Plant Biotechnol Rep (2011) 5:169–175
DOI 10.1007/s11816-011-0170-7

ORIGINAL ARTICLE

The use of JIP test to evaluate drought-tolerance of transgenic rice overexpressing *OsNAC10*

Mark C. F. R. Redillas · Reto J. Strasser · Jin Seo Jeong · Youn Shic Kim · Ju-Kon Kim

Received: 8 December 2010 / Accepted: 1 January 2011 / Published online: 22 January 2011

Abstract In this study, the JIP test was exploited to assess drought-tolerance of transgenic rice overexpressing *OsNAC10*. Two types of promoters, *RCc3* (root-specific) and *GOS2* (constitutive), were used to drive the transcription factor *OsNAC10*, a gene involved in diverse functions including stress responses. Three-month-old plants were exposed to drought for 1 week and their fluorescence kinetics was evaluated. Our results showed that drought-treated non-transgenic plants (NT) have higher fluorescence intensity at the J phase (2 ms) compared to transgenic plants, indicating a decline in electron transport beyond the reduced plastoquinone (Q_A^-). As manifested by negative L bands, transgenic plants also showed higher energetic connectivity and stability over NT plants under drought conditions. Also, the pool size of the end electron acceptor at the photosystem I was reduced more in NT than in transgenic plants under drought conditions. Furthermore, the transgenic plants had higher PI_{total}, a combined parameter that reflects all the driving forces considered in JIP test, than NT plants under drought conditions. In particular, the PI_{total} of the *RCc3:OsNAC10* plants was higher than that of NT plants, which was in good agreement with their differences in grain yield. Thus, the JIP test proved to be practical for evaluating drought-tolerance of transgenic plants.

Keywords Chlorophyll *a* fluorescence · JIP test · Transgenic rice · Drought stress · *OsNAC10*

M. C. F. R. Redillas · J. S. Jeong · Y. S. Kim · J.-K. Kim (✉)
School of Biotechnology and Environmental Engineering, Myongji University, Yongin 449-728, South Korea
e-mail: jukon306@gmail.com

M. C. F. R. Redillas
e-mail: markredillas@gmail.com

J. S. Jeong
e-mail: jsjeong74@gmail.com

Y. S. Kim
e-mail: younshic@gmail.com

R. J. Strasser
University of Geneva, 1254 Jussy/Geneva, Switzerland
e-mail: Reto.Strasser@unige.ch

Introduction

Transcription factors in plants often stimulate gene expressions through binding to specific DNA sequences that are responsive to stress. Whenever plants are exposed to stress these factors regulate stress-related functional genes that protect the plants from excessive and further damage subsequently conferring tolerance to stress. Dozens of transcription factors are involved in the plant response to drought stress (Vincour and Altman 2005; Bartels and Sunkar 2005; Oh et al. 2009). In the field of stress-response biology, these functional genes are activated by overexpressing specific transcription factor genes. Several studies have reported that overexpression of stress-related genes could improve drought tolerance in rice to some extent (Garg et al. 2002; Ito et al. 2006; Xu et al. 1996; Nakashima et al. 2007). In a study done by Jeong et al. (2010), overexpression of *OsNAC10* in the roots of rice plants improved its drought-stress tolerance during both the vegetative and reproductive stages. *OsNAC10* is a member of the NAM-ATAF-CUC (NAC) protein family of transcription factors that contain a highly conserved N-terminal NAC-domain and diverse C-terminal region (Jeong et al. 2009).

Drought stress has multiple effects on plants. Most commonly, plants react by a rapid closure of stomata which reduces the CO_2 diffusion rate into the leaf. This leads to a

reduced CO_2 concentration that ultimately limits photosynthetic activity by direct inhibition of the photosynthetic enzyme Rubisco (Haup-Herting and Fock 2000) or ATP synthase (Tezara et al. 1999). Drought stress also inhibits photosynthesis through alterations in the proportion of photochemical and energy-dependent quenching as a result of inhibition of the enzymatic sites that consume ATP and NADPH (Ismaelov et al. 1998). In brief, plants react to stress by means of adaptive mechanisms that allow the photochemical and biochemical system to cope with stress (Yordanov et al. 2000). Either directly or indirectly, stress affects the photosystems of the plants and as a consequence alters the Chl *a* fluorescence kinetics.

Chl *a* fluorescence kinetics has been widely used to assay the responses of the plants under different types of stress. The effects of stress are manifested in the behavior of the fluorescence transients (Baker et al. 1983; Rohacek and Bartak 1999; Sayed 2003). This method is non-invasive, highly sensitive, fast and easily conducted (Baker and Oxborough 2004). Fluorescence kinetics reflects the photosynthetic efficiency of plants and provide wealth of information on the relationship between structure and function of photosystem II (PS II) reaction center (RC) and core complexes (Govindjee 1995; Krause and Weis 1991). The analysis called JIP test developed by Strasser et al. (1995) translates the fluorescence measurements of the transients (O–J–I–P) into several phenomenological and biophysical expressions that quantify PS II function. Each letter designates a distinct inflection in the curve and this allows the quantification of the structural and functional parameters that ultimately reflects the activity of the whole photosynthetic machinery (Strasser et al. 2004).

