Effects of physiological integration on photosynthetic efficiency of *Trifolium repens* in response to heterogeneous UV-B radiation

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Abstract

Several studies have found the photosynthetic integration in clonal plants to response to resource heterogeneity, while little is known how it responses to heterogeneity of UV-B radiation. In this study, the effects of heterogeneous UV-B radiation (280–315 nm) on gas exchange and chlorophyll fluorescence of a clonal plant *Trifolium repens* were evaluated. Pairs of connected and severed ramets of the stoloniferous herb *T. repens* were grown under the homogeneity (both of ramets received only natural background radiation, *ca.* 0.6 kJ m^{-2} d^{-1}) and heterogeneity of UV-B radiation (one of the ramet received only natural background radiation and the other was exposed to supplemental UV-B radiation, 2.54 kJ m^{-2} d^{-1}) for seven days. Stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and transpiration rate (E) showed no significant differences in connected and severed ramets under homogenous and heterogeneous UV-B radiation, however, net photosynthetic rate (P_N) and maximum photosynthetic rate (P_{max}) of ramets suffered from supplemental increased UV-B radiation and that of its connected sister ramet decreased significantly. Moreover, additive UV-B radiation resulted in a notable decrease of the minimal fluorescence of dark-adapted state (F_o), the electron transport rate (ETR) and photochemical quenching coefficient (q_P) and an increase of nonphotochemical quenching (NPQ) under supplemental UV-B radiation, while physiological connection reverse the results. In all, UV-B stressed ramets could benefit from unstressed ramets by physiological integration in photosynthetic efficiency, and clonal plants are able to optimize the efficiency to maintain their presence in less favourable sites.

Additional key words: environmental heterogeneity; physiological integration; *Trifolium repens*; ultraviolet-B radiation.

Introduction

Ramets of clonal plants connected by rhizomes or stolons can share water, carbohydrates and nutrients through physiological integration (Alpert and Mooney 1986, Marshall 1990, Stuefer *et al.* 1994, Wijesinghe and Hutchings 1997, Saitoh *et al.* 2006). Many studies have shown that clonal integration facilitates establishment of newly produced ramets, improves survival, growth and/or reproduction of adult ramets in stressful environments, and helps genets to occupy open space (Hartnett and Bazzaz 1983, Brewer and Bertness 1996, Yu *et al.* 2002, 2009, Chen *et al.* 2010, Du *et al.* 2010). Plants and habitats are heterogeneously distributed in ecosystems, clonal integration confers a competitive advantage in heterogeneous environments (Wijesinghe and Hutchings 1997). A few studies have expanded the concept of integration in clonal plants to include the responses not only to resource heterogeneous microsites (Zhang and He 2009) but also to stressful heterogeneity, *e.g.* serpentine soils (Roiloa and Retuerto 2006a,b) and sand burial (Yu *et al.* 2004, Chen *et al.* 2010).

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Abbreviations: AQY – apparent quantum yield; C_i – intercellular CO_2 concentration; Chl – chlorophyll; E – transpiration rate; ETR – electron transport rate; $\Delta F/F_m'$ – the PSII photochemical efficiency; F_m – maximum fluorescence of dark-adapted state; F_m' – the steady-state maximum fluorescence yield; F_o – minimal fluorescence of dark-adapted state; F_s – steady-state fluorescence; F_v/F_m – maximum photochemical efficiency of PSII; g_s – stomatal conductance; LCP – light-compensation point; LRC – light-response curves; LSP – light-saturating point; NPQ – nonphotochemical quenching; P_{max} – light-saturated photosynthetic rate; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; PSII – photosystem II; q_P – photochemical quenching coefficient; R_D – dark respiration rate; Φ_{PSII} – actual photochemical efficiency of PSII.

