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Growth and Above-ground Biomass Production of a Willow Clone with High Productivity, *Salix pet-susu* clone KKDShigeru SATOH^{*1}, Kimiharu ISHIZAWA^{*2}, Yu MITSUI^{*2*4} and Kazuya MINATO^{*3}

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The above-ground biomass yield of willow (*Salix* spp.) clones selected in the previous study was evaluated after two-growing seasons in Tohoku and Kinki regions, which had annual mean temperatures of 11°C and 15°C, respectively. In both regions, the *S. pet-susu* Kimura clone KKD exhibited more vigorous growth than the other three clones (*S. pet-susu* Kimura clone HB471, *S. sachalinensis* Fr. Schm. clone SEN and *S. pseudolinearis* Nasarov clone FXM), and its above-ground biomass yield per unit area was largest among four clones tested. In the Kinki region, KKD had an above-ground biomass yield of 26.8 tDM ha⁻¹ two years after planting, suggesting that KKD could attain an acceptable yield even in two years. The root distribution pattern of KKD was an inverted-cone shape, whereas that of SEN and FXM a disk-like shape. Moreover, KKD had more sinker roots. We estimate that such a characteristic root morphology of KKD is closely related to its high biomass productivity.

Key Words

Annual mean temperature, Biomass energy crop, *Salix* species, Short-rotation woody crops, Willow coppice

1. Introduction

Woody crop biomass is becoming popular worldwide as an energy resource. It can be used to provide energy by direct combustion for generation of local heat and electricity, and as a feedstock for synthesis of natural gases and biofuels^{1)~3)}. A woody crop plantation can contribute to reduction of CO₂ in the atmosphere and decreasing fossil fuel consumption.

There has been much research and industrial activity on biomass production using fast-growing woody species, such as willow (*Salix* spp.), poplar and eucalyptus³⁾. In Europe and North America, a willow-based short-rotation coppice has been established for biomass production. In Japan, however, there have been few trials on the production of fast growing woody species including poplar, birch and willows^{4)~6)}. The current abundance of abandoned farm-

lands provides great opportunities for the production of woody biomass as an energy resource in Japan.

Recently, we evaluated willow plants collected in various regions of Japan and selected four promising clones with a high potential as a biomass energy crop in the southern Tohoku region⁴⁾. The above-ground biomass yield was significantly higher in *S. pet-susu* Kimura clone KKD than in the other three clones, *S. pet-susu* Kimura clone HB471, *S. pseudolinearis* Nasarov clone FXM, and *S. sachalinensis* Fr. Schm. clone SEN, which had no significant difference between each other.

The above-ground biomass yield of willow plants should vary depending on environmental conditions, such as air temperature, precipitation, water availability, soil texture, fertility and light conditions, in addition to differences in clones (genotypes). Therefore, it is unclear whether KKD grows more vigorously with higher biomass production than the other three clones in the regions with climate conditions different from those of the southern Tohoku region.

In our previous study⁶⁾, biomass production was determined only on a per-plant basis since the number of cultivated willow plants per clone was small. When a small number of willow plants were cultivated in a small plot, it is sometimes inevitable to harvest plants grown at the edge

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of plots, resulting in an over or under estimation of biomass yield on a per-area basis (edge effect or edge-of-plot effects). It is desirable to cultivate a number of willow plants per clone enough to measure above-ground biomass yield by eliminating the possible edge effects.

In the present study, we focused on the *S. pet-susu* clone KKD and compared its above-ground biomass yield and growth performance with those of the other three selected clones. We examined (1) the above-ground biomass yield of willow clones by eliminating edge effects and taking into consideration the survival rate of the clones at the time of harvest, and (2) the growth performance of the clones under different climate conditions. Furthermore, we examined the root system of each clone to find the cause of the vigorous growth of KKD.

