Isolation of C_4 *Flaveria bidentis* mutants with reduced quenching of chlorophyll fluorescence from heavy-ion-beam-mutagenized M_2 population

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Plants using C_4 photosynthesis exhibit higher CO_2 assimilation rates than plants using C_3 photosynthesis under low CO_2 conditions. This C_4 photosynthesis (except for single-cell C_4 photosynthesis) is usually achieved by the operation of the C_4 metabolic cycle between mesophyll (M) and bundle-sheath (BS) cells, which concentrates CO_2 at the site of ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) in BS cells.[1] With an aim to analyze the regulation of light-energy conversion and metabolic cycle between two cells, we screened mutants with a reduced quenching of chlorophyll fluorescence from a heavy-ion-beam-mutagenized M_2 population of C_4 *Flaveria bidentis* (*Asteraceae*) which carries NADP-malic enzyme-type C_4 photosynthesis.

We first investigated the survival ratio of mutagenized seedlings to evaluate the optimum condition for mutagenesis by the irradiation of a carbon-ion beam. *F. bidentis* seeds were irradiated at different dose levels ranging from 25 to 300 Gy with a linear energy transfer (LET) value of 30 keV/ μm (Table 1). The Number of seedlings forming true leaves/total number of seedlings (true leaf formation ratio), average true leaf length in 13-days-old seedlings, and survival ratio 1 month after germination decreased with the increase of dose level. Although the true leaf formation ratio was only slightly affected at 50 Gy, the survival ratio was lower at 50 Gy than at 25 Gy. Therefore, we determined 25 Gy as an optical condition for mutagenesis. A large number of seeds were mutagenized with a 25 Gy carbon-ion beam (M_1) and grown in a green house, and the next population of seeds (M_2) was corrected for screening.

Table 1. Survival ratio in *F. bidentis* M_1 population at different dose levels. Average ±SD is shown for the true leaf length. $*P < 0.01$.

Dose level, Gy	True leaf formation ratio, % (n=50-70)	True leaf length, mm (n=25)	Survival ratio, % (n=50-70)
Non-irradiation	100	3.4 ± 2.2	100
25	97	2.4 ± 1.8 *	94
50	97	3.4 ± 1.8	83
100	59	1.4 ± 3.1 *	10
150	62	1.3 ± 2.0 *	4
200	58	1.4 ± 2.2 *	0
300	3.9	0.3 ± 0.8 *	0

We used a chlorophyll fluorescence imaging system, Maxi-Imaging-PAM (Walz, Germany), for screening mutants. Chlorophyll fluorescence emitted from photosystem II reflects the photosynthetic electron transport

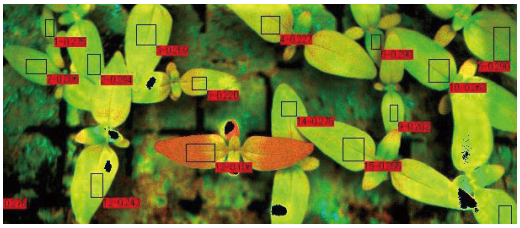

Fig. 1. Chlorophyll fluorescence imaging after exposure to blue light of 500 μmol photons m^{-2} s^{-1} for 1 min. Orange shows lower NPQ levels than green.

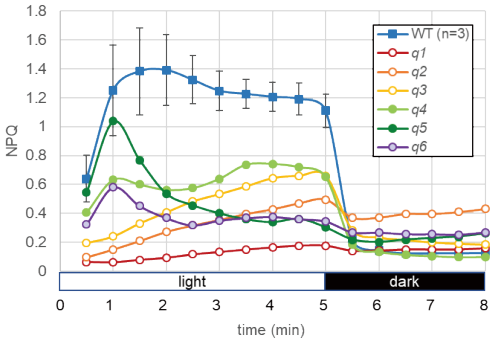

Fig. 2. Time courses of NPQ induction in white-light illumination at 270 μmol photons m^{-2} s^{-1} and relaxation of NPQ in 3 min in dark.

state and light energy dissipation induced by the luminal acidification of the thylakoid membrane, which is monitored as nonphotochemical quenching (NPQ) of chlorophyll fluorescence.[2] In the imaging system, the majority of detected chlorophyll fluorescence was assumed to be derived from chloroplasts in palisade M cells and a minority was assumed to be derived from BS chloroplast because BS cells surround the vasculature immediately beneath the palisade cells within leaves and contain chloroplasts with reduced grana and PSII activity.

Six mutants named *q1*∼*q6* with reduced NPQ were isolated from a population of 860 M_2 plants, which were obtained from 180 M_1 parents (Figs. 1 and 2). *q1*∼*q3* or *q4* and *q5* can possess identical mutation because those were derived from the same parental batch, but at least 3 independent mutants were isolated from this screening system. *q1* and *q4* showed lower CO_2 assimilation rates than the wild type (data not shown). Since coordinated metabolism in M and BS cells is required for C_4 photosynthesis, mutants isolated with this screening method are expected to include defects in not only genes directly related to NPQ induction but also genes related to the regulation of C_4 photosynthesis.

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