Involvement of Gibberellins in the Regulation of Tillering in Welsh Onion
(*Allium fistulosum* L.)

Hiroko Yamazaki¹, Nobutaka Shiraiwa², Akihiro Itai³ and Ichiro Honda⁴*

¹NARO Tohoku Agricultural Research Center, Morioka 020-0123, Japan
²Tottori Horticultural Experiment Station, Hokuei, Tottori 689-2221, Japan
³Faculty of Agriculture, Tottori University, Tottori 680-8553, Japan
⁴Department of Biotechnology, Maebashi Institute of Technology, Maebashi 371-0816, Japan

We investigated the effects of applied gibberellin *A*₃ (GA₃) and uniconazole P (UCP), a GA biosynthesis inhibitor, on tillering in Welsh onion, also known as Japanese bunching onion ‘Hangzhou’, a cultivar with very high tillering capacity. The number of tillers was increased by GA₃ treatment and reduced by UCP treatment. The tillering-inhibitory effect of UCP was counteracted by GA₃ treatment. GAs were considered to be involved in not axillary bud outgrowth but its initiation for the following results: UCP treatment raised the leaf position with the first tiller, and the tillering-promoting effect of GA₃ treatment became apparent about 8 weeks after the treatment, which mostly corresponded to the period during which an axillary bud initiated near the shoot apex develops to become visible. To clarify the relationship between the diversity of tillering capacity in Welsh onion cultivars and GAs, we investigated the levels of endogenous GAs and the responsiveness to GA₃ treatment in several cultivars possessing different tillering capacities. There was a negative correlation between the tillering capacity and the level of GA₄, a major bioactive GA in Welsh onion. On the other hand, there was a positive correlation between the tillering capacity and the responsiveness to GA₃ treatment; GA₃ treatment markedly promoted tillering in ‘Hangzhou’, but not in ‘Raitei-Shimonita’ and ‘Hanemidori-Ipponbuto’, cultivars with very low tillering capacity. These results suggest that the sensitivity to GAs is a factor causing the diversity of tillering capacity in Welsh onion.

Key Words: branching, GA, GA biosynthesis inhibitor, Japanese bunching onion.

Introduction

Welsh onion also known as Japanese bunching onion is an essential vegetable in East Asia. Two types of Welsh onion, green-leaf type and white-leaf sheath type, are cultivated in Japan. Green-leaf type is grown for whole leaves without blanching, while white-leaf sheath type is grown for thick and long leaf sheaths by blanching with soil. There are several kinds of Welsh onion that differ in tillering capacity. Cultivars for the green-leaf type often show tillering capacity to some extent; a certain degree of tillering thins leaves moderately and increases yield. Cultivars for the white-leaf sheath type show low tillering capacity. In culture of the white-leaf sheath type, the occurrence of unexpected tillering is not preferred because it decreases the commercial value by deformation of a round-shaped leaf sheath. For this reason, a cultivation technique to prevent tillering as well as an effective breeding technique to select lines with lower tillering capacity has been anticipated. Elucidation of the mechanism to control tillering in Welsh onion would be useful in developing these techniques.

The pattern of shoot branching (tillering) is a major determinant of plant morphology. Branch development can be separated into three stages: the initiation, dormancy and outgrowth of an axillary bud. Plant hormones are involved in the regulation of branch development, particularly in the regulation of bud outgrowth (Leyser, 2003; Thimann and Skook, 1933). The past 10 years of research have markedly improved our understanding of the mechanisms that control shoot branching. Three classes of plant hormone, auxins, cy-
tokinins, and strigolactones, participate in the control of bud activation (Domagalska and Leyser, 2011; Kebrom et al., 2013; McSteen and Leyser, 2005). In many plant species, the removal of the growing shoot apex, a major site of auxin biosynthesis, allows the growth of dormant axillary buds, and the application of auxins to the cut stump nullifies this effect. However, direct application of auxins to axillary buds does not inhibit their outgrowth, and apically applied auxin is not transported into the axillary bud. Cytokinins and strigolactones, which are mainly produced in roots, are transported acropetally, and move directly into the axillary bud. Cytokinins promote bud outgrowth and strigolactones prevent it. Auxins downregulate cytokinin biosynthesis and upregulate strigolactone biosynthesis.