2. Materials and Methods

2.1 Site characteristics

We cultivated willow plants in two regions in Japan; Maizuru city (35°31.3'N, 135°24.5'E; annual mean temperature, 14.9°C; annual precipitation, 1865 mm) in Kyoto Prefecture in the Kinki region and Ohira village (38°28.3'N, 140°52.4'E; annual mean temperature, 11.4°C; annual precipitation, 1305 mm), Miyagi Prefecture in the Tohoku region. The annual mean temperature and the annual precipitation were calculated from the regional data⁷⁾ over a period of 10 years from 2000 to 2009.

The plot at Maizuru was 40 × 70 m² (0.28 ha), flat and open to the northwest. The plot was originally a paddy field, and had been used for cultivation of leafy vegetables and growing pasture grasses. Therefore, its soil fertility was high. The plot at Ohira was located in Miyagi Prefectural Forestry Technology Institute and 25 × 40 m² (0.10 ha) in area, gently sloping to the west and facing a clump of trees of 15-m high. The plot was left as a wasteland with grasses and shrubs. Its soil was Andosol (fine volcanic ash soil), a predominant soil type in Japanese upland fields, which is low in available P but high in K and Ca. The soil fertility was expected to be lower in the plot at Ohira than at Maizuru.

2.2 Willow clones

The willow clones used in the present study were *Salix pet-susu* Kimura clone KKD, *S. pet-susu* clone HB471, *S. pseudolinearis* Nasarov clone FXM, and *S. sachalinensis* Fr. Schm. clone SEN. They all had been selected as energy crops having the high potential to produce biomass in the southern region of Tohoku district (Ohira village, Miyagi Prefecture)⁶⁾.

2.3 Cultivation and harvesting

2.3.1 Cultivation and harvesting at Maizuru

Willow shoot cuttings (30 cm in length and 1-3 cm in diameter) were obtained on early March 2009 from the plants grown in the plot at Ohira⁶⁾. The cuttings were wrapped with wet newspaper, put in plastic bags, and transported to the planting site in Maizuru city.

Soils were rototilled before planting of willow shoot cuttings. The shoot cuttings were planted in late March 2009. Forty to sixty cuttings were planted in a row at intervals of 0.5 m. The rows were spaced 1 m apart with 2 m margins in each plot. The total number of cuttings planted was 864 in KKD, 121 in HB471, 1974 in SEN, and 909 in FXM. The calculated density was about 20,000 plants per hectare. Each clone was planted together in the same plot without blocking.

The plots were not fertilized at the start of cultivation to prevent vigorous growth of weeds soon after planting of the cuttings. Then, fertilizer was applied to prevent yellowing of leaves. Two briquettes (ca. 32 g in total) of fertilizer (Maruyama No.1, N6:P4:K3, Nihon Ringyo Hiryo Co., Ltd, Tokyo, Japan) were applied per plant in July 2009 (cal. 384 kg N, 128 kg P and 92 kg K per hectare), and then about 300 g dried poultry manure per plant on October in 2009 (180 kg N, 300 kg P and 240 kg K per hectare). The plots were weeded with a power weeder every month, from May to September 2009. The plots were not irrigated and pest control was not conducted. Willow plants were left to grow without cutting-back for two growing seasons until late December in 2010. In 2010, the plots were neither weeded nor irrigated.

Ten trees each of KKD and FXM, 11 trees of HB471 and 20 trees of SEN were harvested at 20 cm above the ground after two growing seasons in December 2010. The plants were randomly harvested, except for the outermost rows to eliminate edge effects on plant growth. The harvested shoots were cut into several pieces, and weighed for fresh weight. Two trees from each clone were used for analysis of chemical contents (data not shown). Remaining trees of respective clones (8 in KKD, 9 in HB471, 18 in SEN and 8 in FXM) were used to measure the dry weights after air-drying outdoors under a shelter for 8 months. Water contents were calculated from the difference between the fresh weight and the dry weight.

