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BRIEF COMMUNICATION

Transgenic rice tolerant to high temperature with elevated contents of dienoic fatty acids

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Abstract

Transgenic rice plants in which the content of dienoic fatty acids was increased as a result of co-suppression of fatty acid desaturase were more tolerant to high temperatures than untransformed wild-type plants, as judged by growth rate and chlorophyll content. When untransformed wild-type and transgenic rice seedlings were incubated at 35 °C, seedlings of the transgenic rice lines showed approximately 1.6 and 2.1 times the growth of untransformed wild-type seedlings, as assayed by shoot and root mass, respectively. The chlorophyll content of the transgenic leaves after 9 d at 35 °C was also higher than that of wild-type rice. The maximum photochemical efficiency of photosystem 2 was also higher in transgenic plants than in wild-type plants upon high temperature stress.

Additional key words: chlorophyll content, fatty acid desaturase, heat stress, photochemical efficiency.

Membrane desaturation is closely linked to many biological and physiological phenomena, including the maintenance of chloroplast function, pollen development, tolerance to cold, and production of the plant growth hormone jasmonic acid (Gibson *et al.* 1994, Kodama *et al.* 1995, McConn and Browse 1996, Routaboul *et al.* 2000). Much research has been performed on the role of membrane desaturation in the tolerance of plants to cold, because cold is a detrimental environmental factor that causes severe damage to many agricultural crops (Raison *et al.* 1979). However, only one report has addressed the possible role of high temperature in membrane desaturation. By investigating transgenic tobacco plants with a reduced trienoic fatty acid content, Murakami *et al.* (2000) revealed that decreased contents of trienoic fatty acids play an important role in high-temperature tolerance. Recently, our group has generated seven independent transgenic rice lines that overexpress the chloroplast-localized ω-3 fatty acid desaturase (*FAD7*) of *Arabidopsis thaliana* under the control of the maize ubiquitin promoter (Song *et al.* 2004). As with the transgenic tobacco lines of Murakami *et al.* (2000), in all of the rice lines the total linolenic acid content (18:3) was reduced by about 7 - 32 % and the linoleic acid content (18:2) was increased as compared to wild type, possibly due to silencing of the endogenous rice *FAD* gene(s) by the *Arabidopsis FAD7* transgene. All of the transgenic rice lines were demonstrated to contain a single integrated copy of the *Arabidopsis FAD7* gene, with progeny showing a 3:1 segregation ratio of hygromycin resistance and sensitivity. Two of the seven lines, F1 and F10, were selected for further study due to their 4.3- and 7.3-fold respective increases in relative amounts of linoleic acid (18:2) as compared to wild type. Homozygous transgenic seeds were produced by self-pollination of F1 and F10 T_1 transgenic seeds. This study investigated the involvement of elevated levels of linoleic acid (18:2) in high-temperature tolerance in monocotyledonous transgenic rice plants.

T_2 seeds harvested from self-pollinated F1 and F10 T_1 transgenic plants were sown on half-strength Murashige and Skoog medium containing 50 mg m^{-3} hygromycin to

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Abbreviations: FAD - fatty acid desaturase; F_0 - minimum fluorescence; F_m - maximum fluorescence; F_v - variable fluorescence (F_v = F_m - F_0); F_v/F_m - maximum photochemical efficiency of photosystem 2; NPQ - non-photochemical quenching; qp - photochemical quenching.

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select for homozygous T_2 lines. Homozygous transgenic and untransformed wild-type rice seeds were husked, surface-sterilized, and sown in a Petri dish containing half-strength MS medium. Plates were placed at 28 °C for 3 d in darkness until germination, and then transferred to 35, 37, or 39 °C for 9 d under continuous light [250 μmol(photon) m^{-2} s^{-1}]. The growth rates, chlorophyll contents and photosynthetic activity of the samples were then measured.

Chlorophyll content was measured according to Lichtenthaler (1987). Tissues harvested from seedlings were soaked for 48 h in 10 cm^{-3} dimethyl sulfoxide (DMSO) in darkness at room temperature. The total chlorophyll content in the extracts was determined spectrophotometrically (*Model UV-2401PC, Shimadzu Corp.*, Kyoto, Japan). The activity of PS 2 (F_v/F_m) was measured *in vivo*, using a pulse amplitude modulation fluorometer (*Handy PEA, Hansatech Instruments,* Norfolk, England), after dark adaptation for 10 min to allow relaxation of fluorescence quenching associated with thylakoid membrane energization as described previously (Jung *et al.* 2004).

Shoot and root mass measurements showed clear differences between wild-type and transgenic rice seedlings (Table 1). After 9 d at 35 °C the average shoot mass of wild-type seedlings was about 7 mg, whereas those of the F1 and F10 transgenic lines were around 10 and 12 mg, respectively, 1.5 and 1.8 times higher than in wild type. At 37 °C, seedling growth was severely retarded in both wild and transgenic rice lines. However, the transgenic lines showed better growth than the wild type. At 39 °C, the growth of both wild-type and transgenic seedlings was completely inhibited. High temperature had very similar effects on the fresh masses of seedling roots and shoots (Table 1). A similar high-temperature tolerance as measured by seedling growth was reported for transgenic *Arabidopsis* plants expressing choline oxidase and producing high levels of glycinebetaine (Alia *et al.* 1998).

