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Changes of Abscisic Acid and Auxin as Related to Dormancy Breaking of *Allium wakegi* Bulblets by Vacuum Infiltration and BA Treatment

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Abstract. Dormant bulblets of the sterile green onion, *Allium wakegi*, broke dormancy and sprouted when infiltrated with water for 1 h at the reduced pressure of 25 kPa or when dipped in 20 ppm of benzylaminopurine (BA) for 1 h at normal (100 kPa) or reduced pressure. One day after the treatment, the abscisic acid (ABA) contents greatly decreased in bulblets treated with BA at both reduced and normal pressure as well as in bulblets treated with water at reduced pressure. The ABA content of bulblets dipped only in water at normal pressure remained relatively high throughout the study. Changes in 2-*trans*-abscisic acid (*t*-ABA) and IAA content of bulblets showed no correlation with breaking of dormancy. Dormancy breaking of *Allium wakegi* bulblets appears to be related to a sharp decrease in ABA content in the bulblets as a result of water infiltration or BA treatment.

Stimulation of germination of dormant seed after treatment with either high (Davies 1926, Erbecke 1944) or low (De Vries 1915) pressure has been studied. De Vries treated *Oenothera* seeds under low pressure and found marked stimulation of germination of dormant seeds. Until now no report has appeared on the effect of pressure on the sprouting of dormant bulbs.

The sterile green onion *Allium wakegi*, an F_1 hybrid between *A. ascalonicum* and *A. fistulosum* (Tashiro 1984), does not form fertile flowers in any season. Under commercial production the green onions form dormant bulblets at the end of spring which sprout in the autumn. After the dormant period, bulblets are planted in fields and, after about 30 days, are harvested and sold at

local markets as green onions. Since dormant bulblets will not sprout if planted in the field during the summer, Hasegawa et al. (1981) studied ways to shorten the dormant period. They found the dormant period could be shortened to some extent by high-temperature treatment (30–40°C). Hasegawa et al. (1981) also found that dormant bulblets sprouted after treatment with cytokinin under vacuum infiltration (unpublished results).

Using bioassay and paper chromatographic technique, Tsukamoto et al. (1969) found that ABA in *Allium cepa* bulbs remained relatively high during the dormant period and decreased during the breaking of dormancy.

Recent progress in instrumental analysis including the use of internal standards has enabled the quantitative determination of IAA and ABA in plant tissues (Akiyama et al. 1983, Sakurai et al. 1984). The present study was undertaken to determine changes in IAA and ABA content in bulblets of *A. wakegi* during the breaking of dormancy after vacuum infiltration treatment with cytokinin.

Materials and Methods

Plant Material and Incubation Conditions

Allium wakegi Araki cv. Kihara-bansei No. 1 was grown in the field at Hiroshima Prefectural Agricultural Experimental Station and harvested on May 10, 1986. Seventeen days after harvest, 100 bulblets (20–25 mm in diameter and 35 mm in height) were dipped in 1 L of 0 or 20 ppm of BA in a 3-L glass desiccator under a reduced pressure of 25 kPa or standard atmospheric pressure (100 kPa) at 22°C for 60 min. The treated bulblets were planted in moistened vermiculite at 24°C in the dark for 1 week. During this period, they were sampled after 1, 2, 4, and 7 days, frozen in liquid nitrogen, and then stored at −70°C until analysis.

To observe the effects of BA and reduced pressure on the growth of bulblets, they were planted in moistened vermiculite for 7 days and transplanted in the field at the Hiroshima Prefectural Agricultural Experimental Station. Twenty days after the bulblets were transplanted, plant height, number of roots, number of tillers, diameter of leaf sheath at the base, fresh weight of leaf and sheath, and fresh weight of root were measured.

For ABA and IAA analysis, four frozen bulbs were cut in half, and the four halves, ~20 g fresh weight, were analyzed. Two analyses were made on each portion, and reproducible results were obtained.

Extraction of IAA and ABA

IAA and ABA in the bulblets were extracted and analyzed according to the method of Sakurai et al. (1985). *t*-ABA, which has less biological activity than ABA, was measured simultaneously. The four half bulblets were blended using an ultrasonic homogenizer (Ika Werk, Breisgau, West Germany, Ultratullax TP 18/10 S2) in 80% ethanol containing 1 nmol indolepropionic acid and 10,500

dpm ^{14}C-ABA (Amersham, Buckinghamshire, U.K., 25.6 mCi/mmol) as the internal standard. The homogenate was filtered, and the residue was again extracted with ethanol. The filtrates were combined and reduced to a small volume (~20 ml).

The pH of the concentrate was adjusted to 3.5 with solid tartaric acid followed by four extractions with petroleum ether. IAA and ABA were extracted from the water layer with diethylether. The diethylether fraction was evaporated, and the residue was dissolved in 10 ml of 5 mM sodium acetate. This solution was combined with 0.5 g of polyvinylpyrollidone. The suspension was stirred for 10 min, filtered, and washed twice with 5 mM sodium acetate. The filtrate was reduced to ~10 ml, after which it was mixed with 17 ml of 100% ethanol.

