大麦における渦遺伝子の内生ジベレリン含量におよぼす影響

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EFFECT OF UZU (*UZ*) GENE ON THE LEVEL OF ENDOGENOUS GIBBERELLINS IN BARLEY

HIROSHI SUGE

National Institute of Agricultural Sciences,
Nishigahara, Kita-ku, Tokyo 114

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The recent introduction of genetic technology into Mexico and several South Asian countries has spawned dramatic changes in their possibilities for increasing agricultural production (Staub and Blase 1971). This so called "green revolution" is tightly associated with the introduction of dwarf or semi-dwarf gene(s) into new varieties of cereal crops.

In Japan, semi-brachytic type called uzu has widely been cultivated in barley acreage especially accompanied with the increased use of nitrogen fertilizer since the varieties of uzu type are, usually, much more resistant to lodging than normal type, called nami in Japan (Takahashi 1964). It was already shown that the uzu varieties have in common a recessive gene for semi-brachytic growth, the so called uzu (*uz*) gene which also exerts pleiotropic effects on the coleoptile, upper most leaf, spike, awns, rachillas and caryopsis (Takahashi 1942; Takahashi and Yamamoto 1951).

Several workers have made efforts for finding a physiological link between the gene deficiencies of dwarf plants and the inhibition of their growth (Phinney 1961; Ogawa 1965; Suge and Murakami 1968; Proano and Greene 1968; Radley 1970; Gotoh 1970). Dwarfness is specially interesting in relation to endogenous gibberellin (GA) metabolism. For these purposes, experiment using two isogenic lines, which differ each other only in single major gene for the dwarfness but are practically the same in the residual genetic constitution or genetic background, will quite be useful. In this report a near isogenic line pair which differs only in the *Uz* and *uz* alleles was used for investigating the endogenous GA relationship in the dwarfness of barley.

MATERIALS AND METHODS

Plant materials: Seeds of a near isogenic line pair were supplied by Dr. R. Takahashi of Okayama University. The near isogenic line pair used here had been developed from a plant heterozygous for the gene pair among the F_8 hybrid population of a cross between Shang-hai No. 3 (nami: normal type) and Kobinkatagi (uzu: semi-brachytic type) by Takahashi and his co-workers (Takahashi *et al*. 1961). Seeds of the isogenic line pair were sown in October 30, 1965 in 1/2000 *are* pots and also in the field of Central Agricultural Experiment Station, Konosu, Saitama. Extraction of GAs from pot grown plants was made 4 times using whole shoots except roots in different growth periods; Nov. 15, Dec. 15, Feb. 24 and Apr. 20. Shoots of 100 g fresh weight material were used for the extraction of GAs. In April 20th, 1966, at the time of flag leaf emergence, field grown plants were harvested and separated into two parts, leafblade and stem (with leaf sheath and young ear) and then 100 g fresh weight material of each plant part was subjected to extraction of GAs.

For studying the effect of 2-chloroethyl trimethylammonium chloride (CCC), an inhibitor of GA biosynthesis (Ninnemann *et al.* 1964), on the growth and GA production in nami isogenic line (*Uz*), seeds were sown in 1/5000 *are* pots in Nov. 2, 1965. In Mar. 16, 1966, 2 g of Cycocel (65% dust of CCC) dissolved in 300 ml of water were poured into the soil. Twenty-five days after the CCC application, plants of 200 g fresh weight were harvested for extraction of GAs.

Extraction and bioassay: Immediately after harvesting, the plants were ground in a mixer with 70% acetone. The resulting brei was shaken for about 8 hr at room temperature and then filtered. The residue was re-extracted once more in a similar manner. The combined filtrates were evaporated under reduced pressure. The resulting aqueous solution, after being adjusted to pH 2.0 with phosphoric acid, was extracted three times with ethyl acetate and the ethyl acetate fraction was then extracted with 1 M phosphate buffer at pH 7.0. This phosphate buffer solution was acidified to pH 2.0 with phosphoric acid and extracted five times with ethyl acetate. The ethyl acetate fraction was dehydrated with anhydrous sodium sulfate overnight and then the solvent was distilled off under reduced pressure. The resulting residue was taken up in a small volume of acetone and was subjected to paper chromatography. Ascending paper chromatography was carried out on Toyo No. 50 filter paper with the mixture of isopropanol/water/ammonia (10:1:1), the solvent front being allowed to travel 30 cm. The developed chromatogram was dried and divided transversely into 15 equal strips. Each strip was eluted with 50% acetone and the eluate was put into a test tube (12.3×