These findings were obtained using Arabidopsis thaliana (L.) Heynh., pea, rice, maize and so on. In these plants, many more axillary buds are initiated than will grow out to become fully developed branches. Therefore, most of the plasticity of branch development arises from the regulation of axillary bud outgrowth. In Welsh onion, however, most of the plasticity of tiller development arises from the regulation of axillary bud initiation because axillary buds of Welsh onion are initiated in a specific axil at a specific time, and most of them are active (Yakuwa, 1963). In addition, gibberellins (GAs) inhibit tillering in grasses (Appleford et al., 2007; Lee et al., 1998; Lo et al., 2008), but they promote tillering in Welsh onion (Murai et al., 1981; Yamazaki et al., 2006). These findings suggest that Welsh onion has a tillering-controlling mechanism that differs from that in A. thaliana and rice.

The purpose of this study is to elucidate the role of GAs in the regulation of tillering in Welsh onion. We examined whether treatment with UCP, a GA biosynthesis inhibitor, inhibits tillering, and whether subsequent GA treatment counteracts this effect. We also examined the levels of endogenous GAs in several cultivars in relation to their tillering capacity, and the correlation between tillering capacity and responsiveness to GA treatment.

**Materials and Methods**

All experiments were conducted at NARO Tohoku Agricultural Research Center in Morioka, Japan (at 39° 45’ 55” north and 141° 8’ 1” east).

**Effects of GA and its biosynthesis inhibitor on tillering**

Welsh onion ‘Hangzhou’ (NIAS Genebank accession no. JP138784), which originates in China and possesses very high tillering capacity, was used. Seeds were sown in a 128-cell tray filled with commercial nursery soil on August 16, 2007, and grown in a greenhouse kept above 15°C under natural photoperiods. Solution of 10 ppm UCP (Sumiseven; Sumitomo Chemical, Osaka, Japan) or distilled water was applied to the soil near seedlings 10 times, at 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 weeks after sowing (WAS). The amounts of UCP applied per seedling were 1 mL until the 3rd treatment, 2 mL in the 4th and 5th treatments, and 5 mL in the 6th and subsequent treatments. Solution of 40 ppm GA3 (Water-soluble GA powder; Kyowa Hakko Bio Co., Tokyo, Japan) or distilled water was applied by irrigation from the bottom 71 days after sowing. The amount of GA3 applied was 10 mL per seedling. Thus, four treatments, water + water (W + W), UCP + W, W + GA, and UCP + GA, were prepared. Three days after GA3 treatment, seedlings were transplanted to a plastic container (65 × 22 × 19 cm) filled with commercial nursery soil at 4 seedlings per container, and grown until 24 WAS.

The number of tillers and the length of the youngest fully expanded leaf were measured at 2-week intervals during 2–24 WAS. Shoot fresh weight and leaf blade width of the youngest fully expanded leaf were measured at 24 WAS. The leaf position where the first tiller developed was recorded. The experiment was set up with 20 seedlings (5 containers) for each treatment.

**Endogenous GA levels in cultivars possessing different tillering capacities**

Experiments were conducted in 2007 and 2008. In the experiment in 2007, six cultivars, ‘Raitei-Shimonita’ (tillering capacity: very low; Sakata Seed Co., Yokohama, Japan), ‘Hanemidori-Ipponbuto’ (very low; Tohoku Seed Co., Ltd., Utsunomiya, Japan), ‘Shonan’ (low; Sakata Seed Co.), ‘Koharu’ (medium; Takii & Co., Ltd., Kyoto, Japan), ‘Asagikei-Kujo’ (high; Takii & Co., Ltd.), and ‘Hangzhou’ (very high), were used (Fig. 1A). In the experiment in 2008, two cultivars, ‘Hanemidori-Ipponbuto’ and ‘Koharu’, were used. ‘Hanemidori-Ipponbuto’ and ‘Koharu’ are F1 hybrids, and the others are incomplete fixed lines. ‘Raitei-Shimonita’
Shimonita’, ‘Hanemidori-Ipponbuto’, and ‘Shonan’ are white-leaf sheath types, and ‘Koharu’, ‘Asagikei-Kujo’, and ‘Hangzhou’ are green-leaf types. In regards to characteristics other than tillering capacity, ‘Raitei-Shimonita’ is marked by a stumpy plant form (Fig. 1A) and ‘Hanemidori-Ipponbuto’ is marked by late bolting.