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Photosynthesis is a very important process in the overall plant performance. To understand the ecology of clonal plants, it is important to reveal the ways in which they colonize space and overcome establishment risks (Roiloa and Retuerto 2006b). Photosynthesis and photosynthetic capacity are progressively reduced under various stressful conditions, *e.g.* water stress (Flexas *et al.* 2004, Lawlor and Tezara 2009), salinity (Lawlor 2009) and serpentine soils (Roiloa and Retuerto 2006a). In recent years, Chl fluorescence measurements have become a widely used method to study the functioning of the photosynthetic apparatus and are a powerful tool to study the plants' response to environmental stress (Pankovic *et al.* 1999, Maroco *et al.* 2002, Ennahli and Earl 2005, Massacci *et al*. 2008). In the previous studies of physiological integration, integration effects were generally often assessed in terms of survival and biomass growth, and little has been done to examine the photosynthetic efficiencies (but *see* Roiloa and Retuerto 2006b, Wang *et al*. 2008, Zhang and He 2009, Chen *et al.* 2010). Roiloa and Retuerto (2006b) found that the maximum and effective quantum yields of PSII in parent ramets growing in heterogeneous (offspring ramets in poor-quality soil) environments were significantly higher than in parent ramets growing in homogeneous (offspring ramets in fertile soil) environments, showing that *Fragaria vesca* L. growing in soil heterogeneity (stressed environment) adopt the risk-sharing rather than self-insurant strategy.

UV-B radiation can result in deleterious effects on many plant processes because it is strongly absorbed by many macromolecules and causes their conformational changes, especially in proteins and nucleic acids (Bassman *et al.* 2002). Environmental UV-B radiation is highly variable in spatial and temporal distributions. It is controlled by a great deal of factors, such as changes in the solar elevation with latitude, time of day, season and clouds. The heterogeneity of UV-B radiation exists even in microenvironment (Madronich *et al.* 1998, Grant *et al.* 2005), for example, Caldwell and Pearcy (1994) found millisecond time-resolved changes in light intensity in forest understory spot. To countermine the heterogeneous UV-B radiation in microscale, it might be important for clonal plants to acclimate to this stressful environment by a well regulated physiological integration. We have found the intensity of physiological integration of *T. repens* L. for N and water resources decreased under heterogeneous UV-B radiation in favor of the stressed ramets and UV-B stressed ramets benefited from unstressed ramets by physiological integration (changes in physiological parameters) (Li *et al.* 2011). However, there have been no studies of photosynthetic integration in clonal plants growing under heterogeneous UV-B radiation.

T. repens is a perennial herb that can be distributed patchily in a mixed pasture of grass species (Williams 1987), and shows high sensitivity to ambient southern hemisphere UV-B levels in the field, and cultivars bred for agricultural performance were sensitive to UV-B (Lindroth *et al.* 2000, Hofmann *et al.* 2003). In this paper, the homogeneity and heterogeneity of UV-B radiation were simulated and two experiments, connecting and severing, were established to determine how the photosynthetic efficiencies of a clonal plant like *T. repens* response to deleterious heterogeneous environment when its ramets are located at different UV-B patches.

Materials and methods

Plants and propagation: The experimental material was obtained from original plants (*T. repens*, cv. C615) collected from the campus of Northwest University in Xi'an (34.3°N, 108.9°E, 397 m a.s.l.). These plants, that might or might not differ in genotype, were vegetatively propagated in a glasshouse, at least three vegetative generations to minimize potential carry-over effects of their previous environments.

Experimental design: The experiment used a factorial design involving heterogeneous radiation (with or without) and stolon connection (intact or severed). Pairs of new, similarly sized ramets that were adjacent to each other along a stolon were rooted in plastic trays (60 × 40 × 20 cm, length × width × height) filled with a soil mixture of 25% sand, 25% organic matter, and 50% peat. Plants were grown at 25°C, 40–60% relative humidity, 14/10 h day/night rhythm with a photosynthetic photon flux density (PPFD) of 200 μmol m^{-2} s^{-1} provided by *Osram* lamps. Trays were watered once every other week, each tray was divided into two sections, nutrients, water and roots in the two sections were the same and did not interfere with each other. The clonal ramets were randomly assigned (Table 1). The experiment lasted from March 2009 to June 2010.

Intact, ramets in a pair left connected by the stolon between them; severed, ramets disconnected by cutting the stolon. Both ramets of the group of homogeneous UV-B radiation were kept under no additional UV-B conditions. For the group of heterogeneous UV-B radiation, one of the ramets (HeterCII and HeterSII) was exposed to supplemental UV-B radiation (8 hours per day from 9:00 to 17:00 for 7 days) centered around solar noon using *Q-PANEL* UV-B fluorescent lamps (40 W, *Qing*, Baoji, China) following the procedure outlined in Yue *et al.* (1998). Lamps were suspended above and perpendicular to the planted rows (rows oriented in an east-west direction to minimize shading) and filtered with 0.13 mm cellulose diacetate foil to remove UV-C radiation. 0.13 mm polyester plastic films (absorbing all radiation below 320 nm) were set vertically between the two ramets to avoid scattered UV-B radiation. The