The principle of the test is based on the "Theory of Energy Fluxes in Biomembranes" (Strasser 1981) and on the basic concept that the fluorescence yield of PS II is determined by the state of the RC, i.e. open or closed. This follows the dogma that, when Q_A in a RC is reduced (Q_A^-), the RC is closed and the chlorophyll fluorescence of the antenna is high, whereas when Q_A is in the oxidized state, the RC is open and the fluorescence of the antenna is quenched, i.e. oxidized Q_A quenches fluorescence. The closing of RCs can be induced by actinic light on dark-adapted plants. Under strong actinic light (e.g., 3,000 μmol photons m^{-2} s^{-1}), the fluorescence intensity F_P (P for the peak) is equal to the maximum fluorescence F_M when all Q_A is reduced (all RCs are closed). The sequential events reflected in the fluorescence rise proceed with different rates and, concomitantly, the rise is polyphasic designated by the letters O–J–I–P (Strasser et al. 2004). In this paper, the Chl *a* fluorescence transients using the JIP test was used to assess drought-tolerance of transgenic plants overexpressing *OsNAC10*.

Materials and methods

Plant materials

The transgenic plants analyzed here were the T_6 generation of plants produced and used previously (Jeong et al. 2010; Jang et al. 1999).

Measurement of the fast chlorophyll *a* transients

Chlorophyll *a* fluorescence transients were measured using the Handy-PEA fluorimeter (Plant Efficiency Analyzer; Hansatech Instruments, King's Lynn, Norfolk, UK) during night time (2100–0300 hours) to ensure sufficient dark adaptation. Two plants were chosen for each of the three independent T_6 homozygous lines. The tallest and the visually healthy-looking leaves were selected for each plant and measured at their apex, middle and base parts. The readings were averaged using the Handy PEA Software (version 1.31).

The Handy-PEA fluorimeter was set using the following program: the initial fluorescence was set as O (50 μs), J (2 ms) and I (30 ms) are intermediates, and P as the peak (500 ms–1 s). The transients were induced by red light at 650 nm of 3,500 μmol photons m^{-2} s^{-1} provided by the 3 light-emitting diodes, focused on a spot of 5 mm in diameter and recorded for 1 s with 12-bit resolution. Data acquisition was set at every 10 μs (from 10 μs to 0.3 ms), every 0.1 ms (from 0.2 to 3 ms), every 1 ms (from 3 to 30 ms), every 10 ms (from 30 to 300 ms) and every 100 ms (from 300 ms to 1 s). The Biolyzer 4HP software (v4.0.30.03.02) was used to analyze the O–J–I–P transients and OriginPro 8 SR0 v9.0724 (B724) was used for the graphs of the transients. The fluorescence O–J–I–P transients were analyzed according to the equations of the JIP test (for review, see Strasser et al. 2004; Yusuf et al. 2010).

Results and discussion

Chlorophyll *a* fluorescence transients and their averaged values of the dark-adapted plants are shown in Fig. 1a, b, respectively. The transients exhibited the typical polyphasic rise O–J–I–P having the same variable fluorescence ($F_M - F_O = F_V$), indicating that the photosynthetic systems of plants were functioning. Double normalization at F_O and F_P (V_{OP}) revealed changes in the shape of the transients between the two end points shown in Fig. 1c. After the onset of illumination of the dark-adapted plants, a rapid increase in the fluorescence at the OJ intermediate steps (2 ms) were observed in plants exposed to drought as shown in Fig. 1d. Note that the non-transgenic (NT) plants had the highest intensity as compared to *GOS2:OsNAC10*

