UV-B injury in rice plants: A genetic study

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ABSTRACT

Application of an inhibitor of gibberellin biosynthesis alleviated the UV-B injury in rice seedlings when tested for chlorophyll content per unit fresh and dry weight. A genetic study was conducted by crossing Tan-ginbozu, a gibberellin-deficient mutant, against two floating rice cultivars that presumably contain large amounts of endogenous gibberellins. The heritabilities of plant height, and chlorophyll content per unit fresh and dry weight were high in the F₂ plants in non UV-B irradiated control populations. When those plants were treated with UV-B irradiation, the F₂ population tended to shift to the lower range in the chlorophyll content per unit weight, and the value of heritability fluctuated depending on the cross and characters measured. Selection of plants that are highly resistant to UV-B irradiation can be made possible by finding the range of UV-B energy that increases the difference between sensitive and resistant, while also increasing the value of the heritability.

1. INTRODUCTION

Recent ecological studies suggest that stratospheric ozone depletion will cause an increase in the amount of solar ultra violet (UV) radiation that reaches the earth's surface. Since UV-B radiation is known to cause injury and harmful effects to biological systems, it is important to study the biological consequences of an increase in terrestrial UV radiation (Urbach, 1989).

In particular, accumulation of fundamental information about the effect of increased UV radiation on growth and development of cereal crops is a particularly urgent task (Tevini and Teramura, 1989). This task can be divided into two parts. First, it is important to study the basic biological processes, which are damaged by UV-B, and to investigate mechanisms of reducing damage to those processes. Second, the genetic sources of cereals that may survive in, or may adapt to the future environments of the earth must be explored. Tevini and Teramura (1989) noted that genetic selection of varieties that adapt to areas where UV radiation will be the greatest is important. From this viewpoint, rice plants that are adapted to growth in tropical areas are of world-wide interest. Currently, nothing is known about how rice will be affected either by enhanced UV-B or increased temperature and CO₂ (Tevini and Teramura, 1989).

On the other hand, it may be possible to reduce UV injury by the application of

growth retardants which are known to inhibit gibberellin (GA) biosynthesis, and which have been reported to reduce injuries caused by environmental stresses such as drought, frost, low temperature, excess fertilizer, extreme pH and photochemical air pollutants (Cathey, 1964; Cathey and Heggstad, 1972). To explore this possibility, we first examined the effect of an inhibitor of GA biosynthesis on UV-B injury in rice seedlings. Since we found that the application of the inhibitor reduced the UV-B injury, we surveyed the genetic behavior of UV-B sensitivity in F_2 segregants in two rice crosses between a rice mutant deficient in endogenous GAs, and two floating rice cultivars with presumably normal amounts of endogenous GAs (Suge, 1987).

2. MATERIALS AND METHODS

Two floating rice cultivars from Bangladesh, Aswina and Habiganj Aman II (HA II); and a dwarf mutant Tan-ginbozu, which is deficient in GAs (Suge and Murakami, 1968), were used. Seeds of the two floating rice cultivars were soaked in water for 48 hr at 32°C and sown at 15 seeds per cup in plastic cups (8.5×12.5 cm) filled with a commercial soil mixture containing 0.5 g N, 1.5 g P and 0.5 g K per kg of soil (Kureha Engei Baido, Kureha Chemicals, Tokyo). Plants were grown in a green house. When plants reached the two-leaf stage, 6 different concentrations of S-327D, (E)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol, an inhibitor of GA biosynthesis (Izumi et al., 1984), were applied to the soil. Eight days later, plants were subjected to UV-A and UV-B irradiation for 10 days in growth cabinets (Koito-KG type).

Rice plants were grown under 12 h light-12 h dark, at 27° and 17°C, respectively. The light sources and measurement of fluence were as follows: The white light was supplied by a combination of twenty-two 80-watt fluorescent lamps (Toshiba), nine 400 watt extra high pressure mercury lamps (metal-haloid "Yoko", Toshiba) and twelve 200 watt reflector lamps (Toshiba). Altogether, they provided about 9.8 w/m² at plant level.

Each monochromatic band of UV-A and UV-B supplementing the white light was provided by 20 watt tubes of BLB (Toshiba, 20S-BLB) and SE (Toshiba, 20-E). The fluence rate of each light in the region of UV-A (320–400 nm), UV-B (280–320 nm) and UV-C (250–280 nm) was 750, 33 and 4 mw/m² for the white light; 900, 87 and 6 mw/m² for the white light supplemented with BLB; and 755, 192 and 8 mw/m² for the white light supplemented with SE, respectively. The spectral distribution of the latter two lights is shown in Fig. 1. Fluence rate and spectral distribution were measured by a radiospectrometer with a multichannel detector (Hamamatsu Photonics Co.) and a CT-10C diffracting grating monochromator (Japan Spectroscopic Co.) devised by Japan Spectroscopic Co. (Tokyo), allowing measurement of the fluence of light between 250–800 nm with accuracy of 0.01 mw/m²/mm.