Table 1. Experimental design. The ramets of *Trifolium repens* were randomly divided into four treatments, two intact and two severed ones (intact, ramets in a pair left connected by the stolon between them; severed, ramets disconnected by cutting the stolon). Homogeneity represents that both ramets in a clonal fragment (ramet pair) were grown under natural background radiation. Heterogeneity means that one ramet in a ramet pair (I) was grown under natural background radiation, and the other (II) was exposed to supplemental UV-B radiation. + indicates supplemental treatment; – means without the respective treatment.

Group		Treatment UV-B
Homogeneity+Connected (HomoC)	I	–
	II	–
Homogeneity+Severed (HomoS)	I	–
	II	–
Heterogeneity+Connected (HeterC)	I	–
	II	+
Heterogeneity+Severed (HeterS)	I	+
	II	+

spectral irradiance from the lamps was determined with a UV radiometer (*Instrument Company of Beijing Normal University*, Beijing, China). The spectral irradiance was weighted with the generalized plant response action spectrum (Caldwell 1971) and normalized at 300 nm to obtain UV-B_{BE}. The UV-B dose was 0.6 kJ m^{-2} d^{-1} during clear sky conditions on the summer solstice in homogeneous UV-B radiation, while the enhanced UV-B dose was 2.54 kJ m^{-2} d^{-1}, and the radiation was guaranteed by varying the distance between lamp and the plants canopy.

Photosynthetic characteristics: Light-response curves (LRC), measuring the change in net photosynthesis as a response to changes in photosynthetic photon flux density (PPFD), were measured in young fully expanded leaves on three individual plants. The response of photosynthesis to eight differing PPFD levels (1,200; 1,000; 800, 600, 400, 200, 100, and 0 μmol m^{-2} s^{-1}) using the *Li-Cor 6400* internal red + blue light source (*LiCor*, Lincoln, NE, USA) were measured. Gas-exchange parameters, net photosynthetic rate (P_N), stomatal conductance (g_s), the intercellular CO_2 concentration (C_i), and the transpiration rate (E) were measured at saturating PPFD (600 μmol m^{-2} s^{-1}). During all measurements, CO_2 concentration in the reference chamber was set to 400 μmol mol^{-1}, the leaf block was set at 25°C, and flow rate of air was 400 μmol s^{-1}.

The minimal (F_o), the maximum fluorescence yield (F_m), the maximum quantum yield of PSII (F_v/F_m), photochemical quenching (q_P), and nonphotochemical quenching (NPQ) of Chl fluorescence parameters and the electron transport rate (ETR) were measured for a fully developed, healthy leaf on *T. repens* ramet using a portable *Li-Cor 6400* internal fluorescence source (*LiCor*, Lincoln, NE, USA) with the saturation pulse method. The plants were placed in a dark room for 20 min to determine the background fluorescence signal (F_o), the maximum fluorescence (F_m), the maximum photochemical efficiency of PSII (F_v/F_m) at an irradiance of 0.5 μmol m^{-2} s^{-1} and a frequency of 500 Hz. Steady-state fluorescence (F_s) was determined at an irradiance of 0.5 μmol m^{-2} s^{-1} and a frequency of 20,000 Hz. The PPFD incident on the leaves was always higher than 1,000 μmol m^{-2} s^{-1}, which was above photosynthesis saturation in these plants. To obtain the steady-state maximum fluorescence yield (F_m'), saturation pulses of about 10,000 μmol m^{-2} s^{-1} and 0.8-s duration were applied. The PSII photochemical efficiency ($\Delta F/F_m'$), then the ETR and NPQ were estimated ($\Delta F/F_m' = (F_m' - F_s)/F_m'$; ETR = $(\Delta F/F_m') \times$ PPFD $\times 0.5 \times 0.84$, PPFD is the photosynthetic photon flux density incident on the leaf, 0.5 is the factor that assumes equal distribution of energy between the two photosystems, and the leaf absorbance used was 0.84 because is the most common value for C_3 plants; NPQ = $(F_m - F_m')/F_m'$).

