PHOTOSYNTHETICA 35 (1): 13-19, 1998

Changes in net photosynthetic rate and chlorophyll fluorescence in potato leaves induced by water stress

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Abstract

Net rates of photosynthesis (P_N) saturated by irradiance of >500 μmol m^{-2} s^{-1} (PAR) significantly decreased in water-stressed potato (*Solanum tuberosum* L. cv. Kufri Sindhuri) plants. The quantum yield of photochemical energy conversion (F_v/F_m), relative electron transport rate (ETR), and photochemical quenching (Q_p) exhibited a parallel decline at high irradiance. A slight decrease in relative water content (RWC) was accompanied by a drastic decline in leaf water potential (Ψ_w) from -0.2 to -1.0 MPa. Dehydrated leaves showed an increase in the amount of total soluble sugars per unit leaf area which inhibited the photosynthesis in a feedback manner. After rewatering, P_N and F_v/F_m were restored to the values of control plants within 24 h, and the restoration was accompanied by a proportionate lowering of content of total soluble sugars in the leaves.

Additional key words: drought; electron transport; feedback inhibition; photochemical quenching; osmotic potential; quantum yield; *Solanum tuberosum;* water potential.

Introduction

Potato is highly sensitive to water stress as the decline in photosynthesis is fast and substantial even at relatively high Ψ_w (*ca.* -0.5 MPa, Sukumaran *et al.* 1989). Vos and Oyarzún (1987) reported an almost complete inhibition of photosynthesis in potato at Ψ_w -1.2 MPa. Ezekiel *et al.* (1987) found a mid-day depression of photosynthesis in a well-irrigated potato crop. One of the conspicuous effects of drought is the decrease in stomatal conductance (g_s) that affects P_N under water stress. However, Ackerson *et al.* (1977) did not find any significant decrease in g_s in

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Received 25 July 1997, *accepted* 1 October 1997.

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Acknowledgements: Authors are thankful to the Indian Council of Agricultural Research, New Delhi, India for providing financial support, and to Dr G.S. Shekhawat, Director, CPRI, Shimla for providing facilities for this work.

potato upto $\Psi_w \geq 1.0$ MPa. Schapendonk *et al.* (1989) report that stomatal closure is not the cause of reduction of P_N in potato under moderate water stress, and indicate the possible nonstomatal factors causing such inhibition. Vos (1986) observed a cultivar difference in the sensitivity to water stress in potato, although their water relation characteristics such as Ψ_w, osmotic potential, and stomatal response to drought did not differ significantly. Water deficits also differentially affect photosynthesis in different potato cultivars (Shimshi and Susnoschi 1985). The present work is an attempt to identify possible factors responsible for high sensitivity of photosynthesis to water stress in potato.

Materials and methods

Plants: Potted plants of potato (*Solanum tuberosum* L.) cv. Kufri Sindhuri were raised in a glasshouse using recommended doses of nitrogen, phosphorus, and potassium fertilizer. 35- to 40-d-old nontuberised plants growing under maximum/minimum temperature of 25/15 °C and 12 h photoperiod at Shimla (31° 06'N, 77°10'E) were used for the experiment.

Water stress treatment: Irrigation was withdrawn for some period to induce water stress. Leaf water potentials were determined using a *Wescor HR-33T* microvoltmeter in the dew point mode. Droughted leaves were allowed to attain full turgidity by saturating with water, and then put into liquid nitrogen. Leaves were subsequently stored at –20 °C for 2-3 h. Osmotic potentials of the sap extracted from frozen leaf samples of turgid and stressed leaves were measured with the *Wescor* model *5100C* vapour pressure osmometer.

P_N was measured at constant temperature of 20 °C and relative humidity of 60 % using the *LI-6400* (*LI-COR*, USA) device. Leaves were exposed to different irradiances with centre wavelength 660 to 675 nm by using the *6400-02 LED* light source installed over the sensor head. PAR was measured using quantum sensor mounted inside the leaf chamber.