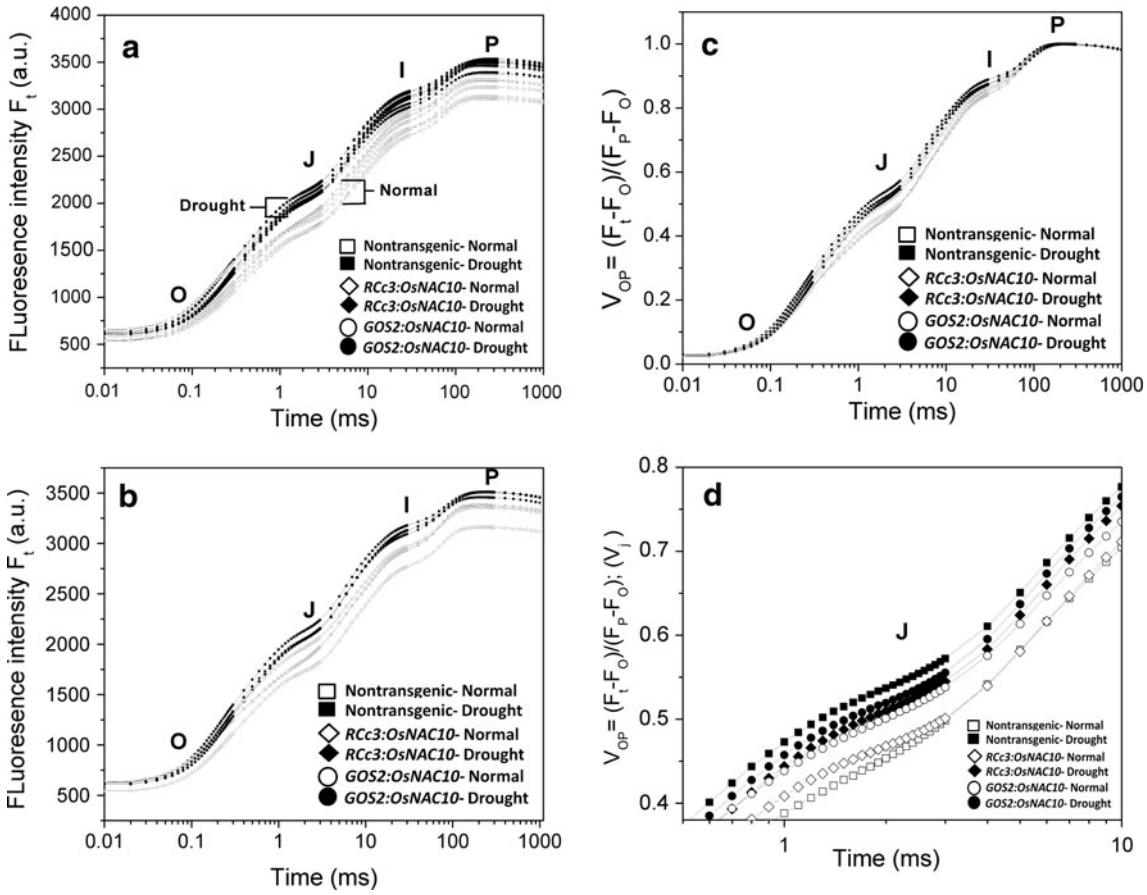

Fig. 1 Polyphasic fluorescence OJIP of chlorophyll *a* exhibited by dark adapted transgenic (*RCc3:OsNAC10* and *GOS2:OsNAC10*) and non-transgenic (NT) plants. **a** Raw fluorescence with *brackets* showing transients of plants under drought conditions (*filled symbols*) plants under normal conditions (*unfilled symbols*). **b** Averaged fluorescence transients for each plant. **c** Double normalization at F_O to F_P phase; $V_{OP} = (F_t - F_O)/(F_P - F_O)$. **d** Differences in fluorescence intensity at the J phase (2 ms) of the V_{OP}

and *RCc3:OsNAC10* plants. Under normal conditions, however, the *GOS2:OsNAC10* plants had the highest intensity followed by the *RCc3:OsNAC10* with NT plants at the bottom. The change in the fluorescence intensity of each plant is interpreted as differences in the reduction of Q_A to Q_A^-, i.e. a single turnover event in the OJ phase. Specifically, high fluorescence intensity occurs due to the decrease in electron transport beyond Q_A^- (Haldiman and Strasser 1999) which results from the accumulation of a fraction of reduced Q_A^- (Munday and Govindjee 1969; Strasser et al. 1995; Lazár et al. 1997). This behavior is a typical response of a plant since the PS II is drought tolerant compared to photosystem I (PS I) and the effects of drought stress is only usually observed in the lowered efficiency of electron transport towards the PS I. At this point, the JIP test was able to discriminate plants grown under normal conditions from those grown under drought conditions and also reflected the degree of effect on the electron transport when the plants are exposed to drought.

To further elucidate the differences between samples, subsequent normalizations and subtractions were carried out. Starting within the OJ phase, differences in the plants fluorescence trace were compared through normalization between F_O (50 μs) and F_K (300 μs). The comparison of the traces of two samples revealed a negative peak on its difference kinetics at about 150 μs (ΔV_{OK} at 150 μs) called the L-band (in alphabetical order I, J, K, L). As shown in Fig. 2, all plants had a negative value confirming the presence of energetic connectivity (or grouping) between the antennas of PS II units (Strasser 1978). Under drought stress, the negative band of *RCc3:OsNAC10* and *GOS2:OsNAC10* plants were more pronounced than NT plants. Many studies indicated that higher energetic connectivity in the photosynthetic machinery leads to a better utilization of the excitation energy and a higher stability of the system (Strasser et al. 2007; Zhu et al. 2005; Chernev et al. 2006; Oukarroum et al. 2007). The increased connectivity is a partially protective mechanism by diverting more excitations energy to photochemistry. This is similar to the photoprotective role of non-photochemical quenching that diverts more excitation energy for heat dissipation (Horton et al. 1996), since at high-light flux, the excited