2.3.2 Cultivation and harvesting at Ohira

Shoot cuttings of KKD, SEN and FXM were prepared in late April 2008 from their stock plants at Ohira⁴⁾. Their sizes were the same as described above. Soils were rototilled and furrowed to 0.7 m in width. The ridges were covered with black 90 cm-wide plastic mulching sheets (0.03

mm thick) with 10 cm buried margins to prevent the growth of weeds and grasses. The distance between centers of adjacent ridges was 1 m, leaving a 0.3 m-wide aisle in the middle. Planting density was the same as described above; 1 m between rows and 50 cm between plants. About sixty cuttings were planted in a row. The total number of cuttings planted was 90 in KKD, 363 in SEN and 351 in FXM. The calculated density was about 20,000 plants per hectare. Each clone was planted in the same plot without blocking. No fertilizer was applied during cultivation. Aisles between rows were weeded with a power weeder every 2 months, from May to September in 2008. The plots were not irrigated and pest control was not conducted. Willow plants were grown until March in 2009, then cut back to a 20 cm height to leave stumps. The stumps were left to resprout and grow for two successive growing seasons until late autumn in 2010. The plots were neither weeded nor irrigated during the two successive growing seasons.

Ten trees of each clone grown for two growing seasons were harvested at 20 cm above the ground in December 2010. Harvesting procedures were similar to the case at Maizuru as described above. Briefly, ten plants per each clone were randomly selected and harvested. The harvested shoots were cut into several pieces and weighed for fresh weight. Values of water contents obtained for each clone in the previous report⁶⁾ were used to calculate the dry weights of willow plants shown in Table 2.

2.4 Root system of willow clones

We examined the root growth of the willow plants cultivated in the plot at Ohira by planting the cuttings at the density of 1 m between rows and 1 m between plants to ensure sufficient individual root growth. In October 2008 and 2009, plants grown from the cuttings planted in late April 2008, were excavated by digging a ditch 0.3 m deep in a circle with a radius of 0.5 m from the center of the

willow base. Roots extending outside the circle were cut off. The original shoot cutting (20 cm) remained at the base of the growing willow plant. The below-ground part was horizontally divided into 4 zones at 5 cm intervals.

Roots developing from each zone were separately collected to evaluate the three-dimensional distribution of root system. Shoots and roots separated from a whole tree were used for determination of fresh weight and dry weight. Four trees were selected from each clone. The dry weights of the harvested plant parts were determined after drying in an oven at 70°C for 3 days. Data are shown by the mean $\pm$ SE of four replicates.

2.5 Statistical analysis

Statistical analyses were carried out by Tukey's multiple range test or Student's t-test using an on-line statistical analysis program MEPHAS (<http://www.gen-info.osaka-u.ac.jp/testdocs/tomocom/>).

3. Results and Discussion

3.1 Above-ground biomass yield

At Maizuru, shoots of willow plants grown for two growing seasons without cutting-back were harvested. Table 1 shows the survival rates of respective clones at the time of harvest (two years after planting), which ranged from 82.1% to 85.1% depending on the clones, and the fresh and dry weights per plant and water contents calculated from them. Both average fresh and dry weights per plant were highest in KKD, followed by HB471, SEN and FXM. However, although there was no significant difference among four clones by Tukey's test at 5% level, the difference in the dry weight between KKD and FXM was significant (Table 1). Using the dry weight per plant, the survival rates, and the plant density of 20,000 plants per hectare, the dry matter yields per hectare were calculated as 26.8, 23.1, 18.0 and 14.3 tDM ha⁻¹ in KKD, HB471, SEN and

Table 1 Above-ground biomass yield of willow plants grown for two growing seasons after planting at Maizuru

