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Intrinsic changes in photosynthetic parameters of carrot leaves under increasing CO$_2$ concentrations and soil moisture regimes

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Abstract

A controlled growth chamber experiment was conducted to investigate the short-term water use and photosynthetic responses of 30-d-old carrot seedlings to the combined effects of CO$_2$ concentration (50–1 050 µmol mol^{-1}) and moisture deficits (–5, –30, –55, and –70 kPa). The photosynthetic response data was fitted to a non-rectangular hyperbola model. The estimated parameters were compared for effects of moisture deficit and elevated CO$_2$ concentration (EC). The carboxylation efficiency (α) increased in response to mild moisture stress (–30 kPa) under EC when compared to the unstressed control. However, moderate (–55 kPa) and extreme (–70 kPa) moisture deficits reduced α under EC. Maximum net photosynthetic rate (P_{Nmax}) did not differ between mild water deficit and unstressed controls under EC. Moderate and extreme moisture deficits reduced P_{Nmax} by nearly 85 % compared to controls. Dark respiration rate (R_{D}) showed no consistent response to moisture deficit. The CO$_2$ compensation concentration (Γ) was 324 µmol mol^{-1} for –75 kPa and ranged 63–93 µmol mol^{-1} for other moisture regimes. Interaction between moisture deficit and EC was noticed for P_{N}, ratio of intercellular and ambient CO$_2$ concentration ($C_{\mathrm{i}}/C_{\mathrm{a}}$), stomatal conductance (g_s), and transpiration rate (E). P_{N} was maximum and $C_{\mathrm{i}}/C_{\mathrm{a}}$ was minimum at –30 kPa moisture deficit and at C_{a} of 350 µmol mol^{-1}. The g_{s} and E showed an inverse relationship at all moisture deficit regimes and EC. Water use efficiency (WUE) increased with moisture deficit up to –55 kPa and declined thereafter. EC showed a positive influence towards sustaining P_{N} and increasing WUE only under mild moisture stress, and no beneficial effects of EC were noticed at moderate or extreme moisture deficits.

Additional key words: *Daucus carota*; model; stomatal conductance; transpiration; water use efficiency.

Introduction

Several research findings have proposed that elevated ambient CO$_2$ concentrations (EC) can improve the photosynthesis and water use efficiency (WUE) of plants (Cure and Acock 1986, Druță 2001) under abiotic stress, including drought (Prior *et al*. 1991, Azam and Farooq 2003). This beneficial interaction is a consequence of increased turnover from Calvin cycle achieved through a saturated supply of CO$_2$ to ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) enzyme and a moderate decrease in photorespiration rate (Bowes 1996). In contrast, some studies have shown a negative influence of EC on the photosynthetic efficiency of plants. For instance, Baker *et al*. (1997) saw a decline in photosynthetic capacity of rice (*Oryza sativa*) in response to EC. Therefore, the nature of this influence still remains inconclusive. Downward regulation of leaf net photosynthetic rate (P_{N}) to EC is the fundamental mode that governs this crucial process. Several postulates are available to explain this phenomenon. De-allocation of N sources to other functional processes other than photosynthetic enzymes for better balance in N utilization is suggested by Bowes (1996), while Sage (1994) proposed that plants acclimate to EC and lose their photosynthetic capacity.

A mathematical model based on biochemical processes is a representation of a real biological system (Farquhar *et al*. 2001) and it can provide means to elucidate the impact on food production of global

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Abbreviations: C_{a} – ambient CO$_2$ concentration; C_{i} – internal CO$_2$ concentration; E – leaf transpiration rate; g_{s} – stomatal conductance; P_{N} – net photosynthetic rate; R_{D} – dark respiration rate; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase; WUE – water use efficiency; Γ – carbon dioxide compensation concentration.

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atmospheric changes (Wolf 1993). The non-rectangular hyperbola model proposed by Cannell and Thornley (1998) utilizes a set of parameters which are reflective of the key biochemical processes involved in photosynthesis. This feature allows revelation of the impact of imposed stress in a biological context. Photosynthetic responses of several crops including cucumber, sweet pepper (*Capsicum annum* L.) (Nederhoff and Vegter 1994), and carrots (Kyei-Boahen *et al.* 2003) were successfully evaluated using this model.

A relative increase in WUE and production efficiency of several plant species under dry conditions are reported by Idso and Idso (1994). Kimball *et al.* (1995) reported a 21 % increase in grain yield of wheat grown with EC under moisture limiting conditions. The authors attributed this behaviour to a combination of factors including reduced stomatal conductance (g_s), decrease in transpiration rate (E), and/or decrease in C_i. However, Wu *et al.* (2004) found that an increase in P_N of wheat plants occurred only under ample water supply. Carrots represent an important horticultural crop that is extremely sensitive to moisture deficit. Although the photosynthetic responses of carrots were studied against EC (Kyei-Boahen *et al.* 2003), no information is available on the interaction between EC and moisture deficits in carrots. Accordingly, this study was performed with the following objectives: (*a*) to identify the nature and extent of the influence of EC on the leaf P_N, g_s, E, and WUE of carrots under a range of moisture deficits, and (*b*) to construct the P_N/C_i relationship curve using a non-rectangular hyperbola model, estimate the model parameters, and evaluate the effects of moisture deficits and EC in terms of the model parameters.