Chlorophyll fluorescence of the leaves was measured by using PAM-fluorometer (*Mini-PAM, Walz*, Germany) according to Schreiber *et al.* (1986). Intact leaf or leaf disc was dark adapted for 5 min using a dark leaf clip holder. Minimal fluorescence F_0 of the dark adapted leaves was measured by exciting the leaf with weak modulated radiation (LED 655 nm) of 0.15 μmol m^{-2} s^{-1} at frequency of 0.6 kHz. Thereafter, saturation pulse of 4500 μmol m^{-2} s^{-1} was applied through fibre optic cable for 400 ms to obtain maximal fluorescence, F_m. Quantum yield (F_v/F_m) was calculated automatically by using the formula $(F_m - F_0)/F_m$. Soon after the F_v/F_m measurement in dark-adapted leaf, samples were irradiated by actinic radiation (8 V/20 W halogen lamp). The leaves were gradually exposed to higher irradiance and were allowed to acclimate at each irradiance for 5 min. Saturation pulse was triggered at the end of the 5 min acclimation in correspondence to each irradiance at different time intervals to obtain response of F_v/F_m to irradiance.

Relative electron transport rate (ETR) of the leaf sample was determined by using the *MINI-PAM* attached by leaf clip holder *2030-B* with the PAR sensor, and was calculated using the following formula:

ETR = quantum yield × PAR × 0.5 × ETR factor,

where ETR factor = 0.84. This factor corresponds to the fraction of incident radiation absorbed by various leaf species.

Photochemical quenching coefficient (Q_p) was calculated according to Van Kooten and Snel (1990) after dark adaptation and subsequently at constant irradiance of 1300 μmol m^{-2} s^{-1} at every 10 s interval.

Estimation of total soluble sugars, reducing sugars and saccharose: Leaf saccharose was estimated by the method of Van Handel (1968), total reducing sugars by the method of Nelson (1944), and total soluble sugars according to Yemm and Willis (1954).

Statistical analysis: The two factor factorial, completely randomized design was applied for determining the least significant difference (LSD) between the treatments.

Results and discussion

Irradiance-saturated P_N (irradiance >500 μmol m^{-2} s^{-1}) significantly decreased in water-stressed leaves (Ψ_w −1.1 MPa) as compared to control plants (Ψ_w −0.2 MPa; Fig. 1*A*). This reduction in P_N was associated with simultaneous decline in the photosynthetic electron transport rate, ETR (Fig. 1*B*), quantum yield (F_v/F_m) of photosystem 2, PS2 (Fig. 2), and photochemical quenching, Q_p (Table 2). The reduction in P_N and quantum yield due to water stress was not significant at low irradiance ($I \leq 100$ μmol m^{-2} s^{-1}) (Figs. 1*A*, and 2). This agrees with the findings of Wong *et al.* (1985).

Gradual lowering of F_v/F_m even in light-adapted leaves of control plants (Fig. 2) with increasing irradiance indicated that more of the Q_A pool are in reduced state when irradiated. Water-stressed leaves showed relatively lower F_v/F_m values at all irradiances above 100 μmol m^{-2} s^{-1}, and F_v/F_m declined to almost zero at PAR I >300 μmol m^{-2} s^{-1} (Fig. 2). The decrease in F_v/F_m in water-stressed leaves at high irradiance may be due to photoinhibition and increase of heat dissipation. Both an increase of F_0 and a decrease of F_m in water-stressed leaves contributed to the observed decrease in F_v/F_m. Prange (1986) reported a change in F_0 and F_m in water-stressed leaves of potato due to the reduction in CO_2 fixation and electron transfer.

The observed decline in F_v/F_m in light-adapted water-stressed leaves (Fig. 2) was associated with a decrease in photosynthetic ETR (Fig. 1*B*). The relative quantum yield of PS2 in photosynthesising plants exposed to saturating PAR depends upon the state of Q_A reduction and its subsequent reoxidation which is influenced by P_N (Schreiber *et al.* 1995). Under normal turgidity when leaf is irradiated, Q_A is kept oxidised by transferring electron to NADP and finally to CO_2 *via* Q_B, plastoquinone pool, and PS1, and thus F_v/F_m remains fairly high (Walker 1987). On the contrary,

F_v/F_m may decrease if reoxidation of Q_A in light is limited by decrease or partial block of electron transport from PS2 to PS1 by some stress factor.