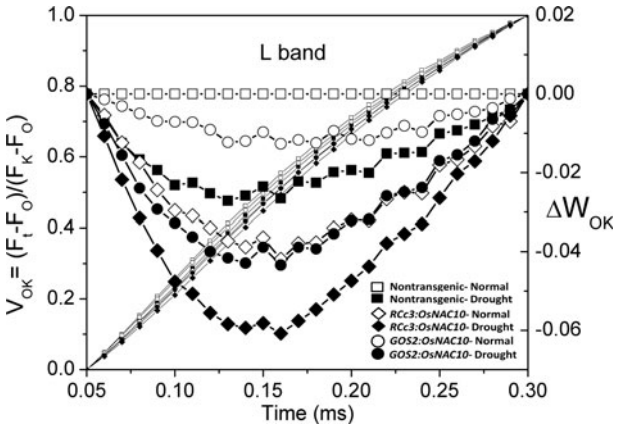

Fig. 2 Double normalization at F_O and F_K; $V_{OK} = (F_t - F_O)/(F_K - F_O)$; *left axis*. The difference kinetics was computed through the equation $\Delta W_{OK} = V_{OKsample} - V_{OKcontrol}$ and plotted at the *right axis* showing the L band (150 μs). *Filled* and *unfilled symbols* represent plants grown under drought and normal conditions, respectively

chlorophylls in core antenna of closed RCs can potentially generate radicals leading to photoinhibition (Long et al. 1994). These findings suggest that the *RCc3:OsNAC10* plants were most stable under drought conditions, followed by the *GOS2:OsNAC10* plants and NT plants as the least.

To characterize PS I from PS II, double normalization at the F_O (50 μs) and F_I (30 ms) was plotted in Fig. 3a. The area formed versus time, between the F_I and F_M of the IP phase, is a measure of the size of the end electron acceptor pool reduced by the PS I. It can be observed that the effect of stress can be manifested through a decline in pool size. In general, drought-treated plants had lower pool sizes compared to plants grown under normal conditions (Fig. 3b). The NT plants, however, was found to be the most sensitive to drought stress due to the drastic decline in pool size compared to transgenic plants.

To quantify the rates of reducing end electron acceptor pool in each plant, the transients were double normalized at F_I and F_P (V_{IP}) as shown in Fig. 4. The rate constants of *RCc3:OsNAC10* plants were higher under normal than in drought conditions. This was further elucidated at the right axis by looking at the difference kinetics (ΔV_{IP}) between F_I and F_P. The amplitude of the negative band around 100 ms of illumination (which represents a given light dose) becomes an estimate of the K_m value for the reduction of end acceptors of PS I. The negative band is proportional to the time and light dosage needed to reach 50% of the rise in the IP phase. Drought-treated *RCc3:OsNAC10* showed the highest amplitude followed by NT while *RCc3:OsNAC10* plants under normal conditions showed the lowest amplitude indicating fastest reduction rate. Collectively, the double normalizations at V_{OI} and V_{IP} collectively illustrated the influence of stress applied on the reduction rates

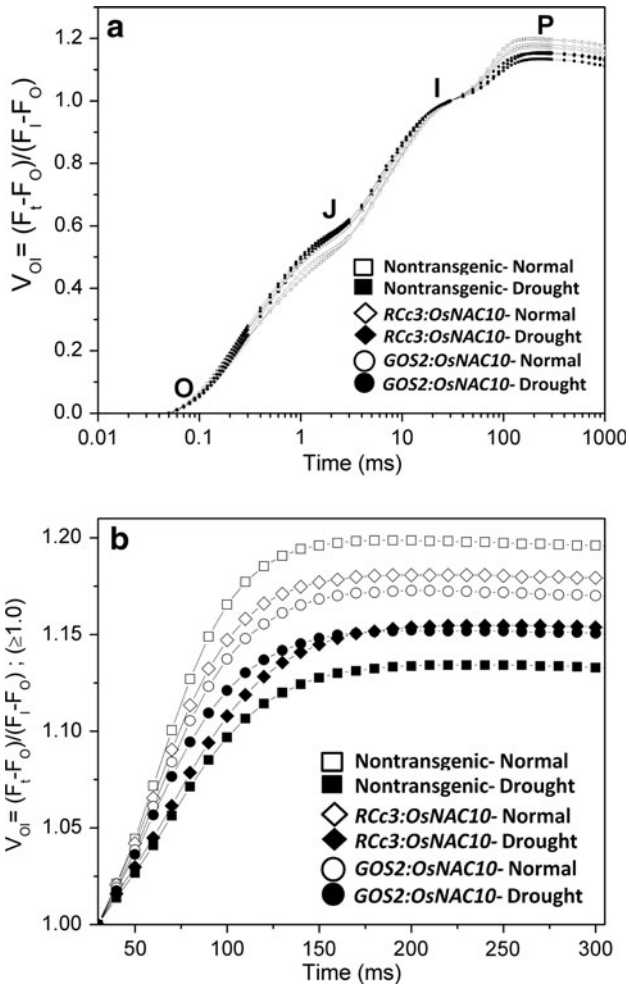

Fig. 3 Double normalization at the F_O to F_I; $V_{OI} = (F_t - F_O)/(F_I - F_O)$. **a** Individual events starting from the photons absorbed (ABS) followed by exciton trapping (TR), electron transport (ET), and to the plastoquinone (PQ) reduction (O–I phase, from 0 to 1) by the PS II; then from the PS I-driven electron transport to the reduction of end electron acceptors (RE) on the PS I acceptor region that started at PQH_2 to Cyt bf complex to PC. **b** Events for $V_{OI} \geq 1.0$ illustrating the differences in the pool size of end electron acceptors

and pool size of the end electron acceptor at the PS I region. From these, the regulation of the reduction of the end electron acceptors was found to be independent from the regulation of the pool size which agreed on the observations reported by Yusuf et al. (2010).