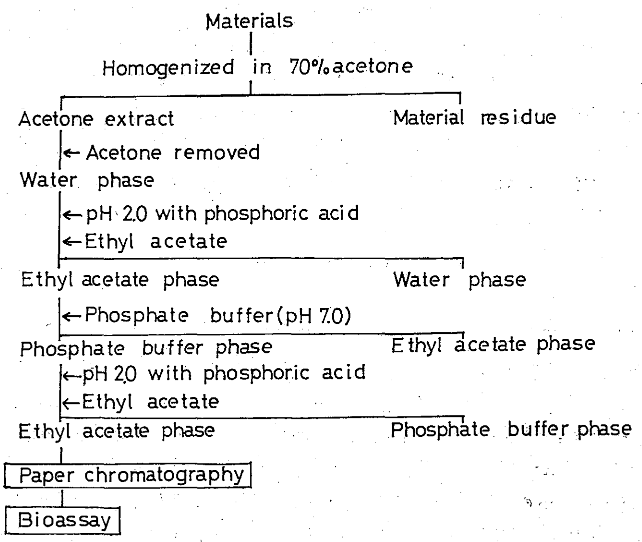

Fig. 1. Method of extraction of gibberellins.

6 cm). Then acetone was dried off and 0.5 ml of water was added. In this test tube, five rice seedlings (dwarf variety "Tan-ginbozu"), whose coleoptile attained about 1 mm, were placed and allowed to grow under artificial light (4000 lux) conditions at 30°C. They were supplied with 0.5 ml water after 3 days. The length of the second leaf sheath was measured after 5 days. Procedure of extraction is shown in Fig. 1.

RESULTS

Actual measurements of coleoptile and first leaf in seedlings with or without GA_3, grown under artificial light (4000 lux) conditions at 30°C for 5 days, were plotted in Fig. 2. Results of Fig. 2 and Fig. 3 demonstrate that the elongation of first leaf of uzu barley was stimulated by GA_3 although that of coleoptile was not influenced by GA_3.

As shown in Fig. 4 amounts of endogenous GAs in uzu (*uz*) plants were found to decrease in about a half to that of nami (*Uz*) plants throughout entire growth period when comparison was made on whole shoots. Analysis of GAs in the time of flag leaf emergence has revealed that there was no detectable difference of the amount in the leaf blades but in stems (including leaf sheath and young ear) amount of GAs was found to decrease in uzu plants about one-third to that of nami plants (Fig. 5). Histograms showing the GA activity in the stems of the isogenic line pair are presented in Fig. 6.

As shown in Fig. 7 and Fig. 8 application of CCC to nami plants reduced both the stem growth and the level of GAs. Plant height of nami plants was decreased to the level of uzu plants by CCC treatment as shown in Fig. 8.

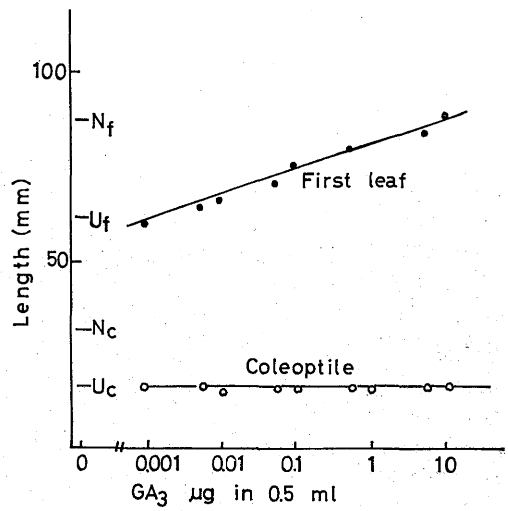

Fig. 2. Effect of gibberellin A_3 on the elongation of coleoptile and first leaf in uzu isogenic line.
U: Uzu isogenic line. N: Nami isogenic line. f: First leaf. c: Coleoptile.

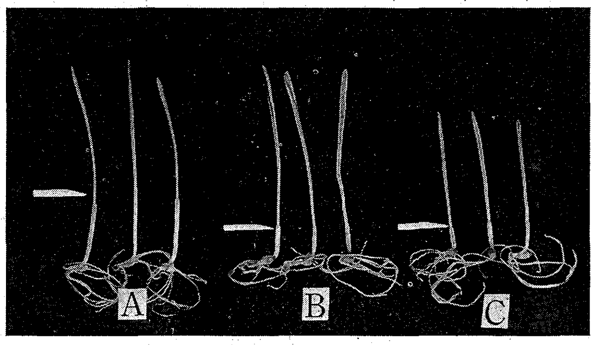

Fig. 3. Fffect of gibberellin A_3 on the elongation of coleoptile and first leaf in uzu isogenic line. White arrows indicate the terminal of coleoptiles.
A: Nami isogenic line. B: Uzu isogenic line grown with gibberellin A_3 (0.5 μg/0.5 ml). C: Uzu isogenic line.