<i>Salix</i> spp.	Clone	Survival rate* ¹ (%)	Fresh weight* ² (kg plant ⁻¹)	Dry weight* ² (kg plant ⁻¹)	Water content (%)	Estimated yield* ³ (t DM ha ⁻¹)
<i>S. pet-susu</i>	KKD	83.7	2.77 $\pm$ 0.40	1.60* ⁴ $\pm$ 0.22	42.2 ^a * ⁵ $\pm$ 1.8	26.8
<i>S. pet-susu</i>	HB471	84.3	2.24 $\pm$ 0.50	1.37 $\pm$ 0.29	39.8 ^a $\pm$ 3.3	23.1
<i>S. sachalinensis</i>	SEN	85.1	1.92 $\pm$ 0.26	1.06 $\pm$ 0.14	45.1 ^b $\pm$ 0.8	18.0
<i>S. pseudolinearis</i>	FXM	82.1	1.83 $\pm$ 0.24	0.87* ⁴ $\pm$ 0.12	52.4 ^c $\pm$ 0.6	14.3

Willow plants were 2-year old for both roots and shoots (above-ground portions).

*1 Survival rates were determined at the time of harvest.

*2 Data are the mean $\pm$ SE of 8 plants for KKD, 9 plants for HB471, 18 plants for SEN and 8 plants for FXM.

*3 Yields were calculated by assuming that the planting density is 20,000 plants per hectare.

*4 Dry weights between KKD and FXM are significantly different by Student's t-test ($p < 0.05$).

*5 Figures with different letters in this column are significantly different by Tukey's multiple range test ($p < 0.05$).

FXM, respectively.

Table 2 shows the results of cultivation study at Ohira, where willow plants were grown for two growing seasons after cutting-back of one-year grown plants, and then harvested to measure the growth. In this case, the above-ground portion (shoot) was 2 years old, and the below-ground portion (root) 3 years old. In this plot, the number of planted KKD was only 90 because of shortage of cutting in the spring of 2008. The KKD plants were cultivated in two neighboring rows, only one side being next to the row of FXM. Therefore, we harvested KKD plants from the row next to that of FXM to avoid edge effects. The survival rate at the time of harvest was 96.7, 90.0 and 96.7% in KKD, SEN and FXM, respectively (Table 2). In this study, the above-ground fresh weight yield was highest in KKD, followed by FXM and SEN, and the value in KKD was significantly higher than those in FXM and SEN by Tukey's test at 5% level. The dry matter yield per hectare was calculated from water contents obtained in the previous study⁶⁾ and survival rates observed in this study. They were 26.5, 12.9 and 7.7 tDM ha⁻¹ in KKD, FXM and SEN, respectively.

This order in above-ground biomass yield among three clones agreed with the results obtained in the previous study at Ohira⁶⁾. However, the biomass obtained in the previous study was larger than that in the present study⁶⁾. This was probably caused by the difference in soil texture and fertility between two independent plots used for the studies. Moreover, the previous cultivation study was conducted on a smaller scale; only 24-48 cuttings for each clone. Because of this small scale cultivation, edge effects on plant growth might have caused an over-estimation of fresh weight per plant in the previous study⁶⁾.

At Maizuru, where the annual mean temperature was 15°C, the order of yield per plant after two growing sea-

sons without cutting-back was KKD > HB471 > SEN > FXM (Table 1). At Ohira, where the annual mean temperature was 11°C, the order of yield per plant was KKD > FXM > SEN (Table 2). Moreover, cultivation trials subsequent to the present study have been conducted with KKD, HB471, SEN and FXM at the other two sites, Tsuchiura city (36° 07.5'N, 140° 08.6'E; annual mean temperature, 15°C; annual precipitation, 1154 mm) (unpublished observation) and Ogata village, Akita prefecture (40° 00.2'N, 139° 57.2'E; annual mean temperature, 11°C; annual precipitation, 1354 mm) (personal communication from Professor S. Hidaka of Akita Prefectural University). The results obtained until now showed that the growth of KKD surpassed the other three clones, HB471, SEN and FXM. These results revealed that KKD showed the highest growth and biomass production per plant basis among four selected clones irrespective of difference in cultivation conditions.