The structural and functional parameters affecting photosynthetic behavior of the plants were also characterized from the fluorescence transients shown in Fig. 5. Here, all the fluorescence traces of the plants were normalized to NT plants grown under normal conditions. Structural parameters are the ratio of rate constants and/or the energy flux ratios expressed per ABS, e.g. as quantum yields (TR_0/ABS), or per electron transport between the two photosystems (ET_0/ABS), or per electron transport reducing the end electron acceptors (RE_0/ABS) of PS I (Fig. 5a). On the

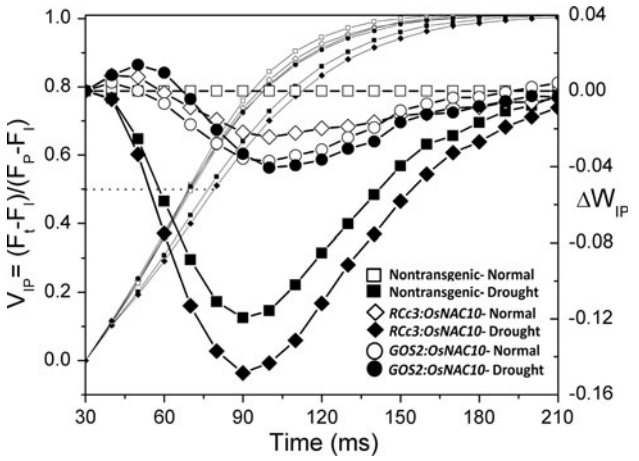

Fig. 4 Comparison in the reduction rates in pool of the end electron acceptor through double normalization at the F_I and F_P (*left axis*); *horizontal dashed line* at 0.5 indicates half time. The difference kinetics at F_I and F_P ($\Delta W_{IP} = V_{IPsample} - V_{IPcontrol}$) is shown at the *right axis*

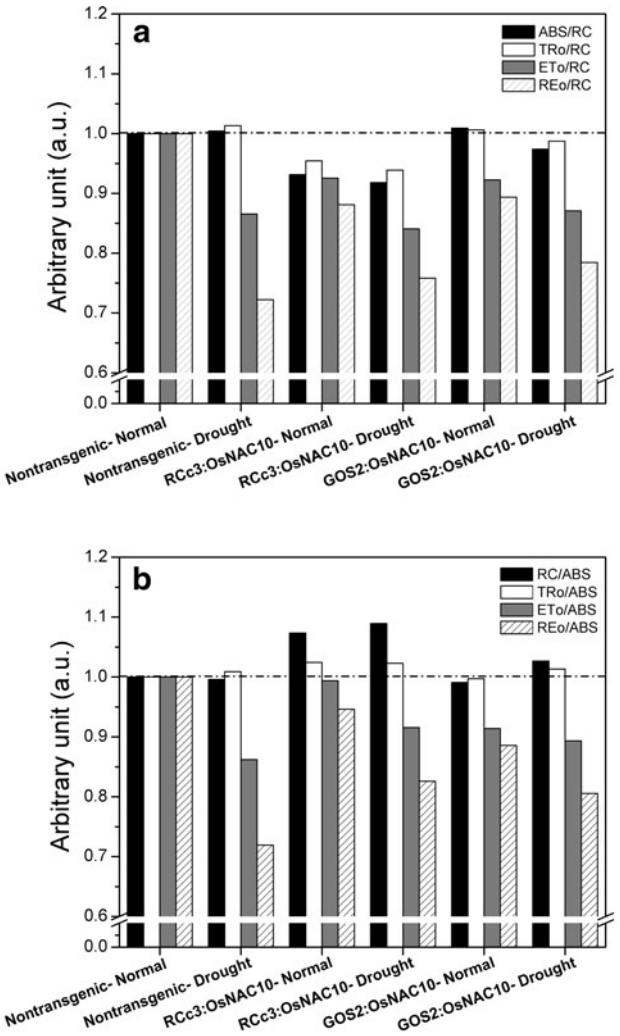

Fig. 5 Photosynthetic parameters derived from the JIP analysis relative to the non-transgenic control plants grown under normal conditions. **a** Specific energy fluxes (per RC) considered as functional parameters: absorption flux (*ABS/RC*), trapping flux (TR_0/RC), electron transport flux (ET_0/RC) and electron flux for reducing end electron acceptors at PS I region (RE_0/RC). **b** Ratio of energy fluxes representing the structural parameters; quantum for primary photochemistry (TR_0/ABS), the quantum yield for the conversion of excitation energy to electron transport (ET_0/ABS) and the quantum yield for the reduction of end acceptors (RE_0/ABS)