In both experiments, eight-week-old seedlings were transplanted to an open field in late May at 5 cm intrarow spacing and 1 m interrow spacing. The seedlings were harvested on August 7 and September 10 in 2007 and 2008, respectively. For GA analysis, 5-cm-long basal leaf sheaths were sampled in 2007 and unexpanded leaves less than 5 cm in length including true stem and shoot apex in 2008 (Fig. 1B). Basal leaf sheaths (fresh weight about 25 g per replicate) and unexpanded leaves (fresh weight about 10 g per replicate) were frozen in liquid N\(_2\) immediately and stored at −30°C until use. We prepared at least 4 replicates. On the sampling day, the state of tillering was investigated for about 25 seedlings of each cultivar.

We quantified GA\(_1\), GA\(_3\), GA\(_4\), GA\(_9\), GA\(_{20}\), and GA\(_{34}\), which had been identified as endogenous GAs in Welsh onion (Shiraiwa et al., 2011). On the basis of previous findings regarding GA metabolism in plants (Yamaguchi, 2008), GA\(_1\), GA\(_3\), and GA\(_4\) among these GAs are considered to function as bioactive hormones, and to be metabolized as shown in Figure 2. The samples were homogenized with 80% cold methanol, and the homogenates were kept at 4°C overnight. After adding polyvinylpolypyrrolidone and [17,17-\(^2\)H\(_2\)]-GAs, obtained from Dr. L. N. Mander at the Australian National University (Canberra, Australia), as internal standards, the homogenates were filtered and evaporated to dryness. The dosage of [17,17-\(^2\)H\(_2\)]-GA\(_1\) and GA\(_{30}\) was 1.2 ng per sample, and that of [17,17-\(^2\)H\(_2\)]-GA\(_4\), GA\(_9\), and GA\(_{34}\) was 12 ng per sample. Since we could not have \(^3\)H-labeled GA\(_3\), [17,17-\(^2\)H\(_2\)]-GA\(_3\) was used as an internal standard for GA\(_3\) quantification. The residues were dissolved in distilled water and adjusted to pH 2.5. The acidic solutions were washed with hexane and partitioned against ethyl acetate. The ethyl acetate fraction was evaporated to dryness. The residues were dissolved in solvent containing 1% acetic acid, and the solutions were subjected to liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) using prominence liquid chromatography ( Shimadzu, Kyoto, Japan) coupled to a 3200 Q TRAP linear ion trap quadrupole mass spectrometer with Analyst software equipped with a Turbo V ion source operated in the ESI mode (Applied Biosystems, Carlsbad, CA, USA). The purified sample was dissolved in 20% methanol containing 0.05% acetic acid, loaded onto the column (Capcell-Pak C\(_{18}\) MGII, 2.1 mm i.d. × 150 mm, Shiseido, Tokyo, Japan) at 40°C and eluted at a flow rate of 0.2 mL·min\(^{-1}\). The mobile phases were as follows: A, 20% methanol containing 0.05% acetic acid; B, 100% methanol. The gradient program was as follows: 0 to 2 min, isocratic elution with 100% A; 2 to 7 min, linear gradient 0% to 60% (v/v) B; 7 to 17 min, linear gradient 60% to 100% (v/v) B; 17 to 23 min isocratic elution with 100% B. The mass spectrometer was run in negative ion mode. The parent and fragment ions used for quantification of GA\(_3\) on MS/MS were 345 and 239, while those for other GAs were the same as described by Fukuda et al. (2009).

