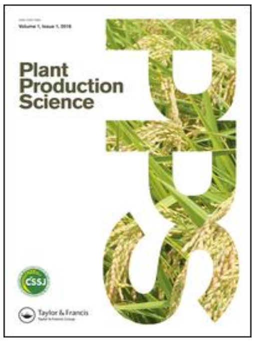

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【Short Report】

Suppression of Tillering in *Erianthus ravennae* (L.) Beauv. Due to Drought Stress at Establishment

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Abstract: We investigated the effect of drought stress on biomass productivity of newly established *Erianthus ravennae* (L.) Beauv. This species has recently drawn great attention as a novel cellulosic energy crop because of its excellent tolerance against various environmental stresses, but the shoot dry weight of the newly established *E. ravennae* was significantly decreased under drought compared to irrigated condition. A significant correlation between shoot dry weight and stem number suggested that the drought-induced decrease in stem number was ascribable to the reduced shoot dry weight in the drought condition. Decrease in soil water content was coincident with mid-day decrease in stomatal conductance, suggesting that limitation of CO_2 diffusion into leaf due to lower stomatal conductance in the drought condition caused decrease in photosynthesis followed by suppression of stem number. The present study suggested that *E. ravennae* was susceptible to drought, at least, in the first establishment year.

Key words: Biomass, Cellulosic bioethanol, Drought stress, Energy crops, *Erianthus ravennae* (L.) Beauv.

Japan is highly dependent on imported energy. Therefore, a new energy source needed to diversify the energy sources, even though the price of petroleum is recently decreasing after being on the increase for five years. Bioethanol is being considered as a possible new energy source for Japan and it can also contribute toward reduction of greenhouse gases because it is carbon neutral (Morita, 2008; Hattori, 2008). Ministry of Agriculture, Forestry and Fisheries of Japan has intended to increase production of biofuel including cellulosic bioethanol from energy crops (Biomass NIPPON General Innovation Board, 2007). In addition, in March 2008, the Biofuel Technology Innovation Conference presented the Biofuel Technology Innovation Plan with a roadmap for the development of technologies to produce cellulosic bioethanol, including large scale production of cellulosic energy crops abroad (Biofuel Technology Innovation Conference, 2008). Such energy crops for cellulosic bioethanol should be grown on non-arable land with lower energy input to avoid food-fuel competition and to contribute toward prevention of global warming.

Erianthus spp. is the wild relative of sugarcane and has been grown domestically and internationally as breeding material for sugarcane (Sugimoto, 2004; Aitken et al., 2007). Its members recently been receiving much attention in Japan as novel energy crops suitable for Japan-led large scale cellulosic biomass production abroad (Biofuel Technology Innovation Conference, 2008). This is because *Erianthus* spp. has been reported to show better growth even under unfavorable environmental conditions such as submerged condition and acidic soil (Matsuo et al., 2002), as well as under soil drought during the dry season (Matsuo et al., 2002). However, there is a big pitfall in the production of *Erianthus* spp. on a large scale in arid or semi-arid areas abroad, because the information on the drought tolerance has been limited to that after ratooning and is not available for the establishment year.

We found that growth of *Erianthus ravennae* (L.) Beauv., a member of the *Erianthus* spp. that is currently gaining much attention in Japan, was greatly suppressed by drought stress in the first establishment year. In this paper, we report on the drought stress-induced decrease of biomass productivity of newly established *E. ravennae* and discuss possible relevant physiological factors.

Materials and Methods

In the present study, *Erianthus ravennae* (L.) Beauv. (accession name; KO-1) was used as plant material. Stubbles of several-year-old *E. ravennae* were provided from National Agricultural Research Center for Kyushu

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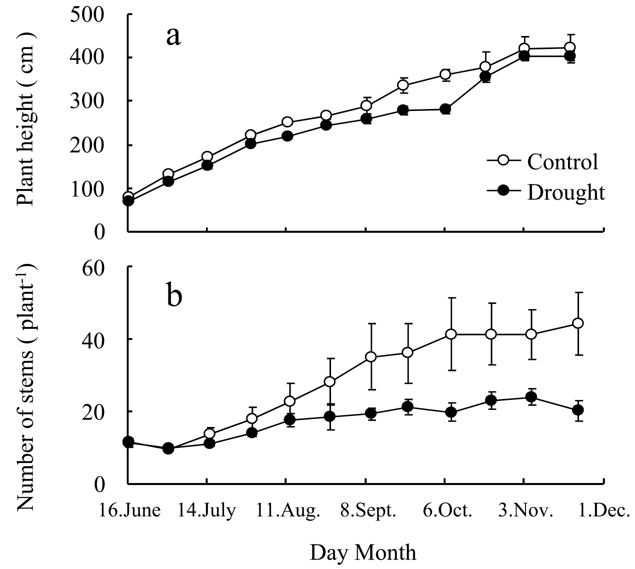