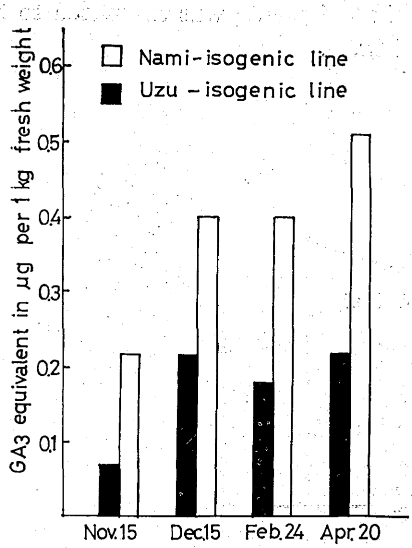

Fig. 4. Comparison of gibberellins in shoots of nami- and uzu-isogenic line at different growth periods.

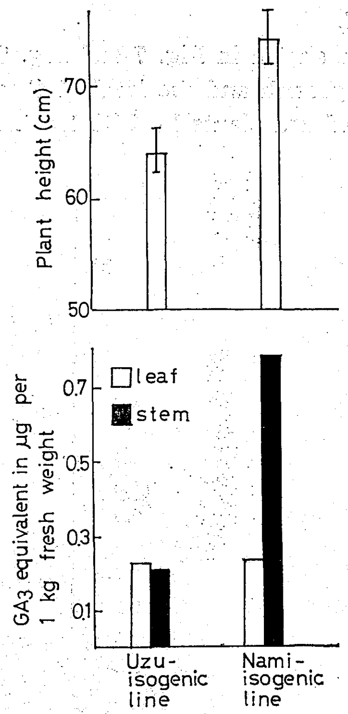

Fig. 5. Comparison of plant height and that of gibberellins in leaves and stems of nami- and uzu-isogenic line at the stage of flag leaf emergence.

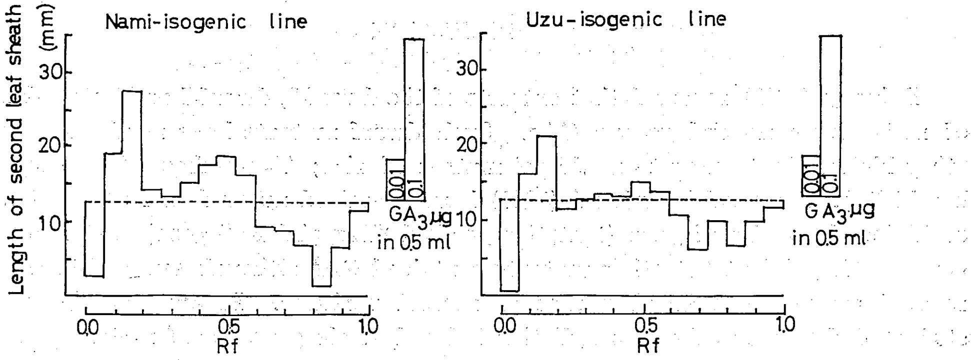

Fig. 6. Histograms indicating gibberellin activities in extracts from stems (including leaf sheaths and young ears) in nami- and uzu-isogenic lines. Dotted lines indicate water control. Extracts from 100 g fresh weight materials.

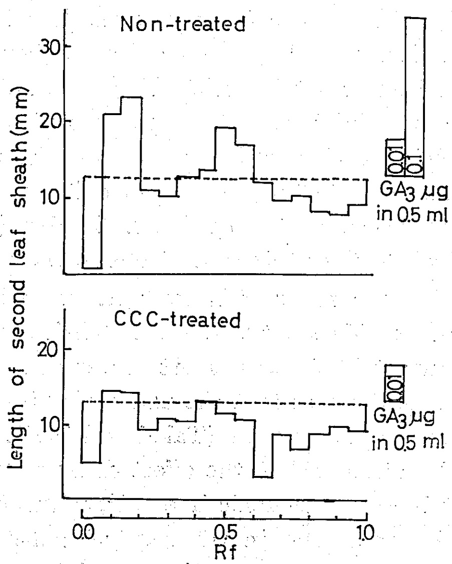

Fig. 7. Histograms indicating gibberellin activities in nami-isogenic line treated with CCC, an inhibitor of gibberellin biosynthesis.