Responsiveness to GA treatment in cultivars possessing different tillering capacities

The six cultivars described above were used (Fig. 1A). Seeds were sown in a 128-cell tray filled with commercial nursery soil on April 1, 2009, and grown in a greenhouse kept above 15°C under natural photoperiods. Solution of 40 ppm GA\(_3\) was applied by irrigation from the bottom at 10 WAS. The amount of GA\(_3\) treatment was about 10 mL per seederling. Control seedlings were treated with distilled water. Five days after the treatment, 112 GA\(_3\)-treated and control seedlings of each cultivar were transplanted to an open field at 5 cm intrarow spacing and 1 m interrow spacing. About 12 weeks after the treatment, the number of tillers of all seedlings was measured.

Results

Effects of GA and its biosynthesis inhibitor on tillering

The seedlings had no tiller until 10 WAS, but the number of tillers during 12–24 WAS was changed by the treatment with UCP, GA\(_3\), or both (Figs. 3 and 4). Seedlings in W + GA had about twice as many tillers as those in W + W at 24 WAS. This tillering-promoting effect became apparent at 20 WAS, about 10 weeks after GA\(_3\) treatment. On the other hand, UCP treatment inhibited tiller development; the number of tillers per
seedling in UCP + W was less than half that in W + W at 24 WAS. The reduced number of tillers in UCP + W was restored by GA$_3$ treatment. Recovery became apparent at 18 WAS, about 8 weeks after GA$_3$ treatment, and seedlings in UCP + GA had more tillers than those in W + W at 24 WAS.

More than 85% of the seedlings in W + W and W + GA had the first tiller at axils of the 6th or 7th leaf (Fig. 5). On the other hand, 50% in UCP + W and 70% in UCP + GA had the first tiller at axils of the 8th–12th and the 9th–11th leaves, respectively. All seedlings in W + W, W + GA, and UCP + GA had tillers, but 20% in UCP + W had no tiller until 24 WAS.

Leaf elongation was also influenced by UCP and GA$_3$ (Fig. 3). UCP treatment inhibited leaf elongation, and this effect was apparent at 4 WAS, 2 weeks after the first UCP treatment (Fig. 6). GA$_3$ treatment partly restored leaf elongation of UCP-treated seedlings, and this effect became apparent at 12 WAS, about 2 weeks after GA$_3$ treatment. On the other hand, in UCP-untreated seedlings, GA$_3$ treatment did not promote leaf elongation. UCP treatment gave thicker leaves and reduced shoot fresh weight, while GA$_3$ treatment thinned leaves (Table 1; Fig. 3).

*Endogenous GA levels in cultivars possessing different tillering capacities*

Table 2 shows the tillering in six Welsh onion cultivars on the day of sampling for GA analysis, and Figure 7 shows the endogenous GA levels in basal leaf sheaths. In all cultivars, the levels of C-13-non-
hydroxylated GAs, GA$_4$, GA$_9$, and GA$_{34}$, were much higher than those of C-13-hydroxylated GAs, GA$_1$, GA$_3$, and GA$_{20}$. The level of GA$_4$ was the highest among examined bioactive GAs, GA$_1$, GA$_3$, and GA$_4$. The level of GA$_4$ was 14–33 times higher than that of

![Fig. 6. Effects of UCP and GA$_4$ treatment on leaf elongation of Welsh onion 'Hangzhou'. A, length of the youngest fully expanded leaf; B, its percentage to W + W treatment. Data in A represent means ± SE (n = 20, the SE is within the data point).](image)

![Table 1. Effect of UCP and GA$_3$ treatment on growth of Welsh onion 'Hangzhou' at 24 WAS.](table)

| Treatments | Width of leaf blade (mm) | Shoot FW (g) |
|------------|--------------------------|--------------|
| W + W      | 14.8                     | 123          |
| W + GA     | 12.8                     | 108          |
| UCP + W    | 25.4                     | 55           |
| UCP + GA   | 17.6                     | 64           |

** ANOVA  
UCP treatment  **  **  
GA$_3$ treatment  **  ns  
Interaction  **  *

ns, *, and ** indicate a non-significant difference and significant differences at $P < 0.05$ and 0.01, respectively.

