials are available in UTokyo Repository.

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