Fig. 1. Plant height (a) and number of stems (b) of *E. ravennae* grown under enough (Control) and limited (Drought) irrigation.
Data are means of 3 plots and vertical bars indicate standard errors.

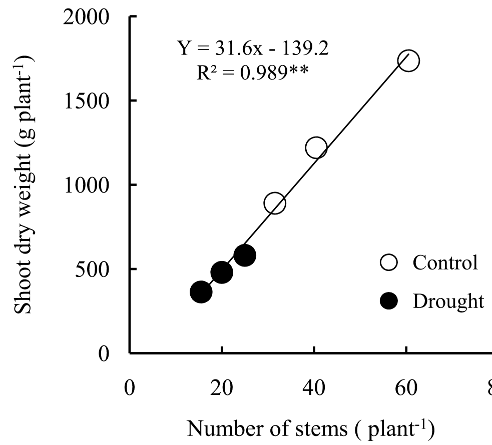

Fig. 2. Relationship between shoot dry weight and number of stems in *E. ravennae*.
Open and closed symbols represent the mean of each individual plot under enough (Control) and limited (Drought) irrigations, respectively. ** represents statistical significance at P<0.01.

Okinawa Region (KONARC) of National Agricultural Research Organization (NARO). Each stubble was divided into 6 through 8 parts of equal size, each of which had about 10 buds. They were planted in 2 greenhouses established at the Field Production Science Center of Graduate School of Agricultural and Life Sciences, the University of Tokyo (35°43´N, 139°32´E. Nishitokyo, Japan) on 6 June 2008 after application of N, P and K at a rate of 72, 108, 96 kg ha^{-1}, respectively. During experimental period, mean temperature, mean daily maximum and minimum temperatures in the greenhouse were 23.8, 33.7 and 18.3°C, respectively, while those outside of the greenhouse were 22.8, 27.8 and 18.9°C, respectively. The soil in the greenhouse was a humic andosol which had dark and humic silty loam in topsoil layer and red-brown silty clay loam in subsoil layer. The hydraulic properties of the soil were previously reported (Kato et al., 2007). Each greenhouse had 24 plots (1.5 m×3.6 m) inside, and the divided stubbles of *E. ravennae* were planted in 3 plots of them at a rate of 6 plants plot^{-1} in each greenhouse.

In both greenhouses, seedlings were irrigated (15 mm) on the 1st and 4th d after planting. After that, one greenhouse was used for drought treatment and another one was for the control. In the control treatment, 12 mm irrigation was applied every 3 d until 13 July and then every 6 d until 17 September, and there was no irrigation thereafter. In drought treatment, 10 mm irrigation was applied in every 6 d until 13 July and then irrigation was stopped. Two plants with ordinary growth were selected from each plot and their plant height and number of stems were recorded every 2 wk until harvest. On 23 August and 2 September during the growing period, soil sample was taken from 4 soil layers (0, 30, 60 and 90 cm from the soil surface) in each plot and soil water content (g water g^{-1} fresh soil) was measured gravimetrically. On 27 August, 45 d after stopping irrigation in the drought treatment, stomatal conductance of the upper-most fully-developed leaf was measured in mid-day time (1100-1300) using leaf porometer (SC-1, Decagon Devices, Inc., WA, USA). Three plants from each plot were used for measurement of stomatal conductance. On 19 November, two plants of which plant height and number of stems had been monitored were harvested to determine final plant height, number of stems, basal diameter of each stem, and other morphological parameters. Then, shoots were divided into leaf, stem and panicle, and they were dried in oven at 80°C for at least 4 d to determine dry weight.