![Table 2. States of tillering in six Welsh onion cultivars on the sampling day for GA analysis.](table)

| Cultivars                  | Seedlings with tillers (%) | No. of axes per seedling |
|----------------------------|----------------------------|--------------------------|
|                            | 2007$^\text{z}$ | 2008 | 2007 | 2008 |
| Raitei-Shimonita           | 0                  | —    | 1.0      | —    |
| Hanemidori-Ipponbuto       | 0                  | 0    | 1.0      | 1.0   |
| Shonan                     | 8                  | —    | 1.1      | —    |
| Koharu                     | 92                 | 100  | 1.9      | 3.0   |
| Asagikei-Kujo              | 96                 | —    | 2.2      | —    |
| Hangzhou                   | 100                | —    | 7.2      | —    |

$^z$ The year of the experiment.
$^y$ Not investigated.

![Fig. 7. Endogenous GA levels in basal leaf sheaths of six Welsh onion cultivars possessing different tillering capacities. Cultivar 1, ‘Raitei-Shimonita’ (tillering capacity: very low); 2, ‘Hanemidori-Ipponbuto’ (very low); 3, ‘Shonan’ (low); 4, ‘Koharu’ (medium); 5, ‘Asagikei-Kujo’ (high); 6, ‘Hangzhou’ (very high). Different letters indicate significant differences by Tukey’s multiple range test at $P < 0.05$.](image)
Table 3. Endogenous GA levels in unexpanded leaves of Welsh onion ‘Hanemidori-Ipponbuto’ and ‘Koharu’ (pg·g\(^{-1}\) FW).

| Cultivars          | Tillering capacity | C-13-non-hydroxylated GAs |       | C-13-hydroxylated GAs |       |
|--------------------|--------------------|-----------------------------|-------|-----------------------|-------|
|                    |                    | GA\(_9\) | GA\(_4\) | GA\(_34\) | GA\(_20\) | GA\(_1\) | GA\(_3\) |
| Hanemidori-Ipponbuto| Very low           | 11188    | 10168    | 13026     | 343     | 108      | 17      |
| Koharu             | Medium             | 8488     | 6084     | 9753      | 336     | 78       | 8       |

\(t\)-test * * * ns * *
ns and * indicate a non-significant difference and a significant difference at \(P<0.05\), respectively.

Table 4. Tiller development in GA\(_3\)-treated and control seedlings of six Welsh onion cultivars about 12 weeks after GA treatment.

| Cultivars          | Seedlings with tillers* (%) | No. of axes per seedling | Seedlings with defective tillers (%) |
|--------------------|-----------------------------|--------------------------|-------------------------------------|
|                    | GA Control GA Control       | GA Control               | GA Control |
| Raitei-Shimonita   | 6 1                          | 1.1 1.0                 | ns 4 0 |
| Hanemidori-Ipponbuto| 3 1                          | 1.0 1.0                 | ns 0 0 |
| Shonan             | 48 15                        | 1.6 1.1                 | ** 12 0 |
| Koharu             | 92 94                        | 2.4 2.2                 | * 5 0  |
| Asagikei-Kujo      | 99 98                        | 3.4 2.8                 | ** 6 0  |
| Hangzhou           | 100 100                      | 15.7 9.7                | ** Not investigated |

* Not including defective tillers consisting of one leaf.
ns, *, and ** indicate a non-significant difference and significant differences between GA-treated and control seedlings by \(U\)-test at \(P<0.05\) and 0.01, respectively.

GA\(_4\), and 24–480 times higher than that of GA\(_2\). Except for GA\(_3\), the levels of all of the GAs in ‘Hangzhou’ (very high tillering) were equal to or lower than those in the other cultivars. In particular, the levels of GA\(_4\), GA\(_9\), and GA\(_34\) in ‘Hangzhou’ were the lowest among the 6 cultivars. The difference in GA level among the five cultivars except for ‘Hangzhou’ was relatively small, but the levels of GA\(_4\) in ‘Asagikei-Kujo’ (high tillering) and ‘Koharu’ (medium tillering) tended to be lower than those in the remaining three cultivars with low or very low tillering capacity.

