Short-term Low Oxygen Treatment Immediately After Planting Alters *Asparagus officinalis* var. UC157's Harvest Timing

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Abstract. To control asparagus harvest timing, we investigated the effects of short-term low (5%) oxygen (O₂) treatment in the cultivation area on asparagus growth and yield using a closed cultivation system. During 120 days of cultivation, low O₂ treatments were initiated at 0 to 4, 20 to 24, and 40 to 44 days after planting (DAP). The sprouting spears and control crown yield gradually decreased with increasing DAP. However, low O₂ treatment at 0 to 4 DAP significantly delayed the decrease until 80 DAP, although the total yield did not change during cultivation. In contrast, low O₂ treatments at 20 to 24 and 40 to 44 DAP did not affect yield performance. Taken together, short-term low O₂ treatment immediately after planting can change the harvest timing of white asparagus and can be used for effective asparagus culturing in a closed system, such as a plant factory.

Asparagus (*Asparagus officinalis* L.) is a popular stem vegetable consumed worldwide. It is a source of functional substances including rutin and protodioscin (Wang et al., 2003). A recent statistical report suggested that China is the largest asparagus-producing country in the world (FAO, 2017). In the past, most asparagus produced in China was processed and canned for export. Recently, domestic consumption of Chinese fresh green asparagus has been increasing (Zhang and Araki, 2017). Most asparagus produced in

Japan is usually supplied as a fresh vegetable; fresh white asparagus has become increasingly popular in China and Japan owing to food diversification in both countries.

Several studies have attempted to optimize forcing cultivation systems for fresh white asparagus production by incremental addition of functional substances (Maeda et al., 2012), to elucidate yield performance differences between male and female plants (Uragami et al., 2016) and develop yield estimation technology (Uragami et al., 2017). In addition, in terms of profitability, it is important to control the harvest timing of white asparagus; thus, studies have investigated the relationship between white asparagus sprouting and environmental conditions surrounding the crown. Heißner et al. (2006) examined the effect of soil temperature on white asparagus yield, and high temperature treatments surrounding the crowns have been shown to control white asparagus sprouting (Paroussi et al., 2002; Watanabe et al., 2019).

Kitazawa et al. (2014) suggested that <14% O₂ in soil surrounding the crowns was effective in controlling sprouting, and sprouting inhibition was caused by the inhibition of metabolism due to the reduction in respiration in the crowns. Recently, a closed cultivation system for white asparagus production has been proposed as a typical model of plant factories (Motoki et al., 2012). In such a system, the O₂ concentration surrounding the crowns can be easily controlled.

In our previous study (Kitazawa et al., 2014), the low O₂ treatment was continuous throughout the cultivation period. However, distinct periods of low O₂ treatment may be cost-effective and labor-saving in cultivation management. In addition, if low O₂ treatment during cultivation can control sprouting, then the harvest period range could be expanded. However, there is no information regarding the effects of short-term low O₂ treatment surrounding the crowns during cultivation on growth and yield performance.

Therefore, the objective of this study was to examine the effect of short-term low O₂ treatments, administered in the cultivation area, at different periods on the spear growth and yield of white asparagus.

Materials and Methods

Plant materials. All experiments were carried out using 9-month-old *Asparagus officinalis* var. UC157 seedlings, which were grown in black polyethylene pots in a glasshouse in the Institute of Vegetable and Floriculture Science, National Agriculture and Food Research Organization, Tsukuba, Japan. The seeds were sown on 25 July 2016. The pots were 120 mm in diameter, 100 mm in height, and 830 mL in volume, and were filled with commercial culture soil (Nippi Engei Baido No. 1; pH: 5.8–6.5, particle size: 0.5–3.0 mm, N: 200 mg·L⁻¹, PO₃: 2500 mg·L⁻¹, K: 200 mg·L⁻¹, and Mg: 200 mg·L⁻¹; Nihon Hiryo, Tokyo, Japan). Four female and four male plants were used for each treatment, as a previous study suggested that the growth of male and female asparagus plants is different (Koizumi et al., 2002). To identify seedling sex, we observed the flowers and/or used a loop-mediated isothermal amplification method, according to Shiobara et al. (2011).

Cultivation system for different periods of short-term low O₂ treatment. Before the experiment, all shoots from the potted plantlets were cut just above the soil, and the crowns were transplanted into new pots of the same size and filled with the same culture soil to renew their physical and fertilizer conditions. The average weight of the crowns was ≈70 g. Next, 200 mL of water was added into each pot. Thereafter, eight pots containing a crown each were placed in a gas-tight acrylic chamber [inside: 340 mm × 380 mm × 375 mm (width × wide × height)] coupled with a gas-supply system (Fig. 1) on 26 Apr. 2017. Each chamber was placed in a dark room at 20 °C during the experiment for 120 d.

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Fig. 1. Details of the layout of the gas-tight chambers coupled with a low O₂-supply system.