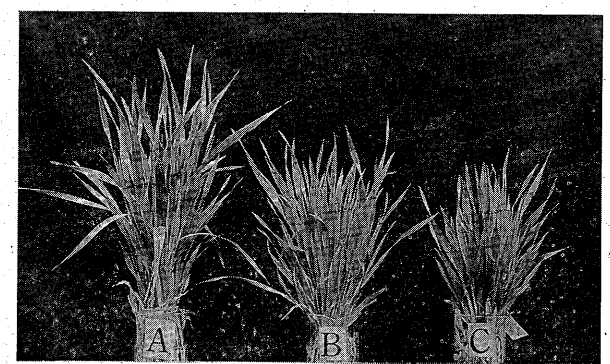

Fig. 8. Effect of CCC application on the growth of nami-isogenic line. A: Nami-isogenic line. B: Uzu-isogenic line. C: Nami-isogenic line treated with CCC.

DISCUSSION

Phinney (1961) reported that extracts of the dwarf-3, dwarf-5 and anther-1 mutants of maize have no endogenous GAs. Such dwarf mutants have not been reported in other higher plants except one dwarf mutant of rice; Tan-ginbozu (Suge and Murakami 1968). Suge and Murakami (1968) have examined many dwarf rice mutamts and could found one dwarf gene (tentatively called "Tan-ginbozu" gene) that makes endogenous GA deficient but other mutants contained GAs although some of them produce reduced amount of GAs than that of normal varieties. Proano and Greene (1968) studied endogenous GAs of a radiation induced single gene dwarf mutant of bean and concluded that the single gene mutation causes a block either in GA precursor formation or in production of non-acidic fraction of GA-like substances.

Dramatic increase of wheat production in Mexico during recent years is tightly associated with the introduction of dwarf genes from "Norin-10", a Japanese wheat cultivar, to new varieties. Radley (1970) compared a number of dwarf wheat cultivar of the "Norin 10" type with several tall forms. Applied GA_3 markedly stimulated the growth of seedlings of the tall cultivars but not the growth of dwarf seedlings. GA_3 caused an increase in soluble carbohydrate in the leaves of the tall cultivars but not in those of the dwarfs. Addition to this, the dwarf cultivars contained more endogenous GA-like activity than those of tall cultivars. Radley (1970) suggested that the dwarf cultivars have a block to the utilisation of GA in the shoot.

In Japan, there are a large number of barley varieties which have in common a recessive gene for uzu or semi-brachytic growth. Their common characteristics are short and thick culms, compact heads with short coarse awns and high resistance to lodging. Most interesting is the fact that the uzu barley is cultivated very widely in Japan occupying 80% of the barley acreage (Takahashi 1964). Reduced production of GAs in uzu barley can be attributed to the effect of *uz* gene since the material used in the present experiment is a near isogenic line pair, which differs only in the *Uz* and *uz* alleles but is practically the same in the residual genetic constitution or genetic background (see Takahashi *et al.* 1961). Application of GA_3 to uzu barley stimulated seedling growth and that of CCC, an inhibitor of GA biosynthesis, to corresponding nami (normal) barley reduced both of stem length and endogenous GA production as shown in the Fig. 7 and Fig. 8. It is very interesting to note that 20 to 25% of the wheat acreage in Germany and Austria is treated with CCC for increasing yield. Treatment of wheat with CCC results in plants of darker green, more upright leaves and thicker, stiffer, and shorter stems that resist lodging. Lodging losses range up to 50% in western Europe since it is related to the high fertility levels and the considerable rainfall during the ripenning-harvesting period, along with the intensity of cultivation practices that produce tall leafy stalk and a tendency to lodge (Wittwer 1971).

It is not known how uzu (*uz*) gene originated and spread widely in Japan but it is plausible to suppose that the uzu barley has arisen spontaneously by mutation from the normal (nami) type barley and happend to be picked up and isolated by some careful, fore-sighted farmer (Takahashi 1964).

Dwarfness in varieties of uzu type, however, may not simply attribute to the reduced production of endogenous GAs since applied GA_3 did not restore of the reduced elongation of coleoptiles in uzu type but brachytic growth habit of this type may partly be due to the interference on GA metabolism. Because of the practical importance and scientific interest of recent introduction of dwarf or semi-dwarf gene(s) into new crop varieties, efforts for finding a physiological link between the dwarf or semi-dwarf gene(s) and the inhibition of growth will be important to search for more effective way to increase crop production.

SUMMARY

A near isogenic line pair which differs only in the *Uz* and *uz* alleles was used for investigating the effect of uzu (*uz*) gene on the level of endogenous gibberellins in barley. Amounts of endogenous gibberellins in uzu plants were found to decrease in about a half to that of nami (normal) plants. Application of gibberellin A_3 to uzu plants stimulated seedling growth and that of 2-chloroethyl trimethylammonium chloride, an inhibitor of gibberellin biosynthesis, to corresponding normal (nami) plants reduced both of stem length and endogenous gibberellin production.

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