Table 3 shows the endogenous GA levels in unexpanded leaves of two Welsh onion cultivars. The level of GA\(_4\) was much higher than those of GA\(_1\) and GA\(_3\). Except for GA\(_20\), the levels of all of the GAs in ‘Hanemidori-Ipponbuto’ (very low tillering) were higher than those in ‘Koharu’ (medium tillering). The levels of GAs in unexpanded leaves were generally higher than those in basal leaf sheaths.

Responsiveness to GA treatment in cultivars possessing different tillering capacities

Table 4 shows the effect of GA\(_3\) treatment on tillering of six Welsh onion cultivars possessing different tillering capacities. In ‘Raitei-Shimonita’ and ‘Hanemidori-Ipponbuto’, there was no significant difference in the number of tillers per seedling between control and GA\(_3\)-treated seedlings, although GA\(_3\) treatment slightly increased the percentage of seedlings with tillers. In ‘Shonan’, GA\(_3\) treatment increased both the percentage of seedlings with tillers and the number of tillers per seedling. In ‘Koharu’, ‘Asagikei-Kujo’, and ‘Hangzhou’, GA\(_3\) treatment increased the number of tillers per seedling, although most of the seedlings had tillers without GA\(_3\) treatment. The tillering-promoting effect of GA\(_3\) treatment appeared in ‘Hangzhou’ the most markedly. In this experiment, a few seedlings had defective tillers consisting of one leaf (Fig. 8). Such defective tillers were observed only in GA\(_3\)-treated seedlings.

Discussion

Promotion of leaf elongation is a well-known physiological response to GAs (Jones, 1973), and generally application of UCP, a GA biosynthesis inhibitor, inhibits leaf elongation. However, contrary to expectations,
GA₃ applied to the UCP-untreated Welsh onion seedlings was almost ineffective in promoting leaf elongation (Fig. 6). There have been some reports that GA₃ application has no effect on leaf elongation in onion seedlings (Fukuda et al., 2012; Mita and Shibaoka, 1984a), although Shiraïwa et al. (2011) reported that GA₃ application to 10-day-old Welsh onion seedlings promoted elongation of the first leaf. In our preliminary experiment, GA₃ applied to potted young Welsh onion seedlings slightly promoted leaf sheath elongation, but had no effect on leaf blade elongation (unpublished data). On the other hand, GA₃ applied to the UCP-treated dwarf seedlings was effective in promoting leaf elongation (Fig. 6). Mita and Shibaoka (1984b) reported that application of S-3307, a chemical substance almost identical to UCP, to young onion seedlings grown under long-day conditions oriented cortical microtubules in leaf sheath cells longitudinally or obliquely to the cell axis, which caused swelling of leaf sheaths, and that simultaneous GA₃ treatment reversed the effect of S-3307 on swelling. According to Shibaoka (1993), GAs promote longitudinal expansion of plant cells by orienting cortical microtubules transversely to the cell axis. Thus, the elongation-promoting effect of GAs may be weak in the plant leaves with microtubules naturally arranged transversely to the cell axis. According to Mita and Shibaoka (1984a), microtubules in leaf sheath cells of onion seedlings are arranged transversely or nearly transversely to the cell axis, but unfortunately, the arrangement of microtubules in the leaf blade cells has not been investigated.

In the present study, the application of GA₃ promoted tillering in Welsh onion as reported previously (Murai et al., 1981; Yamazaki et al., 2006). In addition, treatment with UCP reduced the number of tillers, the effect of which was counteracted by the application of GA₃ (Fig. 4). A tillering-inhibitory effect of UCP treatment was also obtained in another experiment, where UCP was applied to two Welsh onion cultivars, ‘Asagikei-Kujo’ and ‘Hangzhou’ (Yamazaki et al., 2007). These results suggest the involvement of GAs in axillary bud initiation. On the other hand, the leaf position with the first tiller in W + GA was almost the same as that in W + W (Fig. 5). This may be because most of the seedlings in W + W had already initiated the first axillary buds when GA₃ was applied at about 10 WAS.