Table 1. Effect of high temperature on the fresh masses [mg] of shoot and root, chlorophyll (Chl) contents [mg g^{-1} (f.m.)] and Chl fluorescence of wild-type and two transgenic rice lines, F1 and F10. Following germination after 3 d in darkness at 28 °C, the seedlings were maintained at temperature 35, 37 or 39 °C under continuous light (250 μmol m^{-2} s^{-1}) for 9 d and then the above mentioned parameters were measured. The data presented are means of the masses of 10 seedlings with standard error or of the results of three independent experiments with duplicates (Chl). WT - untransformed wild type rice, F1 and F10 - transgenic lines, Chl - chlorophyll content, F_0 - minimum fluorescence, q_p, - photochemical quenching of Chl fluorescence, NPQ - non-photochemical quenching. Within each row of the same temperature, means with the same letter are not significantly different at $P < 0.05$ by Duncan's multiple range test.

	35 °C			37 °C			39 °C		
	WT	F1	F10	WT	F1	F10	WT	F1	F10
Shoot	6.90^a	10.70^b	12.60^b	1.90^a	2.20^a	3.80^b	0.19^a	0.18^a	0.19^a
Root	1.40^a	2.40^b	3.70^c	0.90^a	1.10^a	2.20^a	0.30^a	0.30^a	0.30^a
Chl	0.03^a	0.11^b	0.17^c	0.005^a	0.02^b	0.03^b	0.0001^a	0.0002^a	0.0002^a
F_0	280.00^a	380.00^b	400.00^b	-	-	-	-	-	-
q_P	0.30^a	0.39^b	0.51^c	-	-	-	-	-	-
NPQ	2.81^a	3.28^b	3.85^c	-	-	-	-	-	-

The transgenic lines had also much higher chlorophyll content than the wild type at 35 °C (Table 1). The chlorophyll contents in F1 and F10 lines were 0.11 and 0.17 mg g^{-1}(f.m.), respectively, whereas in wild-type shoots only 0.03 mg g^{-1}(f.m.). In other words, the chlorophyll content of the F1 and F10 transgenic lines was 4.4- and 6.8-fold higher than that of the wild type, respectively, suggesting that inhibition of seedling growth at high temperatures is closely associated with chlorophyll content. Chlorophyll fluorescence parameters of rice leaves were also measured. Minimum fluorescence (F_0) was higher in transgenic lines than in wild type which suggested higher levels of fluorescence occurring when all photosystem 2 reaction centers are open. Photochemical quenching (q_p) and non-photochemical quenching (NPQ) were also higher in transgenic lines relative to those found in wild type.

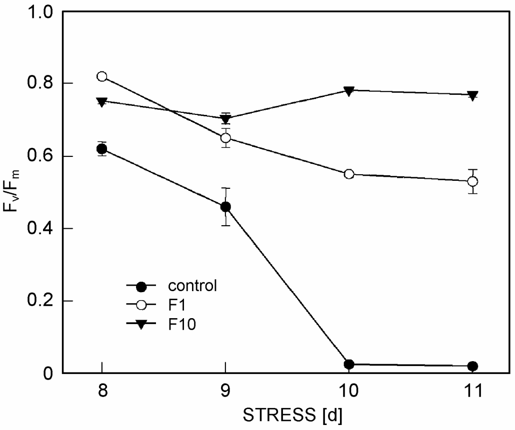

Fig. 1. Changes in variable to maximum fluorescence ratio (F_v/F_m) in the wild-type and transgenic plants upon high temperature stress. 3-d-old seedlings grown at 28 °C were exposed to a high temperature (35 °C) for 8, 9, 10 and 11 d. Data represent the mean ± SE of three samples.

Additionally, the photochemical efficiency of photosystem 2 (F_v/F_m) was measured to show that transgenic plants were resistant to high temperature stress. Wild-type seedlings showed a significant drop in the F_v/F_m, but transgenic plants were not affected by high temperature stress (Fig. 1). These data clearly show that the transgenic lines exhibit greater tolerance to high temperatures than wild type.

Increased amounts of linoleic acid have been reported to be related to the tolerance of dicotyledonous plants to high temperatures, including transgenic tobacco lines expressing *Arabidopsis FAD7* and the *fad7fad8* double mutant line of *Arabidopsis*. The involvement of elevated levels of linoleic acid in high-temperature tolerance in rice plants demonstrates that the mechanism of high-temperature tolerance is conserved between monocotyledonous and dicotyledonous plants. To our knowledge, this is the first report that high-temperature tolerance can be achieved in a monocotyledonous rice plant by elevating the levels of dienoic fatty acids.

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