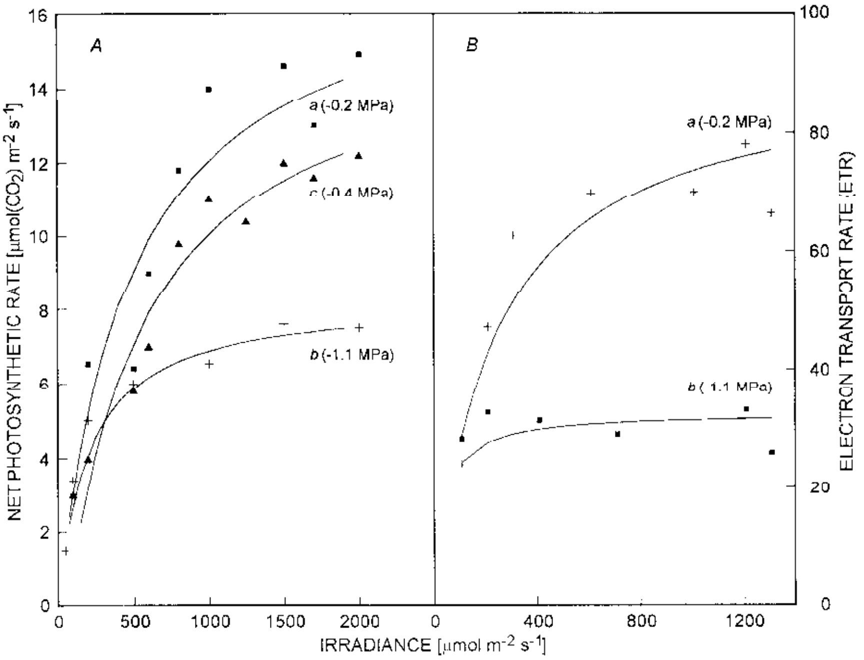

Fig. 1. Response of net photosynthetic rate, P_N (*A*) and electron transport rate, ETR (*B*) to irradiance in potato leaves of (*a*) control plants (Ψ_w = -0.2 MPa), (*b*) water-stressed plants (Ψ_w = -1.1 MPa), and (*c* in *A*) after recovery from water stress (Ψ_w = -0.4MPa). Lines fitted into regression equation in A, *a*: 1/y = 0.06 + 26.93/x, r^2 = 0.93, and *b*: 1/y = 0.12 + 24.69/x, r^2 = 0.94; in *B*: *a*: 1/y = 0.12 + 2.64/x, and *b*: 1/y = 0.31 + 1.13/x. For *B*, $LSD_{0.01}$ for effect of irradiance = 3.4, for difference between control and stress =2.8, for irradiance × stress level = 6.8.

Table 1. Effect of water stress on contents of soluble sugars in leaf. RWC = relative water content, Ψ_w = water potential , Ψ_s = osmotic potential, TSS = total soluble sugars, RS = reducing sugars. Each value represents mean of ten replicates. Significant difference in the sugar content: $LSD_{0.01}$ = 0.176, $LSD_{0.05}$ = 0.132.

Treatment	RWC [%]	Ψ_w [MPa]	Ψ_s [MPa]	Sugar content in leaf [g m^{-2}] TSS	RS	sucrose
Control	96	-0.2	-0.75	3.5	0.4	0.6
Stressed	88	-1.1	-0.90	9.2	1.3	3.6
Rehydrated	94	-0.4	-0.82	2.2	0.5	0.8

Water capacitance of potato is not large, and as a result small decrease in RWC (less than 10 %) in the leaf tissue causes large changes in the water potential (decline of Ψ_w to about -1.1 MPa, Vos and Groenwold 1988). This decrease in Ψ_w was accompanied by a significant increase in contents of total soluble sugars and saccharose (Table 1). The accumulation of sugars in dehydrating leaves occurred rapidly after withdrawing irrigation. Soluble sugars accumulated in response to water stress may contribute partly to osmoregulation. However, both the increase in sugar contents as well as faster decline in Ψ_w accompanied by cell shrinkage manifold concentrated these sugars per unit leaf area. This sugar accumulation and further increase in their concentration due to dehydration may cause feedback inhibition of photosynthesis. Shekhar and Iritani (1979) report inhibition of photosynthesis due to saccharide accumulation in potato subjected to water stress. Goldschmidt and Huber (1992) found a strong end product inhibition of photosynthesis due to sucrose accumulation and its subsequent hydrolysis into hexose (reducing sugars) in leaves.