Statistical analyses: Maximum photosynthetic rate (P_{max}), apparent quantum yield (AQY), dark respiration rate (R_D), the light-saturating point (LSP) and light-compensation point (LCP) were determined using the *Photosynthetic Assist* software (*Dundee Scientific*, Dundee, UK). Data obtained from measurements have been evaluated statistically at factorial level by means of variance analysis (*ANOVA*) and significant differences between parameter estimates were tested with *Duncan*'s multiple range test ($p<0.05$ or $p<0.01$, $n = 5$). Analysis was performed with *STATISTICA* software *version 6.0* (*Statsoft*, Tulsa, OK, USA).

Results

Gas exchange: Net photosynthesis increased up to PPFD of 600 μmol m^{-2} s^{-1} and decreased at PPFD of 1,200 μmol m^{-2} s^{-1} in all treatments (Fig. 1).

P_N, g_s, C_i, and E were measured at saturating PPFD (600 μmol m^{-2} s^{-1} as observed in Fig. 1) and are shown in Table 2. No significant differences were observed in g_s, C_i, and E among HomoCI, HomoCII, HomoSI, HomoSII HeterCI, HeterCII, HeterSI, and HeterSII. The severing treatment resulted in a notable decrease of P_N under homogeneous UV-B environment (Table 2, HomoSI, HomoSII *vs.* HomoCI, HomoCII). In the case of their paired ramets located under a different UV-B microenvironment (heterogeneous UV-B radiation), supplemental UV-B radiation brought about a descent of P_N in severed group (HeterSII *vs.* HeterSI). However, in connected group, P_N increased in HeterCII (under

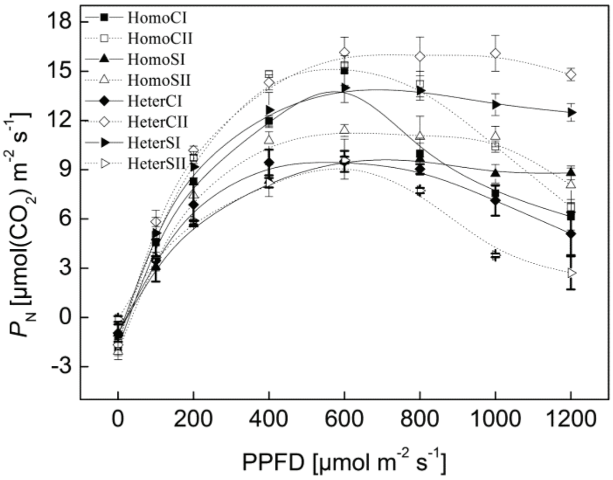

Fig. 1. Light-response curves of net photosynthesis (P_N) on photosynthetic photon flux density (PPFD) in connected and/or severed ramets under homogeneous (HomoC and HomoS) or heterogeneous UV-B radiation (HeterC and HeterS). Error bars show SE (n = 5). *See* Table 1 for experimental design.

enhanced UV-B radiation), and HeterCI (no UV-B radiation but its connected sister ramets exposed to additive UV-B radiation) was significantly lower than that in group HomoC (also no supplemental UV-B radiation and no severing treatment).

P_{max}, AQY, R_D, LSP, and LCP determined from light-response curves are displayed in Table 3. AQY, R_D, LSP and LCP showed no significant differences among HomoCI, HomoCII, HomoSI, HomoSII HeterCI, HeterCII, HeterSI and HeterSII. P_{max} also exhibited no significant changes under homogenous UV-B radiation (HomoCI, HomoCII, HomoSI, HomoSII), while under heterogeneous UV-B radiation, P_{max} in HeterSII (exposed to enhanced UV-B radiation) was lower than that of groups under homogenous UV-B radiation (HomoCI, HomoCII). It is worth pointing out that although the UV-B exposure level was the same, P_{max} in connected ramet HeterCII increased and HeterCI (only its connected sister ramet exposed to supplemental UV-B radiation) was significantly lower than that of HeterSI (disconnected ramet) and HomoCI, HomoCII (connected but did not expose to enhanced UV-B).

Table 2. Gas-exchange parameters - net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO$_2$ concentration (C_i), and transpiration rate (E) in leaves of *Trifolium repens* under homogeneous and heterogeneous UV-B radiation for connected (CI, CII) and severed (SI, SII) ramets. *Different letters* indicate significance (mean ± SE, n = 5, p<0.05). *See* Table 1 for experimental design.