Fig. 1. Spectral distribution of the white light in growth cabinets supplemented with either UV-A(upper) or UV-B(lower).

For the control, plants with no application of inhibitor were also subjected to UV-A irradiation. A preliminary test revealed that no injury was detected under the UV-A irradiation. Ten days after the start of UV-B irradiation, the plants were harvested for measurement of chlorophyll content. Ten plants were har-

vested for each dosage of the inhibitor as well as 10 control plants grown under UV-A irradiation. Each plant was cut at ground level and immediately measured for height and fresh weight. The plant was then cut into small pieces and transferred into a test-tube containing 10 ml of 80% ethanol. Chlorophyll in the cut pieces of plant was extracted by gentle boiling, and the extract was made up to the standard volume with 80% ethanol. The optical density of the chlorophyll extract was measured at the wavelength of 673 nm against a blank of 80% ethanol using a Shimadzu UV-120-02 spectrophotometer. The selection of 673 nm for the extract of plants was based on the preliminary test, which showed the highest peak of absorbance at 673 nm.

After drying, the residue was taken up and used for the measurement of dry weight. One sample of HA II (5 mg S-327D) was accidentally lost, so the data lacks the value of this plant.

In the genetic study, F_2 segregants from two crosses, Tan-ginbozu $\times$ Aswina, and Tan-ginbozu $\times$ HA II were used. F_2 seeds were sown in plastic boxes, 15.5 $\times$ 11.0 cm, filled with the same soil mixture mentioned above, with 20 seeds per box. Seeds of parent varieties were also shown as well. Plants were grown in the green house for 10 days before being transferred into the growth cabinet for the UV-B irradiation. UV-B irradiation was started at the five leaf stage and continued for 14 days. Chlorophyll extraction was performed for 142 control and 117 UV-B irradiated F_2 segregants for the cross Tan-ginbozu $\times$ Aswina; For the Tan-ginbozu $\times$ HA II cross, 168 and 154 F_2 segregants in control and UV-B irradiated plants, respectively, were extracted. For the parental varieties, the extraction was done for 25 UV-B irradiated plants in all three varieties, and for 19, 18 and 19 plants in Tan-ginbozu, Aswina and HA II, respectively for the non-irradiated controls.

3. RESULTS

When plants were treated with S-327D, shoot height was significantly reduced (Fig. 2, 3 and 4). S-327D-treated plants resembled Tan-ginbozu, a GA deficient mutant (Suge and Murakami, 1968), especially when the dose of S-327D was increased. Furthermore, UV-B irradiation had the same effect on plant height, without the chemical treatment (Figs. 2, 3 and 4; compare the control and 0 dose in Figs. 3 and 4).

As shown in the photograph (Fig. 2), UV-B irradiation induced both chlorosis and necrosis in rice seedlings. However, application of S-327D, particularly in high dosages, ameliorated these UV-B injuries. Further, neither fresh weight nor dry weight were affected by the application of S-327D in either of two cultivars, although UV-B irradiation alone caused a decrease in both traits. An exception was found in Aswina's dry weight which did not decrease under UV-B irradiation. The chlorophyll content per unit fresh or dry weight was greatly

Fig. 2. UV-B injuries as affected by an inhibitor of GA biosynthesis (S-327D) in two floating rice varieties Aswina (upper) and HA II (lower). From left to right: control plants grown under UV-A irradiation; UV-B irradiated plants treated with S-327D 0, 0.1, 0.5, 1.5 and 10 mg/cup, respectively.

reduced under UV-B irradiation compared with that obtained in plants grown under UV-A irradiation. However, as shown in Fig. 3. the chlorophyll content in Aswina increased with incremental doses of S-327D, to the point when it exceeded

Aswina

Fig. 3. Plant height, fresh or dry weight, chlorophyll content per unit fresh or dry weight and fresh/dry weight ratio as affected by the inhibitor S-327D in UV-B irradiated plants of the floating rice variety Aswina. C: UV-A irradiated only.

the control, i.e., the value in UV-A irradiated plants when the chlorophyll content was calculated on fresh weight basis. When calculated on a dry weight basis it was almost the same for all S-327D doses (Fig. 3). In HA II, the result was different; the degree of UV-B injury was greater in HA II than in Aswina and the chlorophyll content did not recover to the level of the control with the application of S-327D (UV-A irradiation) (Fig. 4). The fresh weight/dry weight ratio was almost constant, that is, it was not affected by the increased doses of S-327D in either cultivars. However, the ratio decreases with UV-B irradiation (Figs. 3 and 4).