The survival rate of willow plants at harvest varied with the colony, and it was from 90.0% to 96.7% at Ohira, and from 82.1% to 85.7% at Maizuru. The survival rate was lower at Maizuru than at Ohira. This may be attributed to the mistaken cutting of young plants during weeding with a power weeder. In the plot at Maizuru without mulching sheets, weeds grew vigorously and a power weeder was used every month in the first growing season.

When we compared the dry weight yield per unit-area (hectare) by taking the survival rate (Tables 1 and 2) into consideration, it was highest in KKD at both Maizuru and Ohira, irrespective of the cutting-back of plants. KKD cultivated at Maizuru was estimated to have a dry matter yield of 26.8 tDM ha⁻¹ at the end of two growing seasons. Usually in the first cutting cycle of biomass production willow short-rotation coppices are harvested 3-5 years after the cutting-back of the plants grown for one year after planting^{8)~10)}. This means that there would be no or little har-

Table 2 Above-ground biomass yield of willow plants grown for two growing seasons after the cutting-back of one-year grown plants at Ohira

<i>Salix</i> spp.	Clone	Survival rate* ¹ (%)	Fresh weight* ² (kg plant ⁻¹)	Water content* ³ (%)	Estimated yield* ⁴ (t DM ha ⁻¹)
<i>S. pet-susu</i>	KKD	96.7	2.70 ^a * ⁵ ± 0.54	49.3	26.5
<i>S. sachalinensis</i>	SEN	90.0	0.90 ^b ± 0.17	52.4	7.7
<i>S. pseudolinearis</i>	FXM	96.7	1.30 ^b ± 0.20	48.6	12.9

Willow plants were 3-year old for roots, but 2-year old for shoots (above-ground portions).

*1 Survival rates were determined at the time of harvest.

*2 Data are the mean ± SE of 10 plants of each clone.

*3 Water contents are data determined in the previous study with plants grown for 2 years after cutting-back of one-year grown plants at the same sites⁴⁾.

*4 Yields were calculated by assuming that the planting density is 20,000 plants per hectare.

*5 Figures with different letters are significantly different by Tukey's multiple range test ($p < 0.05$).

vest for several years in the first cutting cycle. Therefore, the willow clone KKD has an economical merit that it can be harvested only two years after planting in warm regions in Japan.

3.2 Comparison of root system among willow clones

Table 3 shows the biomass yields (fresh and dry weight of whole-tree and root), and water contents of whole-tree and root and root/shoot ratios in FXM, SEN and KKD plants, which were cultivated for one or two growing seasons at Ohira. In this experiment KKD gave largest biomass yields after one and two growing seasons.

The root/shoot ratio has been used for analysis of biomass distribution between below- and above-ground tis-

sues^{11) 12)}. The root/shoot ratio in FXM and SEN remained almost constant after the first and second growing seasons (0.15 vs. 0.10 in FXM and 0.10 vs. 0.12 in SEN). On the other hand, that in KKD markedly increased during the second growing season (from 0.08 to 0.18), showing that investment of resource to roots was stimulated in parallel with enhancement of shoot growth in the second growing season. These findings suggested that in KKD the investment of dry matter to roots was smallest although the growth of whole trees was largest during the first growing season, and most of the mechanical tissues in roots developed during the second growing season.