other hand, energy fluxes expressed per RC are functional parameters, i.e. specific energy fluxes (Fig. 5b). The parameter ABS/RC (inverse of RC/ABS) is a measure of apparent antenna size (total absorption or total chlorophyll per active RC). The ABS/RC of *RCc3:OsNAC10* plants both under normal and drought conditions declined while *GOS2:OsNAC10* and NT plants were relatively similar to the control. The reduction in ABS/RC can be explained in two ways, either, first, the apparent antenna size is reduced (i.e. the antenna that supplies excitation energy to active RCs has decreased in size) or, second, the active RC (i.e. by being transformed to Q_A-reducing centers) is increased. The first reason presents the closest possible explanation for the reduction in ABS/RC in *RCc3:OsNAC10* plants since no K band was observed (data not shown). The presence of a positive K band may indicate an increase in the functional PS II antenna size as reported by Yusuf et al. (2010). It was also stated by Ballottari et al. (2007) that sun leaves are actively managing the flux of electrons by reducing the antenna size in the PS II and enhancing the controlled dissipation of energy. Thus, in this study, the reduction in functional antenna size may have enhanced the passing of energy to active RCs relative to the control and further supports the claim that under drought conditions the *RCc3:OsNAC10* plants possess an advantage over NT plants in terms of stability and efficiency in utilizing energy.

Another key expression of the JIP test is the TR_0/RC as it expresses the rate, per RC, by which excitons are trapped resulting in the reduction of Q_A to Q_A^-. Under both normal and drought conditions, *RCc3:OsNAC10* plants were lower than *GOS2:OsNAC10* plants while the latter is relatively similar to the control. At this point, it can be said that all the plants had a stable association between energy-absorbing chlorophyll, in the form of active RC, and the trapping of this energy for the reduction of Q_A to Q_A^-. The specific electron flux (ET_0/RC) further than Q_A^- of the plants relative to the control is shown in Fig. 5a. Differences between TR_0/RC and ET_0/RC showed the connection between these components in PS II. Under drought conditions, NT plants showed the highest difference followed by *GOS2:OsNAC10* and *RCc3:OsNAC10* while under normal conditions a lower change was observed both for *GOS2:OsNAC10* and *RCc3:OsNAC10*. From these results,

not only the values per specific energy flux were shown but also the connection between one flux to the next suggesting that transgenic plants under drought conditions were more efficient in the utilization of energy than the NT. The specific energy for the reduction of the end electron acceptors (RE_0/RC) at PS I side showed that under drought conditions transgenic plants *RCc3:OsNAC10* and *GOS2:OsNAC10* were comparably higher than NT. The behavior of the specific fluxes in both transgenic and NT plants together with the changes accompanied due to stress were similar, suggesting that the drought stress affected the same part of the photosynthetic system.

The quantum yields of the plants were also ranked in Fig. 5b. The parameter TR_0/ABS or the maximum quantum yield for primary photochemistry, which is also the commonly used F_V/F_M fluorescence parameter, showed that all the plants had the same maximum quantum yield for primary photochemistry suggesting the insensitivity of this parameter for assessing the drought-treated plants. The same observations in using F_V/F_M for studying the fluorescence test for stressed plants have been reported elsewhere (Oukarroum et al. 2007; Van Heerden et al. 2004). In contrast, the quantum yields for electron transport (ET_0/ABS) relative to the control varied more visibly. Results showed that both the drought-treated transgenic plants exhibited a higher quantum yield for electron transport when compared to the drought-treated NT plant. Furthermore, NT plants had the largest difference from its normally grown counterpart. The quantum yields for the reduction of end electron acceptor (RE_0/ABS) at PS I of transgenic plants remained higher than NT under drought conditions for approximately 10%. In summary, transgenic plants showed to have an advantage compared to NT as shown in the quantification of the structural and functional parameters. To express all the parameters mentioned above in a single form the performance index (PI_{total}) of each plant was plotted in Fig. 6.

The parameter PI_{total} by definition considers the following individual effect on the component parameters: the RC-density in the chlorophyll bed (RC/ABS), the performance due to the quantum efficiency of primary photochemistry $[\varphi_{Po}/(1 - \varphi_{Po})]$, the performance due to the quantum efficiency of the conversion of excitation energy to electron transport $[\psi_{Eo}/(1 - \psi_{Eo})]$ and the performance due to the quantum efficiency of the reduction of end acceptors $[\delta_{Ro}/(1 - \delta_{Ro})]$ (Smit et al. 2009). The PI_{total} of *RCc3:OsNAC10* plants were higher than the control under normal conditions, and during drought conditions it reduced by 22% while the NT plants reduced by as much as 42%. Results suggest that the advantage of *RCc3:OsNAC10* over the NT plant were already present under normal conditions and were able to "utilize" it when exposed to drought stress. On the other hand,