One gram of DEAE Sephadex A-25 (Pharmacia Fine Chemicals, Uppsala, Sweden) was mixed with 60% ethanol, and the resulting slurry was packed in a glass column (1.0 cm in diameter and bed height of 6 cm). The packed column was washed successively with 0.5, 0.05, and 0.005 M sodium acetate in 60% ethanol. The sample solution (~27 ml) was loaded on the column which was washed with 10 volumes of 5 mM sodium acetate in 60% ethanol before elution of IAA and ABA with 70 ml of 0.5 M NaCl in 60% ethanol.

The eluate from the DEAE A-25 column was evaporated, and the pH of the reduced solution was adjusted to 3.5 with 1 M HCl. A SEP-PAK C-18 cartridge (Waters Associates Co., Milford, Mass., USA) was immersed in 100% methanol for 10 min, then 10 ml of deionized water was passed through the cartridge, which was then equilibrated with 20 mM sodium acetate buffer (pH 3.5) before the sample solution was injected into it. Next, the cartridge was washed with 10 ml of deionized water and eluted with 100% methanol.

HPLC and GLC-ECD Chromatography for Assay of IAA and ABA

IAA was assayed using an HPLC system (Japan Spectroscopic Co., Tokyo, TRI ROTOR) equipped with a fluorometric detector (Japan Spectroscopic Co., FP-100) as reported previously (Akiyama et al. 1983). The HPLC column was a Radial Pak μ-Bondapak C-18 (Waters Associates). For IAA detection and ABA fractionation, the sample was eluted with 25% CH_3CN solution (pH 3.5, 20 mM sodium acetate buffer) at a flow rate of 2 ml/min. The endogenous IAA content was calculated from the ratio of the peak area of indolepropionic acid to that of IAA.

The eluate corresponding to the retention time for ABA was passed through a SEP-PAK C-18 cartridge. After washing the cartridge with deionized water, ABA was eluted with 100% methanol, the methanol was evaporated, and the dried sample was methylated with diazomethane generated with a diazomethane generator (Wheaton Scientific) in ether. After evaporation of the ether, the sample was dissolved in 100 μl of methanol. A 50-μl portion of the methanol solution was injected in the HPLC system to measure the radioactivity of the methylated ABA.

For the HPLC separation, the starting eluant was 25% CH_3CN (pH 3.5, 20 mM sodium acetate buffer) and increased to 50% CH_3CN (pH 3.5, 20 mM

Table 1. Time of sprouting of dormant bulblets of *A. wakegi* after vacuum infiltration and ABA content of solution after bulblet infiltration.

Treatment			
Pressure (kPa)	BA (ppm)	Time of sprouting (days)	ABA content (pmol g^{-1} fresh weight ± SE)
100	0	17.1	10.0 ± 0.4
100	20	6.5	10.0 ± 0.9
25	0	8.5	12.5 ± 0.6
25	20	4.5	14.7 ± 0.6
LSD 1%		1.8	

sodium acetate buffer) using a gradient for 8 min, and then 50% CH_3CN was run isocratically until methylated ABA was eluted. The fraction that corresponded to the retention time (14 min) for methylated ABA was collected and mixed with 10 ml of the scintillation cocktail (Amersham, ACS-II), and the radioactivity was measured using a scintillation counter (Aloka, Tokyo, LSC-701).

A portion of the methylated sample in methanol was introduced into a GLC system (Yanagimoto Manufacturing Co., Kyoto, G-180) equipped with a ^{63}Ni electron capture detector. The temperature of the column was increased from 150 to 230°C at a rate of 5°C/min, and the column was packed with Gaschrom Q (100/120 mesh) coated with 1.5% OV-101. The injector and detector temperatures were 250°C. The nitrogen carrier gas flow rate was 40 ml/min, and the ionized gas (N_2) flow rate was 80 ml/min. *t*-ABA was eluted 1 min after ABA and was effectively separated from ABA.

Results

Dormancy Breaking and Growth of A. wakegi *After Vacuum Infiltration*

Vacuum infiltration and BA treatment both significantly shortened the average number of days for bulblet sprouting (Table 1). The growth of *A. wakegi* for 20 days after planting in the field is shown in Table 2. Plants from bulblets dipped in only water at normal atmosphere pressure showed the least growth and root number among treatments. The number of tillers was not markedly affected by BA or reduced pressure treatments. The diameter of the leaf sheath at its base and the fresh weight of the leaf, the sheath, and the root were significantly increased by reduced pressure treatment either in water or BA solution. Plants from bulblets imbibed with BA solution at normal atmospheric pressure also showed a marked effect of treatment on plant height, number of roots, diameter of leaf sheath at the base, and fresh weight of leaf, sheath, and root.

ABA and IAA Content in the Bulblets After the Treatments

Figure 1 shows changes in the content of ABA, *t*-ABA, and IAA after the dormancy-breaking treatments. The minute decrease in ABA was seen imme-

Table 2. Growth responses of *A. wakegi* 20 days after transplanting in the field (27 days after pressure and BA treatment).