In this study, we examined the endogenous GA levels in several Welsh onion cultivars in relation to their tillering capacity. In all cultivars, the amount of GA₃ was the highest among the examined bioactive GAs, GA₁, GA₃, and GA₄ (Fig. 7). This result is consistent with the report by Shiraïwa et al. (2011) that, in Welsh onion, there are both C-13-hydroxylation and non-hydroxylation pathways of GA biosynthesis, with the non-hydroxylation pathway to GA₄ being predominant. Similar results have been reported in other Allium plants such as onion (Nojiri et al., 1993) and Allium × wakegi Araki (Yamazaki et al., 2002). Hence, GA₃ is probably a major bioactive GA in several Allium plants. At the beginning of the experiment, we had expected a positive correlation between the bioactive GA level and tillering capacity in Welsh onion. However, the level of GA₃ in basal leaf sheaths was lower in ‘Hangzhou’ (very high tillering) than in the other five cultivars (Fig. 7). In addition, the levels of most GAs including GA₄ in unexpanded leaves were lower in ‘Koharu’ (medium tillering) than in ‘Hanemidori-Ipponbuto’ (very low tillering). These results suggest that the diversity of tillering capacity in Welsh onion is not attributable to the endogenous GA levels.

The functional expression of a plant hormone is often affected by the plant’s sensitivity to the hormone as well as the endogenous hormone level (Walker-Simmons, 1987). In A. thaliana, pea, and rice, strigolactone-insensitive mutants as well as strigolactone-deficient mutants show enhanced shoot branching (Kebrom et al., 2013). Accordingly, we investigated the responsiveness to GA₃ treatment in cultivars possessing different tillering capacities. We found a positive correlation between the tillering capacity and the responsiveness to GA₃ treatment; GA₃ treatment
markedly promoted tillering in ‘Hangzhou’ (very high tillering), but it was mostly ineffective in ‘Raitei- Shimonita’ and ‘Hanemidori-Ipponbuto’ (very low tillering) (Table 4). These results suggest that the sensitivity to GAs is a factor causing the diversity of tillering capacity in Welsh onion.

Auxins, cytokinins, and strigolactones are considered to play a pivotal role in the regulation of branching or tillering (Domagalska and Leyser, 2011; Kebrom et al., 2013; McSteen and Leyser, 2005). On the other hand, the role of GAs in shoot branching has not attracted much attention. However, the results of this study indicate that GAs play an important role in the regulation of tillering in Welsh onion. According to Yakuwa (1963), the timing and position of axillary bud initiation are common to several *Allium* plants including *A. × wakegi*, chive, Chinese chive, rakkyo, onion, and Welsh onion; an axillary bud is initiated at the axil of the (n − 1)th leaf shortly after the initiation of the (n)th leaf. In addition, the tillering-promoting effect of GA3 treatment has been found in onion as well (Fukuda et al., 2012). These results suggest that *Allium* plants have a common mechanism involving GAs to control tillering.

**Acknowledgements**

We are grateful to Dr. Akio Kojima and Dr. Tadayoshi Wako at NARO Institute of Vegetable and Tea Science for kindly supplying Welsh onion seeds ‘Hangzhou’.

**Literature Cited**

Appleford, N. E. J., M. D. Wilkinson, Q. Ma, D. J. Evans, M. C. Stone, S. P. Pearce, S. J. Powers, S. G. Thomas, H. J. Jones, A. L. Phillips, P. Hedden and J. R. Lenton. 2007. Decreased shoot stature and grain alpha-amylase activity following ectopic expression of a gibberellin 2-oxidase gene in transgenic wheat. J. Exp. Bot. 58: 3213–3226.

Domagalska, M. A. and O. Leyser. 2011. Signal interaction in the control of shoot branching. Nature Rev. Mol. Cell Biol. 12: 211–221.

Fukuda, M., S. Matsuoi, K. Kikuchi, W. Mitsuhashi, T. Toyomasu and I. Honda. 2009. The endogenous level of GA3 is unregulated by high temperature during stem elongation in lettuce through *LsGA3ox1* expression. J. Plant Physiol. 166: 2077–2084.

Fukuda, M., Y. Yanai, Y. Nakano, H. Sasaki, A. Urugami and K. Okada. 2012. Effects of gibberellin treatment and temperature on onion bolting. Hort. Res. (Japan) 13 (Suppl. 1): 377 (In Japanese).