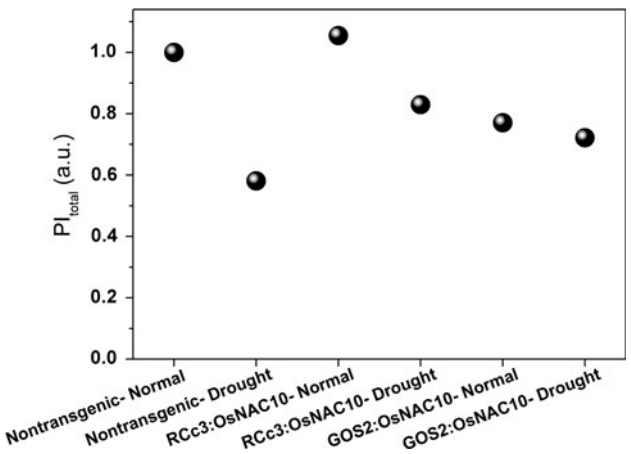

Fig. 6 Performance index (PI_{total}) of the plants relative to the non-transgenic control plants grown under normal conditions

GOS2:OsNAC10 plants under normal conditions had a lower PI_{total} than the control but its drought-treated counterpart, *GOS2:OsNAC10*, were still higher than NT. These findings indicate that NT plants are more sensitive for drought stress compared to the transgenic plants. Studies regarding a strong correlation between physiological parameters such as plant growth, survival rate and PI_{total} have also been reported elsewhere (Strasser et al. 2007; Zubek et al. 2009).

In summary, the use of the JIP test to assay drought-stressed transgenic plants at their vegetative stage was very useful and informative. Both transgenic plants showed an advantage over NT under drought conditions in terms of stability and efficiency in utilizing energy. Considering all the driving forces represented by a single parameter PI_{total}, transgenic plants showed superiority over NT under drought conditions and *RCc3:OsNAC10* plants "outperformed" *GOS2:OsNAC10* plants. In a field test, the overexpression of the transcription factor *OsNAC10* in roots driven by the *RCc3* promoter enhanced the tolerance of transgenic rice under drought conditions compared to the whole-body expresser *GOS2* and to NT plants as reported by Jeong et al. (2010).

Acknowledgments This study was supported by the Rural Development Administration under the research project entitled "Cooperative Research Program for Agriculture Science and Technology Development" (Project No. PJ906910) and the support of the Ministry of Education, Science and Technology through the project entitled "Mid-career Researcher Program" (Project No. 20100026168).

References

Baker NR, Oxborough K (2004) Chlorophyll fluorescence as a probe of photosynthetic productivity. In: Papageorgiou GC, Govindjee (eds) Chlorophyll *a* fluorescence: a signature of photosynthesis. Springer, Dordrecht, pp 65–82

Baker NR, East TM, Long SP (1983) Chilling damage to photosynthesis in young *Zea mays*. J Exp Bot 34:189–197

Ballottari M, Dall'Osto L, Morosinotto T, Bassi R (2007) Contrasting behavior of higher plant photosystem I and II antenna systems during acclimation. J Biol Chem 282:8947–8958

Bartels D, Sunkar R (2005) Drought and salt tolerance in plants. Mol Breed 5:493–503

Chernev P, Golstev V, Zaharieva I, Strasser RJ (2006) A highly restricted model approach quantifying structural and functional parameters of photosystem II probed by the chlorophyll *a* fluorescence rise. Ecol Eng Prot 2:19–29

Garg AK, Kim JK, Owens TG, Ranwala AP, Choi YD, Kochian LV, Wu RJ (2002) Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. Proc Natl Acad Sci USA 99:15898–15903

Govindjee (1995) Sixty-three years since Kautsky: chlorophyll *a* fluorescence. Aust J Plant Physiol 22:131–160

Haldiman P, Strasser RJ (1999) Effects of anaerobiosis as probed by the polyphasic chlorophyll *a* fluorescence rise kinetic in pea (*Pisum sativum* L.). Photosynth Res 62:67–83

Haup-Herting S, Fock HP (2000) Exchange of oxygen nd its role in energy dissipation during drought stress in tomato plants. Physiol Plant 110:489–495

Horton P, Ruban AV, Walters RG (1996) Regulation of light harvesting in green plants. Annu Rev Plant Physiol Plant Mol Biol 47:655–684

Ismaelov MA, Zulfugarov IS, Aliev JA (1998) The effect of water deficit on wheat thylakoid membranes. ISPT congress abstract

Ito Y, Kastura Y, Maruyama K, Taji T, Kobayashi M, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2006) Functional analysis of *DREB1/CBF*-type transcription factors involve in cold-responsive gene expression in transgenic rice. Plant Cell Physiol 47:141–153

Jang IC, Nahm BH, Kim JK (1999) Subcellular targeting of green fluorescent protein to plastids in transgenic rice plants provides a high-level expression system. Mol Breed 5:453–461

Jeong JS, Park YT, Jung H, Park SH, Kim JK (2009) Rice NAC proteins act as homodimers and heterodimers. Plant Biotechnol Rep 3:127–134

Jeong JS, Kim YS, Baek KH, Jung HR, Ha SH, Choi YD, Kim MK, Reuzeau C, Kim JK (2010) Root-specific expression of *OsNAC10* improves drought tolerance and grain yield in rice under field drought conditions. Plant Physiol 153:185–197

Krause GH, Weis E (1991) Chlorophyll fluorescence and photosynthesis—the basics. Annu Rev Plant Physiol Plant Mol Biol 42:313–349