Results and Discussion

Plant height of *E. ravennae* increased with growth and there was no significant difference between the control and drought treatments (Fig. 1a). Although the number of stems also increased with growth, it was fewer in the drought treatment than those of the control after stopping irrigation (Fig. 1b). Morphological analysis at harvest revealed that the number of stems taller than 350 cm was almost the same in the drought treatment and control. However, in the drought treatment, stems with 150–200 cm tall were the most prominent and number of stems taller than 200 cm tended to decrease, while plants in the control had more stems with 200–350 cm tall. Shoot dry weight was significantly decreased by limitation of irrigation; 11.9±2.3 t ha^{-1} in the control and 4.4±0.6 t ha^{-1} in the drought treatment (less than 40% of that in the control treatment). On the other hand, the proportion of

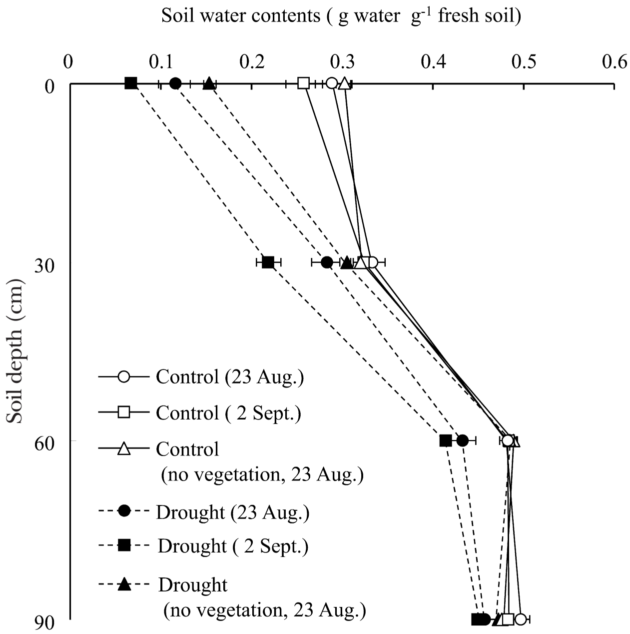

Fig. 3. Changes in soil water content at different soil depth in enough (Control) and limited (Drought) irrigation treatments. Data are means of 3 plots and horizontal bars indicate standard errors.

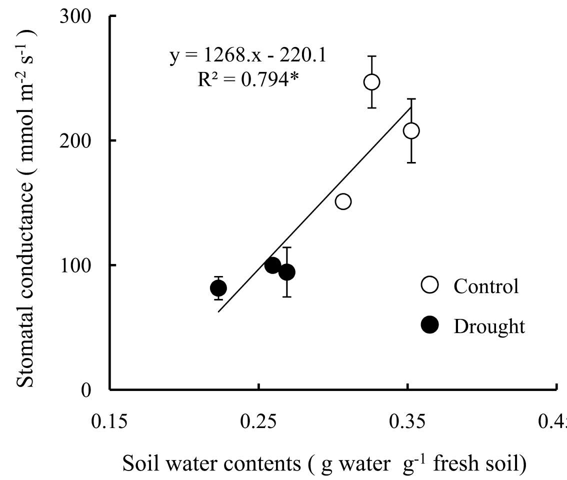

Fig. 4. Relationship between soil water content and stomatal conductance in *E. ravennae*.
Open and closed symbols represent the means of each individual plot under enough (Control) and limited (Drought) irrigations, respectively. Vertical bars indicate standard errors. Stomatal conductance was measured on 27 August. Air temperature and solar radiation during the measurement were 34.4±2.3°C and 2158±134 kJ m^{-2}, respectively. Data of soil water content were means of the values measured on 23 August and 2 September at 30 cm depth (as shown in Figure 3). * represents statistical significance at $P<0.05$.

leaf, stem and panicle in shoot dry weight was 55–56%, 41–42% and 2–4% of shoot dry weight, respectively, in both the control and drought treatment. These results indicated that the lighter dry weight in the drought treatment could be attributed mostly to the decrease in the number of stems. In fact, there was a significant positive correlation, irrespective of the treatments, between shoot dry weight and the number of stems ($r=0.995$, $P<0.01$. Fig. 2). The difference in soil water contents between the drought treatment and control might have caused the difference in the number of stems.

