138. The Change of Amount of Inhibitors Inducing Dormancy in the Dutch Iris Bulb

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Although many studies have been done on flower forcing in Dutch iris (Iris hollandica cv. Wedgwood) by the cooling treatment, references to the problem of endogenous growth regulators in the same plant material have been rather few. It is well known to floriculturists that flower forcing in this plant necessitates a previous break of the dormancy of the bulb before cooling. Kimura and Stuart (1972) carried out experiments to find an appropriate method for breaking dormancy, applying various time-temperature combinations.

The present study was aimed at proving the existence of endogenous growth inhibitors which may regulate the dormancy of Dutch iris bulbs, to identify them chemically, and to clarify the role of high temperature in breaking the dormancy of iris bulbs.

Experiment 1. Bulbs of Dutch iris were obtained from Fukuoka Prefecture on May 30, 1970. They were divided into two lots, each of which was stored at 10°C and 20°C respectively until late August. The bulbs from each lot were bored through the central part containing the main bud, using a cork borer. The bulb columns thus produced were 2 cm in length and 1 cm in diameter. The boring of the bulbs was repeated at weekly intervals from June 7 to the end of August. The total weight of ten columns was about 17 g. These columns were ground into powder while they were cooled with dry ice-methanol. Two grams of the powder were mixed with 90% methanol to extract the inhibitor. The extraction was repeated twice. The ethyl acetate-soluble acidic and neutral fractions were obtained by the procedure shown in Fig. 1. These fractions, which were dissolved in 3 ml of ethyl acetate, were spotted on Toyo No. 51 filter paper and developed with iso-propanol: ammonia: water (10:1:1 v/v). The activities of the endogenous growth inhibitor in 11 segments of the chromatograms were measured using Nitch's straight growth test of Avena coleoptile (Nitch and Nitch 1956) with the modification by an addition of 0.1 γ IAA*) in each test sample. It was shown that the addition of IAA in this test is effective in enlarging the difference among the inhibitions.

*) indole acetic acid
Fig. 1. Extraction procedure for neutral and acidic fractions from iris bulbs.

| Frozen material | extracted with MeOH |
|-----------------|---------------------|
| Extract         | Residue             |
|                 | concentrated in vacuo at 37°C |
|                 | added distilled water |
|                 | adjusted to pH 3.0 with 1N-HCl |
| Aqueous layer   | ethyl acetate layer |
|                 | extracted with 24 sodium bicarbonate |
| Ethyl acetate layer (Neutral fraction) | Aqueous layer |
|                 | adjusted to pH 3.0 with 1N-HCl |
|                 | extracted with ethyl acetate |

Fig. 2. Changes in the amount of inhibitors in the acidic fraction of bulbs of iris stored at 20°C and 10°C.
A comparison of quantitative changes of endogenous acidic inhibitors is shown in Fig. 2. Two inhibitory zones, inhibitors α (Rf. 0.1–0.4) and β (Rf. 0.5–0.7), were clear in both lots until mid-July; then the amount of inhibitor β was slightly greater than that of inhibitor α. A gradual decrease of inhibitor β occurred in the material of the 20°C lot after mid-July, while no changes in inhibitor β were noted in the 10°C lot. In the data on August 9, inhibitor β in the 20°C lot disappeared completely and a small amount of promoter appeared. On the day of assay sampling, bulbs were dissected and the length of the first leaf in the bulb was recorded. The results are shown in Fig. 3. A clear difference between the two lots was recognized. While the first leaf of bulbs stored at 20°C showed remarkable elongation, that of bulbs stored at 10°C elongated only slightly, but with thickening, indicating that new bulbing was occurring. Bulbing continued until the completion of a new bulb formation inside the old scales, which became thin white membranes at 10°C. Newly formed bulbs remained dormant over one year.

The straight growth tests of Avena coleoptile were also carried out with the neutral fraction. The result is shown in Fig. 4. There were also inhibiting substances which decreased completely in the 20°C lot, but did not in the 10°C lot. This means that some inhibitors besides those found in the acidic fraction are involved in the dormancy of the bulb.

Experiment 2. Since it was noticed that inhibitor in the bulbs stored at 20°C, disappeared in early August, and that this disappear-
ance coincided with the elongation of the first leaf formed in Experiment 1, it was tried to obtain inhibitor β from bulb (16 kg) by fractionation with ethyl acetate and the mass paper chromatography in late June, 1971. Crude inhibitor β obtained was purified successively by charcoal and silicic acid-celite adsorption chromatography, and the optical rotatory dispersion of its methanol solution was measured. The result showed that there was a typical cotton effect of ABA.* Then the gas chromatographic analysis was performed, using a Hitachi K53 gas chromatograph. The result of the analysis was that the main inhibitory substance is identified with ABA (Fig. 5).

On the other hand, the inhibitors in the neutral fraction, which was extracted from bulbs (1972) of about 100 kg, were purified by column chromatography. N.M.R., I.R., and Mass spectra were recorded for these substances. The inhibitors in the neutral fraction were identified with capric acid and related compounds.

Discussion and conclusion. Beijer (1952) reported his experimental results obtained from the long term storage of the iris bulb. Bulbs stored at 25.5° and —0.5°C exhibited no growth for a long period. Bulbs stored at a high temperature (25.5°C) were able to be used for forcing without any difficulty, but no flowering resulted

*) abscisic acid
in the bulbs stored at a low temperature (−0.5°C). The data of the present study and of another series of experiments done in the same laboratory (Tsukamoto and Ando, 1973) are in line with that of Beijer. It is interesting to note that the new bulb formation in a mother bulb stored at 10°C for a long period is similar to the pupation of the freesia corm, on which Mansour (1968) conducted an intensive experiment. Aoba (1972) also reported the similar facts found in freesia corm. However, in these papers there was no elucidation of this phenomenon from the standpoint of an endogenous regulator. Judging from the data on the quantitative changes in inhibitors found in the present study, it may be concluded that the breaking of the dormancy of iris bulbs is due to a decrease of inhibitors, which releases the sprouting activity from the dormant state.

The authors clarified that the first inhibitor inducing dormancy is ABA and the second ones are certain fatty acids. Recently, some other inhibitors have been found in the neutral fraction. For example, Hashimoto et al. (1972) reported that neutral inhibitors, bata-tasins, induce the dormancy of bulbils in yam. This is the first information that a fatty acid plays an inhibitory role in dormancy. Tso (1964) studied the inhibitory effect of the methyl ester of a fatty acid on the lateral shoot elongation in the tobacco plant, using a foliar spray. He concluded that the methyl ester of capric acid is the strongest in its inhibitory effect. Poidevin (1965) tested the inhibitory activities of saturated fatty acids on the germination of mustard seeds, and came to the conclusion that only certain fatty acids, which contain 8 to 11 carbon atoms, show inhibitory activity. These results coincide with the data of the present study. Lenton et al. (1972)
reported that there was no relation between the level of ABA and the
dormancy of the sycamore bud. However, the data obtained from our
study show that ABA is responsible for the main influence on the
dormancy of the iris bulb. Of course, the synergistic effect of capric
acid with ABA should also be taken into account. These inhibitors
persisted in the bulbs stored at 10°C, while they decreased in the 20°C
material.

Under natural conditions, the high temperature which causes the
decrease of inhibitors, acts as the essential factor to termination of
dormancy and, successively, to promotion of flower development. This
is generally the case with the autumn sprouting bulbs, such as those
of Dutch iris.

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