The observation of root shape of willow plants cultivated for two growing seasons revealed a distinct distri-

Table 3 Growth, water content, root/shoot ratio and number of sinker roots of FXM, SEN and KKD in the first and second year after planting of cuttings at Ohira

Clone	Whole tree			Root			Root/Shoot*1		Number of sinker roots per tree
	FW (g)	DW (g)	Water content (%)	FW (g)	DW (g)	Water content (%)	FW basis	DW basis	
First year									
FXM	599 ^{b*2} ±72	193 ^b ±27	67.8 ^a ±0.8	178 ^a ±14	23.9 ^a ±2.4	86.6 ^a ±0.4	0.44 ^a ± 0.04	0.15 ^a ±0.02	0
SEN	893 ^{ab} ±178	331 ^{ab} ±64	62.9 ^b ±1.1	216 ^a ±58	30.5 ^a ±9.5	86.3 ^a ±0.7	0.32 ^a ± 0.04	0.10 ^a ±0.02	0
KKD	1692 ^a ±432	658 ^a ±183	62.0 ^c ±1.2	359 ^a ±70	48.1 ^a ±11.1	86.9 ^a ±0.7	0.29 ^a ± 0.09	0.08 ^a ±0.01	0
Second year									
FXM	5166 ^c ±957	2387 ^c ±393	53.8 ^a ±0.5	704 ^c ±158	231 ^a ±53	67.3 ^a ±0.3	0.15 ^c ± 0.01	0.10 ^c ±0.01	1.7
SEN	4994 ^b ±759	2413 ^b ±393	51.9 ^b ±0.7	745 ^b ±88	258 ^b ±31	65.4 ^b ±0.2	0.18 ^b ± 0.01	0.12 ^b ±0.01	4.5
KKD	11054 ^a ±1555	5421 ^a ±745	50.9 ^c ±0.3	1994 ^a ±333	829 ^c ±140	58.5 ^c ±0.4	0.22 ^a ± 0.01	0.18 ^a ±0.01	10

Values for fresh weight and dry weight are the mean ± SE of four independent trees per each clone.

*1 Root/shoot ratio was calculated as root/(whole tree – root).

*2 Figures with different letters in each column are significantly different by Tukey's multiple range test ($p < 0.05$).

Fig. 1 Whole root profiles and three-dimensional distributions of roots, along the axis of shoot cuttings initially planted, in FXM, SEN and KKD after two growing seasons. Asterisks in the photograph of KKD show sinker roots. Horizontal bars with different letters in each panel significant difference by Tukey's multiple range test ($p < 0.05$)

bution. Photographs in Fig. 1 show that FXM roots formed a thin layer at the base of the plants, and SEN roots formed a thicker layer in a cone shape. On the other hand, the shape of the KKD root system was like an inverted cone and it would help increase the tree stability. The three-dimensional pattern (architecture) of woody root system (actually adventitious roots) is an important aspect to assess tree and soil stability, resource acquisition and allocation¹³⁾¹⁴⁾. Moreover, sinker roots were not found in the three clones in the first year, but they developed in all the clones in the second year (Table 3). The number of sinker roots was large (10 roots per tree) in KKD, but small in FXM and SEN after the second growing season. Sinker roots play an important role in tree stability, growth, and acquisition of nutrient and water by elongating vertically to reach a deeper water level in the ground^{13) 15)}. The large number of sinker roots in KKD suggested that KKD trees had firmly settled.

In addition, a distinct distribution of roots among three clones was supported by determining separately dry weights of roots developed from each zone of initially planted shoot cuttings. Histograms in Fig. 1 indicate that root growth of FXM and SEN was largest at the base of cuttings, whereas in KKD it was largest at the sub-top region of cuttings and gradually decreased toward the base of the cutting. The distinct root morphology of KKD is probably a cause for its highest productivity.

4. Conclusions

In the present study, we evaluated the above-ground biomass yield of four previously-selected willow clones after two-growing seasons in Tohoku and Kinki regions, which had annual mean temperatures of 11°C and 15°C, respectively. In both regions, *S. pet-susu* clone KKD exhibited a higher above-ground biomass yield than the other three clones (*S. pet-susu* clone HB471, *S. sachalinensis* clone SEN and *S. pseudolinearis* clone FXM). The distinct root morphology of KKD was estimated to be closely related to its high biomass productivity of KKD.

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