Treatment							
Pressure (kPa)	BA (20 ppm)	Plant height (cm)	Number of roots	Number of tillers	Diameter of leaf sheath at base (cm)	Fresh weight of leaf and sheath (g)	Fresh weight of root (g)
100	0	27.5	29.2	4.5	5.3	10.6	0.96
100	20	34.4	37.8	5.2	9.2	15.2	1.39
25	0	37.8	37.9	5.9	8.8	16.0	1.97
25	20	36.0	35.1	5.6	10.6	14.8	1.23
LSD		3.1[a]	6.4[b]	NS	1.3[a]	2.7[a]	0.4[a]

[a] Significant at 1% level.
[b] Significant at 5% level.

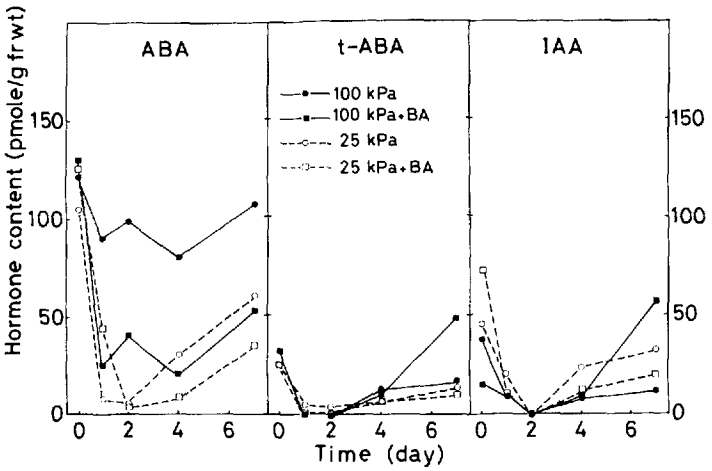

Fig. 1. Changes in ABA, *t*-ABA, and IAA content in bulblets of *Allium wakegi* treated with reduced pressure and BA. Bulbs were treated with 100 (normal) or 25 (reduced) kPa pressure with or without 20 ppm of BA and then planted in moistened vermiculite at 24°C in the dark for 7 days. ABA and IAA contents were measured at 0, 1, 2, 4, and 7 days after the treatment. Standard errors of each measurement were less than 7% of the measured value.

diately after the infiltration at reduced pressure (Fig. 1, at 0 day). One day after treatment, a marked decrease was seen from the BA treatment under both reduced and normal pressure and from the water treatment under reduced pressure.

A gradual increase of ABA in bulblets occurred a few days after the treatment, however. The lowest ABA content was in bulblets treated with BA at the reduced pressure, whereas the ABA content in bulblets treated with water at the normal pressure remained high throughout the experiments.

The finding that *t*-ABA content in the bulblets was far less than that of ABA, along with the fact that treatments did not cause changes in *t*-ABA which did not parallel the degree of breaking dormancy, suggests the importance of *cis*- rather than *trans*-ABA in the phenomenon of breaking dormancy of bulblets. Changes in IAA content in the bulblets did not show a relationship to the physiological effect after the treatment, suggesting a lesser role for IAA in the response.

The ABA content in the infiltration solution immediately after treatment is also shown in Table 1. Only a very minute increase in ABA content was found in the reduced pressure treatment solution.

Discussion

The present study clearly shows that dormant bulblets start to grow immediately after reduced pressure treatment. Furthermore, cytokinin infiltration was evaluated in an effort to apply cytokinins to dormant bud sites which are inter-

nally located in the bulblets. Not only did the cytokinin treatment stimulate the growth of dormant bulbs, but reduced pressure infiltration of water alone also accelerated bud growth. Treatment with cytokinin under reduced pressure gave the most pronounced effect on the breaking of bud dormancy of *A. wakegi*. Dormancy release might be caused by several combinations of effects such as cytokinin-induced bud elongation, decrease in ABA and/or other growth inhibitor in bulblets, and reduced pressure stimulation.

ABA was first shown to be a part of the socalled β inhibitor growth-inhibiting complex (Bennet-Clark and Kefford 1953). Later, Wareing and Saunders (1971) showed that an inhibitor present in dormant buds of the deciduous sycamore and birch trees was correlated with the depth of bud dormancy. Our study using treatment with cytokinin and reduced pressure was successful in breaking *A. wakegi* dormancy and clearly showed a decrease in ABA content concomitant with the stimulation of sprouting. Thus, the treatment of *A. wakegi* bulblets with BA and reduced pressure leads to a decrease in ABA content which, in turn, appears to be associated with breaking of dormancy. The reduced pressure caused only a small increase in the amount of ABA in the treatment solution (Table 1) and a very minute decrease in the ABA content in the bulblets immediately after the vacuum infiltration (Fig. 1). Thus, the marked decrease in ABA content in the bulblets cannot be explained by the transport of ABA from the bulblets into the solution. We concluded that vacuum infiltration with or without BA treatment resulted in by an unknown reaction which is associated with a decrease in the ABA content and dormancy breaking of *A. wakegi*.

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