Treatment	P_N [μmol(CO$_2$) m^{-2} s^{-1}]	g_s [mol(H$_2$O) m^{-2} s^{-1}]	C_i [μmol(CO$_2$) mol^{-1}]	E [mmol(H$_2$O) m^{-2} s^{-1}]
HomoCI	15.04 ± 0.91^{ab}	0.12 ± 0.02^{a}	220.53 ± 26.64^{a}	2.10 ± 0.40^{a}
HomoCII	15.36 ± 0.41^{ab}	0.14 ± 0.04^{a}	213.42 ± 43.60^{a}	2.71 ± 1.01^{a}
HomoSI	9.64 ± 1.22^{c}	0.14 ± 0.02^{a}	220.87 ± 38.58^{a}	2.30 ± 0.42^{a}
HomoSII	11.39 ± 0.36^{c}	0.16 ± 0.08^{a}	209.30 ± 2.64^{a}	2.51 ± 0.92^{a}
HeterCI	9.51 ± 0.64^{c}	0.14 ± 0.12^{a}	238.74 ± 33.20^{a}	2.64 ± 2.26^{a}
HeterCII	16.15 ± 0.93^{ab}	0.25 ± 0.04^{a}	270.00 ± 14.63^{a}	3.82 ± 0.41^{a}
HeterSI	13.99 ± 0.90^{abc}	0.18 ± 0.01^{a}	251.66 ± 13.92^{a}	2.93 ± 0.24^{a}
HeterSII	9.58 ± 0.21 ^{c}	0.25 ± 0.04^{a}	257.40 ± 30.55^{a}	3.87 ± 0.08^{a}

Table 3. Maximum net photosynthetic rate (P_{max}), apparent quantum yield (AQY), dark respiration rate (R_D), light-saturation point (LSP), and light-compensation point (LCP) in leaves of *Trifolium repens* under homogeneous and heterogeneous UV-B radiation for connected (CI, CII) and severed (SI, SII) ramets. *Different letters* indicated significance (mean ± SE, n = 5, p<0.05). *See* Table 1 for experimental design.

Treatment	P_{max} [μmol(CO$_2$) m^{-2} s^{-1}]	AQY [mol(CO$_2$) mol^{-1}(photon)]	R_D [μmol(CO$_2$) m^{-2} s^{-1}]	LSP [μmol(photon) m^{-2} s^{-1}]	LCP [μmol(photon) m^{-2} s^{-1}]
HomoCI	17.95 ± 4.45^{ab}	0.07 ± 0.03^{a}	–1.83 ± 0.69^{a}	256.50 ± 23.33^{a}	25.80 ± 0.14^{a}
HomoCII	17.70 ± 1.84^{ab}	0.07 ± 0.01^{a}	–1.38 ± 0.51^{a}	292.00 ± 86.27^{a}	21.55 ± 11.38^{a}
HomoSI	13.00 ± 1.13^{bc}	0.05 ± 0.01^{a}	–0.97 ± 0.09^{a}	288.50 ± 81.32^{a}	19.90 ± 5.66^{a}
HomoSII	16.05 ± 1.48 ^{abc}	0.06 ± 0.00^{a}	–2.06 ± 0.24^{a}	280.00 ± 24.04^{a}	31.85 ± 3.46^{a}
HeterCI	11.75 ± 3.89^{c}	0.05 ± 0.01^{a}	–0.93 ± 0.57^{a}	278.50 ± 23.33^{a}	22.05 ± 16.62^{a}
HeterCII	19.35 ± 1.06^{a}	0.08 ± 0.01^{a}	–1.62 ± 0.28^{a}	251.50 ± 53.03^{a}	19.60 ± 6.22^{a}
HeterSI	17.65 ± 0.78^{ab}	0.07 ± 0.00^{a}	–1.10 ± 0.15^{a}	255.00 ± 4.24^{a}	15.25 ± 1.63^{a}
HeterSII	12.90 ± 0.92^{c}	0.04 ± 0.00^{a}	–0.90 ± 0.28^{a}	279.50 ± 6.36^{a}	21.30 ± 1.91^{a}