As shown in Fig. 3, soil water contents in the top 60 cm at 40–50 d after stopping irrigation was lower in the drought treatment than the control. The differences in soil water content between the plots with and without *E. ravennae* (no vegetation) in the drought treatment (Fig. 3) indicated that *E. ravennae* absorbed water from a deeper soil layer than those in the control to maintain their growth under no supply of water. On the other hand, a significant correlation between stomatal conductance and soil water contents was observed (Fig. 4), indicating that stomatal conductance in mid-day time depended on the amount of available soil water. Therefore, it was suggested that stopping irrigation decreased available soil water and, hence, lowered the stomatal conductance, although *E. ravennae* tried to maintain stomatal conductance by exploring available water in deeper soil layer.

Lower stomatal conductance generally induces decrease in photosynthetic rate via limitation of CO_2 diffusion into leaf mesophyll tissues. There is a possibility that *E. ravennae* could maintain photosynthetic rate by activation of phosphoenolpyruvate carboxylase (PEPC) under lower stomatal conductance. However, in sugarcane, related C_4 species of *E. ravennae*, such an activation of PEPC under drought stress was not observed (Vu and Allen Jr., 2009), or, even when observed, it has little effects on photosynthetic rate compared to stomatal limitation (Saliendra et al., 1996). Therefore, in the present study, the mid-day decrease in stomatal conductance was suggested to induce concomitant decrease in photosynthetic rate in *E. ravennae* as similarly observed in sugarcane (Vu and Allen Jr., 2009). The decrease in photosynthetic rate will be one of the factors to suppress the number of stems in the drought treatment. Available soil water in the control, in contrast, was enough to maintain stomatal conductance of *E. ravennae* and, hence, active mid-day photosynthesis. Much production of assimilate enabled *E. ravennae* to grow and increase its stem number.

On the other hand, panicles were formed on stems taller than 350 cm irrespective of soil water content (panicles emerged on 6 October in both treatments), and panicle number per plant was not different between the control and drought treatment (2.2±0.4 and 2.5±0.6, respectively). From these results, it was suggested that a decrease in the number of stems in *E. ravennae* grown under drought had contributed to avoid excess water loss by maintaining lower leaf area and, in addition, had enabled *E. ravennae* to allocate limited assimilates and water preferentially to panicle to ensure reproductive growth. In the control, assimilates and water might be enough to allow both panicles and stems to grow.

Erianthus spp. has been reported to be tolerant against soil drought during the dry season (Matsuo et al., 2002) so far. This conclusion might be based on the growth of *Erianthus* spp. in the second year or thereafter. Matsuo et al. (2002) suggested that deeper thick roots of *Erianthus* spp. contributed to water uptake from deeper soil layers during dry season. Sugimoto (2004) also reported that *Erianthus* spp. had thick roots elongating into deeper soil layers, and was considered to be possible breeding material to improve drought tolerance of sugarcane. In our experiment, however, *E. ravennae* might not have such a developed deep root system in the first establishment year possibly due to short growth period after transplanting. This was inconsistent with the fact that soil water content in the soil layer at 90 cm depth was almost unchanged in both drought and control treatments (Fig. 3). There was a possibility that level of nitrogen fertilizer application in the present study was relatively higher for *E. ravennae*, which generally grow in infertile soil, and induced reduction of root development. Further investigation will be required to clarify relationships between root system development and water uptake ability in *E. ravennae* with reference to drought tolerance and nutrition status.

Because *Erianthus* spp. is perennial and has high biomass productivity (more than 30–50 t ha^{-1} after established year), it can be grown for several years without repeated planting and tillage practices (Mislevy et al., 1997). In addition, *Erianthus* spp. has been reported to show high tolerance against many kinds of environmental stresses (Matsuo et al., 2002). With these favorable characteristics, *Erianthus* spp. has drawn attention as a novel cellulosic energy crop. *E. ravennae* is expected to be suitable for large-scale plantations in currently-unused unfavorable environments including semi-arid regions abroad for Japan-led production of cellulosic bioethanol. However, the results of the present study indicated that *Erianthus* spp. including *E. ravennae* would be susceptible to drought at least in the first establishment year and plant growth can be strongly restricted by a long drought period. Thus, attention should be paid to the susceptibility to drought when establishing the plantation of *Erianthus* spp. in a semi-arid condition for production of cellulosic bioethanol.

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