Jones, R. L. 1973. Gibberellins: their physiological role. Annu. Rev. Plant Physiol. 24: 571–598.

Kebrom, T. H., W. Spielmeyer and E. J. Finnegan. 2013. Grasses provide new insights into regulation of shoot branching. Trends Plant Sci. 18: 41–48.

Lee, I.-J., K. R. Foster and P. W. Morgan. 1998. Effect of gibberellin biosynthesis inhibitors on native gibberellin content, growth and floral initiation in *Sorghum bicolor*. J. Plant Growth Regul. 17: 185–195.

Leyser, O. 2003. Regulation of shoot branching by auxin. Trends Plant Sci. 8: 541–545.

Lo, S.-F., S.-Y. Yang, K.-T. Chen, Y.-I. Hsing, J. A. D. Zeevaart, L.-J. Chen and S.-M. Yu. 2008. A novel class of gibberellin 2-oxidases control semidwarfism, tillering, and root development in rice. Plant Cell 20: 2603–2618.

McSteen, P. and O. Leyser. 2005. Shoot branching. Annu. Rev. Plant Biol. 56: 353–374.

Mita, T. and H. Shibaoaka. 1984a. Gibberellin stabilizes microtubules in onion leaf sheath cells. Protoplasma 119: 100–109.

Mita, T. and H. Shibaoaka. 1984b. Effects of S-3307, an inhibitor of gibberellin biosynthesis, on swelling of leaf sheath cells and on the arrangement of cortical microtubules in onion seedlings. Plant Cell Physiol. 25: 1531–1539.

Murai, M., A. Yoshino, S. Jitsukawa and T. Uchida. 1981. Studies on the tillering factors of Welsh onion (*Allium fistulosum* L.). Bull. Chiba Found. Seed & St. Farm 3: 21–42 (In Japanese).

Nojiri, H., T. Toyomasu, H. Yamane, H. Shibaoaka and N. Murofushi. 1993. Qualitative and quantitative analysis of endogenous gibberellins in onion plants and their effect on bulb development. Biosci. Biotech. Biochem. 57: 2031–2035.

Shibaoka, H. 1993. Regulation by gibberellins of the orientation of cortical microtubules in plant cells. Aust. J. Plant Physiol. 20: 461–470.

Shiraiwa, N., K. Kikuchi, I. Honda, M. Shigyo, H. Yamazaki, D. Tanaka, K. Tanabe and A. Itai. 2011. Characterization of endogenous gibberellins and molecular cloning of a putative gibberellin 3-oxidase gene in bunching onion. J. Amer. Soc. Hort. Sci. 136: 382–388.

Thimann, K. and F. Skoog. 1933. Studies on the growth hormone of plants III: the inhibitory action of the growth substance on bud development. Proc. Natl. Acad. Sci. USA 19: 714–716.

Walker-Simmons, M. 1987. ABA levels and sensitivity in developing wheat embryos of sprouting resistant and susceptible cultivars. Plant Physiol. 84: 61–66.

Yakuwa, T. 1963. Studies on tillering and bulb division in the Genus *Allium*. Mem. Res. Fac. Agr. Hokkaido Univ. 4: 130–214 (In Japanese with English summary).

Yamaguchi, S. 2008. Gibberellin metabolism and its regulation. Annu. Rev. Plant Biol. 59: 225–251.

Yamazaki, H., T. Nishijima, M. Koshioka and H. Miura. 2002. Gibberellins do not act against abscisic acid in the regulation of bulb dormancy of *Allium wakegi* Araki. Plant Growth Regul. 36: 223–229.

Yamazaki, H., T. Yano, K. Nagasuga, K. Inamoto and A. Yamasaki. 2006. Reduction of endogenous level of gibberellins inhibits tillering of Japanese bunching onion. J. Japan. Soc. Hort. Sci. 76 (Suppl. 1): 198 (In Japanese).

Yamazaki, H., T. Yano, K. Nagasuga and A. Yamasaki. 2006. Environmental factor to promote the tillering of Japanese bunching onion. 2. Effects of gibberellin treatment. J. Japan. Soc. Hort. Sci. 75 (Suppl. 1): 360 (In Japanese).