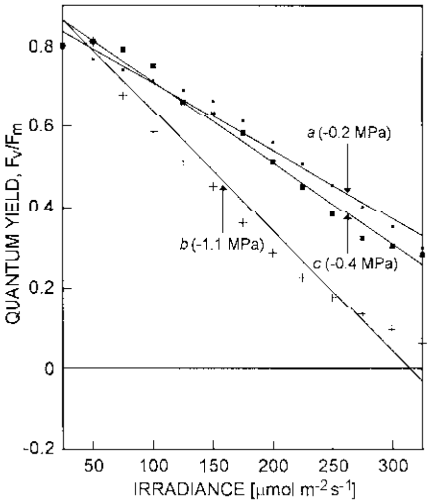

Fig. 2. Changes in the quantum yield (F_v/F_m) with increasing irradiance in leaves of control plants (*a*), water-stressed plants (*b*), and after recovery from water stress (*c*). $LSD_{0.01}$ for effect of irradiance = 0.035, for difference between control and stressed = 0.029, for irradiance × stress level = 0.07. Lines fitted into regression equation for *a* are $y = 0.819 - 0.001x$, $r^2 = 0.98$, and for *b*, $y = 0.747 - 0.002x$, $r^2 = 0.95$.

In accordance with our results, Quick *et al.* (1989) also reported an increase in sucrose content through transient activation of sucrose-phosphate synthase (SPS) in spinach leaves in response to mild water stress. However, as a result of cell shrinkage in dehydrating leaves, they observed that contents of some metabolites in cytosol, *e.g.*, fructose-2,6-bisphosphate, increased considerably under water stress which inhibited photosynthesis in a feedback manner. All the earlier works regarding effect of sugar accumulation in leaves on P_N in plant species such as pea, maize, and spinach, lead to a common conclusion that this process may alter or modify the activation state of SPS or cytosolic metabolites concentration, *e.g.*, the triose-P/P_i ratio and fructose-2,6-bisphosphate content (Stitt *et al.* 1987, 1988). This ultimately restricts the supply of inorganic phosphate (P_i) inside the chloroplast, and photosynthesis is inhibited due to P_i limitation. Water stress may cause a decrease in the Calvin cycle activity in potato (Schapendonk *et al.* 1989). This decrease is also expressed in terms of lowering of quantum yield or ETR when high irradiance is applied to make dark enzymic steps of the Calvin cycle limiting. The depletion of P_i also affects Chl fluorescence as well as photosynthetic electron transport rate (Walker 1987). Therefore the decrease in P_N at high irradiance (Fig. 1) along with observed changes in fluorescence as reflected by the reduction of ETR (Fig. 1*B*) and F_v/F_m (Fig. 2) may be due to P_i limitation leading to feedback inhibition or decrease in the Calvin cycle activity as a result of water stress.

Table 2. Response of photochemical quenching (Q_p) to water stress and after feeding leaf discs with glucose. Leaf discs were fed with 100 mM glucose solution for 5 h at an irradiance of 500 μmol m^{-2} s^{-1}. After initial 5 min dark adaptation, the leaf disc was exposed to actinic irradiation of 1300 μmol m^{-2} s^{-1}. Saturation pulse of 4500 μmol m^{-2} s^{-1} was triggered at every 10 s interval for measuring Q_p.
$LSD_{0.01}$ for treatment A (control, water stress, and glucose) = 0.017, $LSD_{0.05}$ = 0.013.
$LSD_{0.01}$ for treatment *B* (time s) = 0.022, $LSD_{0.05}$ = 0.017.
$LSD_{0.01}$ for interaction *A*×*B* = 0.04, $LSD_{0.05}$ = 0.03.

Time [s]	Q_p control (-0.2 MPa)	water stress (-1.0 MPa)	100 mM glucose (-0.4 MPa)	recovery from stress (-0.4 MPa)
10	0.132	0.050	0.019	0.088
20	0.221	0.082	0.030	0.321
30	0.251	0.087	0.075	0.352
40	0.313	0.112	0.106	0.328
50	0.371	0.149	0.061	0.401

Drought stress significantly decreased Q_p at high irradiance (Table 2). Reduction in Q_p in a similar manner observed in leaf discs from control plants fed with 100 mM glucose supports our view of feedback inhibition of photosynthesis as a result of sugar accumulation (Table 2). Biehler *et al.* (1996) observed a reduction in the Q_p in wheat under water stress. Foyer (1988) reported a decline in the F_v/F_m in turgid leaf after feeding sucrose, glucose, or fructose.

Our results suggest that substantial reduction in P_N even at moderate water stress ($\Psi_w \leq -1.0$ MPa) may be due to the increase in sugar concentration in leaves as a result of decline in RWC. Restoration of normal P_N, F_v/F_m, and Q_p after stress relief indicated that there was no irreversible damage to the photosynthetic system. Decline in the sugar concentration after rewatering of the dehydrated leaf (Table 1) was accompanied by restoration of normal photosynthesis (Fig. 1*A*, curve *c*).

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