Fig. 2. Effect of different periods of short-term low O₂ treatment on initial spear sprouting, mean $\pm$ SE (n = 8). The asterisk indicates a significant difference compared with the control using Dunnett's test ($P < 0.05$).

Fig. 3. Effect of different periods of short-term low O₂ treatment on the time from the sprouting to harvest of spears, mean $\pm$ SE (n = 3–8). The asterisks indicate a significant difference compared with the control using Dunnett's test ($P < 0.05$) within the same investigation period.

Using the previously mentioned gas-supply system, we used three chambers for the low O₂ treatments and one chamber for the control; 5% O₂ was blown in from the inlet port of each chamber at 0 to 4, 20 to 24, or 40 to 44 DAP, at a flow rate of 300 mL·min⁻¹; during the remaining period, the ambient air condition was maintained. For low O₂ treatment, we focused on the early stage after planting because our previous study (Kitazawa et al., 2014) suggested that

low O₂ might inhibit sprouting effectively when the sugar content in the roots was abundant. The control chamber was treated with natural air (20.9% O₂) instead of 5% O₂. The chamber lids were closed during cultivation, except when determining growth and yield ability (described later in this article). Fifty milliliters of water was supplied to each pot when the surface of the soil dried. The water that was not absorbed into the soil and/or the crowns was drained

from a hole at the bottom of the pots and collected.

Measurement of parameters. The spear growth and yield of the crowns in each treatment were determined at 1- to 3-d intervals by opening the lid of the chamber. This process also meant that the inside atmosphere of the chamber was ventilated. The lid of the chamber was opened even during the low O₂ treatment. However, the maximum opening time was 3 min.

To determine the spear growth ability per crown, we recorded the date of initial sprouting, number of days from sprouting to harvest for each spear, and number of sprouts. We defined initial sprouting as the point when the length of the spear emerging from the soil surface was >30 mm.

To determine the yield ability per crown, the date of the initial harvest, number of harvested spears, and yield (g) were recorded. The spears were cut at the surface and harvested when the spear length from the soil surface was >150 mm. To calculate yield per crown, each spear weight was normalized as weight per 150 mm because the length of the harvested spears varied.

The data on both spear growth and yield ability were aggregated by 20-day intervals.

Statistical analysis. Dunnett's test was used to evaluate all measurement items, after confirming the homogeneity of variances using Bartlett's test. Next, we used statistical software to conduct the analyses (Bell Curve for Excel; Social Survey Research Information, Tokyo, Japan). The significance level for both tests was set at 0.05. We only analyzed plants that showed sprouting. Therefore, the replication number for the statistical analyses was not always 8, although eight plants were established per treatment.

Results

Spear growth ability. Initial spear sprouting was significantly delayed compared with that of the control when the low O₂ treatment was carried out at 0 to 4 DAP (Fig. 2). However, no significant differences were observed compared with the control for low O₂ treatments at 20 to 24 and 40 to 44 DAP.

At 1 to 20 DAP, the time from sprouting to harvest was extended following low O₂ treatment at 0 to 4 DAP (Fig. 3). At 101 to 120 DAP, the time from sprouting to harvest was also extended following low O₂ treatment at 20 to 24 DAP.

The number of sprouting spears per crown gradually decreased with increasing DAP. However, low O₂ treatment at 0 to 4 DAP inhibited the decrease within the 61 to 80 DAP (Fig. 4). The total number of sprouting spears per crown under the control and each treatment condition was 18.5 ± 2.3 , 23.4 ± 3.4 , 22.1 ± 3.0 , and 18.0 ± 3.6 , respectively. No significant differences in the total number of sprouting spears per crown were observed between the control and other low O₂ treatments.

Fig. 4. Effect of different periods of short-term low O_2 treatment on the number of sprouting spears per plant, mean $\pm$ SE ($n = 8$). The asterisk indicates a significant difference compared with the control using Dunnett's test ($P < 0.05$) within the same investigation period.

Fig. 5. Effect of different periods of short-term low O_2 treatment on initial spear harvesting, mean $\pm$ SE ($n = 8$). The asterisk indicates a significant difference compared with the control using Dunnett's test ($P < 0.05$) within the same investigation period.

Fig. 6. Effect of different periods of short-term low O_2 treatment on the number of harvested spears per plant, mean $\pm$ SE ($n = 8$). The asterisk indicates a significant difference compared with the control using Dunnett's test ($P < 0.05$) within the same investigation period.

Yield ability. The initial harvest was significantly delayed compared with that of the control when the low O_2 treatment was carried out at 0 to 4 DAP (Fig. 5).

The number of harvested spears per crown gradually decreased with increasing DAP. However, the low O_2 treatment at 0 to 4 DAP inhibited the decrease within 61 to 80 DAP (Fig. 6). A similar trend was observed for yield per plant (Fig. 7). In contrast, low O_2 treatment at 20 to 24 and 40 to 44 DAP did not affect the number of

harvested spears and yield per plant. The total number of harvested spears per crown under the control and each treatment condition was 17.9 ± 2.5 , 22.6 ± 3.7 , 20.5 ± 2.6 , and 17.4 ± 3.3 , respectively. The total yield of harvested spears (g) per crown under the control and each treatment condition was 17.9 ± 2.0 , 16.8 ± 1.9 , 20.2 ± 1.7 , and 15.3 ± 1.5 , respectively. No significant differences in the total number and yield of harvested spears per crown were observed between the control and low O_2 treatments.