Chl fluorescence parameters: Under homogeneous UV-B radiation, HomoCI, HomoCII, HomoSI and HomoSII had no significant difference in Chl fluorescence parameters of F_o, F_m, F_v/F_m, ETR, and q_P, while NPQ in severed ramets (HomoSI and HomoSII) were significantly higher than in connected ramets (HomoCI and HomoCII) (Table 4). However, additive UV-B radiation resulted in a notable decrease of F_o, ETR, q_P and an increase of NPQ under heterogeneous UV-B environment (HeterCII and HeterSII). Stolon connection made ETR and q_P increased and NPQ decreased in HeterCI (no UV-B radiation but its connected sister ramets exposed to supplementary UV-B radiation), which was different from HeterSI (disconnected ramet with no additive UV-B radiation) and HomoCI, HomoCII (connected but did not expose to enhanced UV-B radiation). The ETR in HomoSI and HomoSII were lower than HomoCI which may have been caused by severing.

Discussion

Gas exchange: Many studies have detected that photosynthesis is limited by stomatal (stomatal closure) and nonstomatal factors (metabolic impairment, *e.g.* decreases in Rubisco activity/RuBP regeneration and reduction of ATP production) under stressful environment (Yordanov *et al.* 2000, Bota *et al.* 2004, Grassi and Magnani 2005, Flexas *et al.* 2006, Dias and Brüggemann 2007). Medrano *et al.* (2002) found under moderate water deficit that photosynthesis of grapevines was mainly limited by stomatal conductance, and Dias and Brüggemann (2010) detected limitations of CO_2 assimilation in drought-exposed plants of *Phaseolus vulgaris* L. which could be related to the decline of the enzyme activity involved immediately in RuBP regeneration. Results from Table 2 and 3 reveal that no significant differences of gas-exchange parameters (except for P_N and P_{max}) were found among ramets HomoCI, HomoCII, HomoSI, HomoSII, HeterCI, HeterCII, HeterSI and HeterSII, suggesting that these parameters are not influenced by severing and enhanced UV-B radiation treatment, and the changes of P_N and P_{max} are not determined by stomatal limitations, which are in consistency with results of Dias and Brüggemann (2010).

In this study, under homogeneous UV-B radiation, P_N in HomoSI and HomoSII were lower than in HomoCI and HomoCII, indicating that severing might played an important role, which was in line with Wang *et al.* (2008). When interconnected ramets grown in a heterogeneous environment, ramets may respond not only to their local environment (local response), but also to the different environment experienced by the connected ramets (nonlocal response). The results that HeterSII (suffered from intensified UV-B) retained lower P_N and P_{max} were consequentially due to complementary UV-B radiation and/or severing treatment, just like Bornman's finding that UV-B radiation should be correlated to a reduction in RuBPcase activity and hence net photosynthesis (Bornman 1989). However, in HeterCII (exposed to additive UV-B radiation), P_N and P_{max} were higher than in HeterCI (without UV-B radiation but its connected sister ramets exposed to additive UV-B radiation). Interestingly, P_N and P_{max} in HeterCI were lower than in HomoC and HeterSI. The result could only be ascribed to physiological integration by stolon connection between the ramets. Roiloa and Retuerto (2006b) also found physiological integration in photosynthetic efficiency in serpentine soils. The result showing that photosynthetic rates in HeterCI were lower than in HeterSI and HomoC revealed that under heterogeneous UV-B radiation *T. repens* tend to be more self-insurant.

Chl fluorescence: *In vivo* Chl fluorescence is a nonintrusive indicator to rapidly assess the effects of stress on photosynthesis (Schreiber *et al.* 1994, Wang *et al.* 2008). Higher plants must dissipate absorbed light energy that exceeds the photosynthetic capacity to avoid molecular damage to the pigments and proteins that comprise the photosynthetic apparatus (Gilmore 1997). Based on this, the effects of heterogeneous UV-B radiation on physiological integration of *T. repens* could be ascertained in term of the change of Chl fluorescence parameters within the paired ramets. As shown in Table 4, there is no significant difference in Chl fluorescence parameters (except for NPQ) among HomoCI, HomoCII, HomoSI and HomoSII. The photoprotective heat dissipation is measured as NPQ of the photosystem II (PSII) Chl *a* fluorescence, in the present study (Table 4),

Table 4. Effects of heterogeneous UV-B on chlorophyll fluorescence parameters in the connected and/or severed ramets under homogeneous (HomoC anf HomoS) or heterogeneous UV-B radiation (HeterC and HeterS) in leaves of *Trifolium repens*. *Different letters* indicate significance (mean ± SE, $n = 5$, $p<0.05$). *See* Table 1 for experimental design.