Habiganj Aman II

Fig. 4. Plant height, fresh or dry weight, chlorophyll content per unit fresh or dry weight and fresh/dry weight ratio as affected by the inhibitor S-327D in UV-B irradiated plants of the floating rice variety HA II. C: UV-A irradiated only.

Fig. 5 shows the distribution of plant height in F_2 segregants of two crosses, Tan-ginbozu $\times$ Aswina and Tan-ginbozu $\times$ HA II, for two experimental conditions, control and UV-B irradiated. UV-B irradiation caused the distribution of height to shift and concentrate in a shorter range. Tan-ginbozu differed sharply from the other two cultivars of floating rice. The difference persisted under UV-B irradiation.

The chlorophyll content per unit weight of fresh or dry weight in the F_2 population was obtained for each segregant (Figs. 6 and 7). In the non UV-B irradiated control population, the distribution of Tan-ginbozu was clearly separate

Fig. 5. Effect of UV-B irradiation on the distribution of plant height in F_2 segregants from crosses of Tan-ginbozu and floating rice varieties Aswina and HA II. T, A and H indicate parental varieties.

from that of the floating rice cultivars, Aswina and HA II. Under the UV-B irradiation, the distribution of chlorophyll content shifted and concentrated to a lower and more narrow range than the non-irradiated control. The distribution of Tan-ginbozu and that of other cultivars overlapped, although the peaks of Tan-ginbozu and two other cultivars were still distinguishably different.

Fig. 6. Effect of UV-B irradiation on the distribution of chlorophyll content per unit fresh weight in F_2 segregants from two crosses of Tan-ginbozu and floating rice varieties Aswina and HA II.

Using total and environmental variances, the heritability was calculated for both the non UV-B irradiated, and UV-B irradiated populations of F_2 segregants (Table 1). Heritability for plant height was extremely high. The chlorophyll content per unit fresh or dry weight also showed high heritability in F_2 segregants under non-irradiation of UV-B.

As mentioned, plant height is distinctly different between short stature Tan-

Fig. 7. Effect of UV-B irradiation on the distribution of chlorophyll content per unit dry weight in F_2 segregants from two crosses of Tan-ginbozu and floating rice varieties Aswina and HA II.

ginbozu and the two tall floating rice varieties. Under UV-B irradiation, the heritability for chlorophyll content per unit weight, either of fresh or dry weight, was greatly reduced and varied in different crosses. For example, in the cross of Tan-ginbozu $\times$ Aswina, the heritability for chlorophyll content per unit weight depended entirely on the choice of the fresh weight and dry weight base. The heritability 0.446 was obtained in dry weight base, whereas the heritability was only 0.052 in fresh weight base. However, difference was not as great in the cross Tan-ginbozu $\times$ HA II.

Table 1. Variance and heritability values estimated in F₂ populations from two crosses of Tan-ginbozu with floating rice varieties Aswina and HA II

	Cross	Total variance (V _T)	Environmental variance (V _E)	Heritability (V _T -V _E /V _T)
Control				
Plant height	T×A	13885.2	1470.10	0.897
	T×HA	24692.1	2227.11	0.910
Chlorophyll content/	T×A	0.41408	0.08891	0.785
Fresh weight	T×HA	0.45549	0.05986	0.869
Chlorophyll content/	T×A	26.8429	11.7567	0.562
Dry weight	T×HA	33.8723	6.82758	0.798
UV-B irradiation				
Plant height	T×A	7313.08	756.500	0.897
	T×HA	15611.6	898.779	0.942
Chlorophyll content/	T×A	0.29921	0.28372	0.052
Fresh weight	T×HA	0.16077	0.11687	0.273
Chlorophyll content/	T×A	11.9530	6.61910	0.446
Dry weight	T×HA	7.43734	6.47728	0.129

Environmental variance was estimated using the variance of parents and calculated as follows; $(V_{p1} + V_{p2})/2$. T, A and HA indicate Tan-ginbozu, Aswina and Habiganj Aman II, respectively.

We used the correlation coefficient between the fresh weight and dry weight to analyse the difference between the crosses (Fig. 8). Correlation coefficients were very high in both the non-irradiated and UV-B irradiated populations in the cross Tan-ginbozu×HA II. On the other hand, in the cross Tan-ginbozu×Aswina, the correlation coefficient was high only in non-irradiated segregants, while the value decreased under UV-B irradiation, since some segregants showed abnormal behavior and deviated from the line.

Fig. 9 shows the relationship between the chlorophyll content per unit weight and plant height for the UV-B irradiated and non-irradiated populations in the two crosses. The correlation coefficient was low except in the non-irradiated population of Tan-ginbozu×Aswina, where $r = -0.745$.