Discussion

Initial spear sprouting was delayed when the low O_2 treatment was carried out at 0 to 4 DAP (Fig. 2). This finding demonstrates that a hypoxic atmosphere surrounding plant roots inhibits shoot growth, which is consistent with the results of Smit et al. (1990) and Steffens et al. (2005), and our previous study (Kitazawa et al., 2014).

Previously, we suggested (Kitazawa et al., 2014) that treatment with 5% O_2 surrounding the crowns did not affect the rate of spear elongation when exposed to low O_2 throughout the ≈ 40 d of cultivation. In contrast, the duration between sprouting and harvest at 1 to 20 DAP delayed spear elongation following low O_2 treatment at 0 to 4 DAP (Fig. 3). Nevertheless, considering the entire 120-day cultivation period, it seems that low O_2 treatment during 0 to 4 DAP did not affect the rate of spear elongation, similar to the results of our previous study (Kitazawa et al., 2014). Although low O_2 treatment immediately after the start of the experiment delayed initial sprouting, its effect on spear elongation would be difficult to define if such treatment was continued for more than 40 d. Asparagus spear elongation has been suggested to be regulated by endogenous substances, including plant hormones (Kojima and Sakurai, 1994; Ku and Woolley, 2006). Moreover, it is known that abnormal oxygen conditions induce certain morphological changes in plant organs, such as internode elongation, via the regulation of plant hormones (Fujii et al., 1974; Voesenek et al., 2003). Therefore, low O_2 treatment immediately after the start of cultivation may affect production and accumulation or the activities of such endogenous substances. At 101 to 120 DAP, the time from sprouting to harvest was extended following low O_2 treatment at 20 to 24 DAP. This result also suggests that low O_2 treatment during this cultivation period delays asparagus sprouting. However, as there were no significant differences in the total sprouting among the treatments, growth ability was unaffected by such delays, at least for cultivation within 120 d.

The decrease in the number of sprouting spears per crown was only significantly inhibited by low O_2 treatment at 0 to 4 DAP within 61 to 80 DAP (Fig. 4). This finding suggests that the timing of sprouting could be changed and delayed by inhibiting sprouting during 1 to 20 DAP. Moreover, the number of sprouts following O_2 treatment at 20 to 24 and 40 to 44 DAP did not increase even when the cultivation period was extended.

It is known that the soluble solids, such as sugars and/or organic acids, of unripe fresh products stored under dark conditions are consumed during their respiration (Kartal et al., 2012; Techavuthiporn et al., 2008). However, it is also known that low O_2 conditions reduce such respiration (Caleb et al., 2013; Wang and Long, 2014). In addition, our previous study demonstrated that the consumption of the total soluble solids in the crowns was decreased when a 5% low O_2

Fig. 7. Effect of different periods of short-term low O_2 treatment on spear yield (g) per plant, mean $\pm$ SE (n = 8). The asterisk indicates a significant difference compared with the control using Dunnett's test ($P < 0.05$) within the same investigation period.

treatment was continued during cultivation (Kitazawa et al., 2014). Thus, we hypothesized that the total soluble solids in the crowns of the 20 to 24 and 40 to 44 DAP O_2 treatment groups in this study were consumed before the low O_2 treatment; this may explain the insignificant effects of low O_2 treatment during both periods.

The initial harvest was significantly delayed when low O_2 treatment was carried out at 0 to 4 DAP (Fig. 5). Considering the growth ability results, such a delay could have been caused by both inhibition of sprouting and the delay of spear elongation under low O_2 treatment. At 61 to 80 DAP, the increase in the number of harvested spears and yield per plant occurred only when plants were exposed to low O_2 treatment at 0 to 4 DAP (Figs. 6 and 7). Thus, low O_2 treatment immediately after the start of cultivation leads to a change in the peak of both yield abilities. As no significant differences were observed in the total number of harvested spears and yield per crown, the yield abilities would not have been enhanced even if the cultivation period had been extended.

Conclusions

The current results suggest that short-term low O_2 treatment of asparagus cultivation areas immediately after the start of cultivation delays sprouting of asparagus and the rate of spear elongation and that the treatment could delay the decrease in the number of the sprouting spears and harvest yield. Our results could contribute to controlling the harvest timing of asparagus and possibly other stem vegetables cultivated in a closed system, such as a plant factory. In addition, our results suggest that modified condition of low O_2 concentration below 5% might have a significant influence on controlling the harvest timing of asparagus. Our future research will focus on differences in sex and cultivar on the

growth and yield of asparagus under low O_2 conditions.

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