Lazár D, Llik P, Nauš J (1997) An appearance of K-peak in fluorescence induction depends on the acclimation of barley leaves to higher temperatures. J Lumin 72–74:595–596

Long SP, Humphries SW, Falkowski PG (1994) Photoinhibition of photosynthesis in nature. Annu Rev Plant Physiol Mol Biol 45:633–662

Munday JCM, Govindjee (1969) Light-induced changes in the fluorescence yield of chlorophyll *a* in vivo III. The dip and peak in the fluorescence transient off *Chlorella pyrenoidosa*. Biophys J 9:1–21

Nakashima K, Trans LS, Van Nguyen D, Fujita M, Maruyama K, Todaka D, Ito Y, Hayashi N, Shinozaki K, Yamaguchi-Shinozaki K (2007) Functional analysis of a NAC-type transcription factor OsNAC6 involved in abiotic and biotic stress-response gene expression in rice. Plant J 51:617–630

Oh SJ, Kim Ys, Kwon CW, Park HK, jeong JS, Kim JK (2009) Overexpression of transcription factor *AP37* in rice improves grain yield under drought conditions. Plant Physiol 150:1368–1379

Oukarroum A, Madidi SE, Schansker G, Strasser RJ (2007) Probing the responses of barley cultivars (*Hordeum vulgare* L.) by chlorophyll *a* fluorescence OLKJIP under drought stress and re-watering. Environ Exp Bot 60:438–446

Rohacek K, Bartak M (1999) Technique of the modulated chlorophyll fluorescence: basic concepts, useful parameters, and some applications. Photosynthetica 37:339–363

Sayed OH (2003) Chlorophyll fluorescence as a tool in cereal crop research. Photosynthetica 41:321–330

Smit MF, Van Heerden PDR, Pienaar JL, Weissflog L, Strasser RJ, Kruger GHJ (2009) Effect of trifluoroacetate, a persistent degradation product of fluorinated hydrocarbons, on C3 and C4 crop plants. Plant Physiol Biochem 47:623–634

Strasser RJ (1978) The grouping model of plant photosynthesis. In: Akoyunoglou G, Argyroudi-Akoyunoglou JH (eds) Chloroplast development. Elsevier/North-Holland Biomedical Press, New York, pp 513–524

Strasser RJ (1981) The grouping model of plant photosynthesis: heterogeneity of photosynthetic units in thylakoids. In: Akoyunoglou G (ed) Photosynthesis III. Structure and molecular organization of the photosynthetic apparatus. Balaban International Science Services, Philadelphia

Strasser RJ, Tsimilli-Michael M, Dangre D, Rai M (2007) Biophysical phenomics reveals functional building blocks of plants systems biology: a case study for the evaluation of the impact of mycorrhization with *Piriformospora indica*. In: Varma A, Oelmuler R (eds) Advanced techniques in soil microbiology. Soil biology series, vol I. Springer, Berlin, pp 319–341

Strasser RJ, Srivastava A, Govindjee (1995) Polyphasic chlorophyll *a* fluorescence transient in plants and cyanobacteria. Photochem Photobiol 61:32–42

Strasser RJ, Tsimilli-Michael M, Srivastava A (2004) Analysis of chlorophyll *a* fluorescence transient. In: Papageorgiou GC, Govindjee (eds) Chlorophyll *a* fluorescence: a signature of photosynthesis. Springer, Dordrecht, pp 321–362

Tezara W, Mitchell VJ, Driscoll SO, Lawlor OW (1999) Water stressinhibits plant photosynthesis by decreasing coupling factor andATP. Nature 401:914–917

Van Heerden PDR, Strasser RJ, Krüger GHJ (2004)Reduction of dark chilling stress in N2-fixing soybean by nitrate as indicated by chlorophyll a fluorescence kinetics. Physiol Plant 121:239–249

Vincour B, Altman A (2005) Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. Curr Opin Biotechnol 16:123–132

Xu D, Duan X, Wang B, Hong B, Ho T, Wu R (1996) Expression of a late embryogenesis abundant protein gene, *HVA1*, from barley confers tolerance to water deficit and salt stress in transgenic rice. Plant Physiol 110:249–257

Yordanov I, Velikova V, Tsonev T (2000) Plant responses to drought, acclimation, and stress tolerance. Photosynthetica 38:171–186

Yusuf MA, Kumar D, Rajwanshi R, Strasser RJ, Tsimilli-Michael M, Govindjee Sarin NB (2010) Overexpression of γ-tocopherol methyl transferase gene in transgenic *Brassica juncea* plants alleviates abiotic stress: physiological and chlorophyll *a* fluorescence measurements. Biochim Biophys Acta 1797:1428–1438

Zhu XG, Govindjee, Baker NR, deSturler E, Ort DR, Long SP (2005) Chlorophyll *a* fluorescence induction kinetics in leaves predicted from a model describing each discrete step of excitation energy and electron transfer associated with Photosystem II. Planta 223:114–133

Zubek S, Turnau K, Tsimilli-Michael M, Strasser RJ (2009) Response of endangered plant species to inoculation with arbuscular mycorrhizal fungi and soil bacteria. Mycorrhiza 19:113–123