Treatment	F_o [rel. unit]	F_m [rel. unit]	F_v/F_m	ETR [μmol m^{-2} s^{-1}]	q_P	NPQ
HomoCI	194.36 ± 1.92^{a}	980.38 ± 9.60^{ab}	0.80 ± 0.01^{ab}	97.00 ± 4.50^{a}	0.48 ± 0.06^{a}	0.01 ± 0.01^{c}
HomoCII	192.43 ± 2.72^{a}	1063.91 ± 35.43^{a}	0.82 ± 0.01^{a}	90.76 ± 12.28^{ab}	0.50 ± 0.06^{a}	0.07 ± 0.01^{c}
HomoSI	192.50 ± 2.09^{a}	1032.35 ± 27.13^{ab}	0.81 ± 0.01^{ab}	74.30 ± 21.25^{bc}	0.45 ± 0.02^{ab}	0.50 ± 0.01^{ab}
HomoSII	192.44 ± 2.68^{a}	1074.51 ± 38.15^{a}	0.82 ± 0.01^{a}	73.20 ± 17.20^{bc}	0.52 ± 0.05^{a}	0.54 ± 0.16^{ab}
HeterCI	172.87 ± 2.40^{ab}	912.73 ± 15.67^{b}	0.81 ± 0.01^{ab}	66.85 ± 14.21^{c}	0.41 ± 0.06^{b}	0.55 ± 0.03^{ab}
HeterCII	161.77 ± 1.92^{b}	826.00 ± 9.86^{b}	0.80 ± 0.00^{b}	66.82 ± 4.31^{c}	0.35 ± 0.01^{bc}	0.58 ± 0.33^{ab}
HeterSI	188.01 ± 3.78^{a}	998.38 ± 19.65^{ab}	0.81 ± 0.01^{ab}	82.91 ± 11.02^{b}	0.46 ± 0.02^{a}	0.37 ± 0.22^{b}
HeterSII	154.95 ± 0.19^{b}	816.23 ± 20.02^{b}	0.80 ± 0.00^{b}	55.76 ± 4.97^{c}	0.30 ± 0.03^{c}	0.76 ± 0.03^{a}

severing caused a higher NPQ in severed ramets (HomoSI and HomoSII) than in connected ramets (HomoCI and HomoCII), which is consistent with the result determined by Wang *et al.* (2008).

Several studies have displayed a sustained decrease in F_m, F_v/F_m, ETR, q_P and increase in F_o and NPQ under environmental stresses (Groom and Baker 1992, Epron *et al.* 1992). Larsson *et al.* (1998) found the same changes of Chl fluorescence under UV-B radiation, the stressed plants had a lower capacity for the use of transported electrons and the reduced electron transport chain was insufficient to prevent them from a stronger photoinhibition. In this study, under additive UV-B radiation, F_o, ETR, and q_P decreased and NPQ increased in HeterCII and HeterSII. Different environmental stresses increase F_o, probably due to decreased efficiency of energy transfer from the antenna Chl a to the reaction centre and/or inactivation of PSII reaction centre (Briantais *et al*. 1986). However, in some instances nonfunctional PSII centre may act as dissipative sink, which may be UV-screening pigments. F_o decreased in this study was the same with Kalbin *et al.* (1997). If it connected with its sister ramets under enhanced UV-B radiation, ETR and q_P in the unstressed clone ramets (HeterCI) descended and NPQ ascended compared with group HomoC and HeterSI, the decrease in ETR and q_P of *T. repens* and increase in NPQ were alleviated by stolon connections (Table 4). The results, therefore, suggest that clonal integration markedly reduced the stress effects imposed by UV-B radiation and significantly increased the photosynthetic activity of *T. repens*. Roiloa and Retuerto (2006a) also found that clonal integration significantly buffered the decrease in F_v/F_m of the offspring ramets of *Fragaria vesca* grown in soils contaminated by heavy metals. The present study suggests that under heterogeneous UV-B microhabitat, clonal integration markedly enhanced the photosynthetic activity of *T. repens* under supplemental UV-B radiation, and that the whole clone fragment will benefit, with a cost, from photosynthesis. Physiological integration of *T. repens* in photosynthetic efficiency may be helpful to maintain their presence in less favourable sites.

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