4. DISCUSSION

Teranaka (1975) reported that irradiation with a peak of 310 nm gave inhibition of growth and appearance of necrosis between the leaf veins in rice plants, while irradiation at 360 nm did not.

In our experiments, UV-A irradiation caused no injury while UV-B gave rise to serious injury as shown in Fig. 2. Application of the growth retardant S-327D, an inhibitor of GA biosynthesis, ameliorated the UV-B injuries, particularly in

Fig. 8. Correlation coefficient between fresh weight and dry weight in F_2 segregants from crosses of Tan-ginbozu with two floating rice varieties as affected by UV-B irradiation.

higher concentrations. However, differences exist in the capability of the growth retardant to relieve injury, since UV-B irradiation caused more damage in HA II than Aswina (Fig. 2). The factors responsible for such differences will be the topic of future study.

Application of growth retardants which are known to be inhibitors of GA biosynthesis have also been known to reduce injuries caused by various environmental stresses including photochemical air pollutants (Cathey, 1964; Cathey and Heggestad, 1972). This study revealed that application of an inhibitor of GA biosynthesis also ameliorates the UV-B injury in rice seedlings. An earlier study

Fig. 9. Correlation coefficient between plant height and chlorophyll content per unit dry weight in F_2 segregants from crosses of Tan-ginbozu and two floating rice varieties as affected by UV-B irradiation.

found that application of S-327D blocks endogenous GA production in the variety Aswina (Suge and Türkan, 1990), we concluded that the retardation of plant height might be due to the inhibition of endogenous GA biosynthesis.

These experiments suggest that inhibition of GA biosynthesis might increase the resistance to UV-B damage. Accordingly, we examined the related genetic behavior of this effect. Two cultivars of floating rice from Bangladesh which crossed with GA deficient Tan-ginbozu, were found to produce large amounts of GAs (Türkan and Suge, unpublished).

Fig. 5 indicates that plant height may be controlled by a single gene, since the

short stature of Tan-ginbozu was controlled by a recessive dwarf gene d_{35} (t). Thus heritability for plant height was still high even under UV-B irradiation (Table 1), indicating that expression of this gene might not be affected seriously, even under UV-B irradiation (Table 1).

Chlorophyll content per unit fresh or dry weight in F_2 segregants showed intermediate distribution between the two parent varieties Tan-ginbozu and one of two floating rice cultivars. No plants were found that exceeded the value of Tan-ginbozu.

Heritabilities for chlorophyll content per unit weight decreased under UV-B irradiation. This might be due in part, to the UV-B energy used here. The heritability also varied between crosses. The observed low heritability might depend on the low value of total variance. Since the genetic variation within the F_2 population is small compared to the value of environmental variation, low heritability may be an indication that the genetic mechanisms of UV-B resistance are not simple. Differences in both physiological and morphological characteristics might have some effects on the segregation of UV-B sensitivity. Decrease of endogenous GA content might be only one of many factors that make plants resistant to UV-B irradiation.

If the energy of UV-B irradiation is high beyond the certain limit of value, genetic variance will decrease and the value of this limit must be different between varieties. This might be one reason why heritability value varied between crosses in this experiment. According to Teramura et al. (1990), however, an increase of UV-B radiation corresponding to a 10% ozone depletion at the equator gave no statistically significant change in photosynthesis, yield and total biomass in rice.

For the purpose of selecting resistant plants to UV-B irradiation, it will be necessary to determine a suitable energy range of UV-B irradiation to increase heritability. In the present study, the distribution of chlorophyll content per unit weight, both on the basis of fresh or dry weight in F_2 segregants, was shifted to the lower side. No segregant was found to exceed the limit of range in Tan-ginbozu. Therefore, at the present level of UV-B energy, selection of segregants that are more resistant than Tan-ginbozu may require large numbers of F_2 segregants. The breeding of a UV-B resistant rice line is not an easy task; before doing actual breeding work, it will be necessary to gather more information regarding fundamental resistance mechanisms to UV-B-induced injury (Caldwell et al. 1983; Gold and Caldwell, 1983; Iwanzik et al., 1983; Teramura, 1983; Teramura et al., 1990).

Application of the inhibitor of GA biosynthesis was found to reduce UV-B injury; however, the possibility exists that this effect was the consequence of making the plants dwarfed in stature. If this is the case the high correlation coefficient between height and chlorophyll content per unit weight, using either of fresh or dry weight, is spurious. A comparatively high correlation coefficient

$r = -0.744$ was found only between plant height and chlorophyll content per unit dry weight in the cross Tan-ginbozu $\times$ Aswina, while no significant correlation was found in three other crosses. Further investigation is underway to resolve this problem.

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