Materials and methods

Plant culture: Plastic pots (15 cm diameter) filled with equal quantities of *Pro-mix* (*Premier Horticulture*, Rivière-du-Loup, Quebec, Canada) were irrigated to field capacity. Fifteen seeds of carrot (*Daucus carota var. sativus* L.) cv. Red Core Chantaney were planted per pot. Seedlings were thinned after 7 d of emergence at the first true leaf stage to remain at five per pot. In addition to daily irrigation to field capacity, the seedlings were nourished with 100 cm^3 of nutrient solution (NPK at 15 : 15 : 30 ratio) at weekly intervals. The plants were irradiated with a combination of incandescent, cool white fluorescent, and high pressure sodium lamps providing a photosynthetically active radiation (PAR) of 400±20 µmol m^{-2} s^{-1}. Plants were raised at 20 and 10 °C day/night temperature under a 16-h photoperiod. The ambient CO$_2$ concentration and relative humidity inside the chamber was measured by an infrared gas analyzer (*90DM3A, Vulcain*, New Station, PA, USA) which remained around 375±10 µmol mol^{-1} and 60 %, respectively.

Stress imposition: Moisture deficit was gradually and naturally induced by withholding irrigation beginning 30 d after emergence. Plants that remained well-watered functioned as the controls. Withholding water allowed a decline in soil moisture potentials to –30, –55, and –70 kPa after 24, 48, and 72 h, respectively. Soil moisture potential was measured using *Watermark* soil moisture sensors (*Spectrum Technologies*, IL, USA) daily, prior to gas exchange measurements.

Photosynthesis measurements: P_N, g_s, C_i, and E were measured on the youngest fully expanded intact leaves 30 d after emergence (Fig. 1), using a portable open-flow gas analyzer connected with a Portable Leaf Chamber unit (*LCA-4, Analytical Development Company*, Hoddesdon, UK). The leaves were placed inside the *LCA* unit and the lid was closed carefully to ensure proper air-sealing. The CO$_2$ concentration of the leaf inside the *LCA* unit was controlled using a Leaf Microclimate Control System (LCMS, *Analytical Development Company*, Hoddesdon, UK). The LCMS used a CO$_2$ canister to keep the modified CO$_2$ concentrations (50–1 050 µmol mol^{-1} at incremental steps of 100 µmol mol^{-1}) nearly constant inside the leaf chamber. The analyzer was operated in a differential mode at an airflow rate of 400 µmol s^{-1}. Ample time was allowed to achieve a steady state C_i before each observation was recorded. Readings in replicates of 4 were taken for each observation and the means were used for analysis. Observations on plants at each moisture level were made from 09:00 to 14:00 h on consecutive days. Measurements were taken from three plants for each treatment. Leaf area of the experimental leaves was calculated using an image analysis software program (*CIAS Image Analysis* version *2.0, Jandel Scientific,* California, USA). Photosynthetic parameters were adjusted to reflect their respective leaf area.

Experimental design, statistical analyses, and model fitting: The experiment followed a completely randomized design with 3 replications for each treatment. For statistical analysis, a 4×11 factorial model was used. The first factor was moisture deficit at four levels (–5, –30, –55, and –70 kPa) and the second factor was CO$_2$ at 11 levels (from 50 to 1 050 µmol mol^{-1}). The response variables, P_N, g_s, C_i, E, and the computed values such as C_i/C_a and WUE (P_N/E) were subjected to ANOVA. The analyses were performed using a Mixed Procedure of SAS (SAS Institute 1999) and with a Tukey's range test for separation of treatment means. For modelling the photosynthetic response of carrots, a non-rectangular hyperbola model followed by Kyei-Boahen *et al.* (2003) was used. The model equation originally suggested by Marshall and Biscoe (1980) is as follows:

$$\theta_1 Y^2 - (\theta_2 + \theta_3 X - \theta_1\theta_4)Y + \theta_3 X(\theta_2 - (1 - \theta_1)\,\theta_4) - \theta_2\theta_4 = 0 \quad [1]$$

where Y is P_N, θ_1 is the curvature (convexity) of the photosynthesis-C_i relationship, θ_2 is P_{Nmax}, θ_3 is the initial slope of the C_i response curve at low CO_2, *i.e.* an estimate of carboxylation efficiency, and θ_4 represents R_D.

The model parameters were estimated by iterations invoked by the NLIN procedure of SAS (SAS Institute 1999). The seeding values for estimation were provided from the data collected from the experiment. The effect of moisture deficits on the photosynthetic response curves was evaluated using a sum of square reduction tests between the predictions made by each response equation. Upon significant difference between treatments, for each parameter a nested pair-wise non-linear regression was performed between the full and reduced models (Schabenberger *et al.* 1999) using SAS (SAS Institute 1999). Based on the F-statistic values the differences between the parameters were established.

Results and discussion

Estimating parameters based on mathematical models can reveal the potential associations between the underlying biochemical processes governing photosynthesis (Cannel and Thornley 1998). The non-rectangular hyperbola model employed in our study was primarily built on four parameters such as α, P_{Nmax}, R_D, and θ. The α, initial slope of the curve reflects the unit change in P_N to unit change in C_i, and is a measure of the availability of active exchange sites in RuBPCO enzyme for CO_2 fixation. This ability is commonly referred to as carboxylation efficiency (Caemmerer and Farquhar 1981). The $P_{Nmax,}$ value indicates the maximum net assimilation rate of the photosynthetic apparatus, while R_D represents the dark respiration rate of the plants. The convexity factor, θ in this model, was assumed to an empirical factor as suggested by Cannel and Thornley (1998). Any typical P_N/C_i response predominantly includes two distinctive phases. The linear portions of the curves (Fig. 2) show the CO_2 limited and RuBPCO active phase of photosynthesis and are strongly represented by the α values in our model (Sage 1994). The second stage is the capacity of thylakoid reactions to provide ATP and NADPH for regeneration of RuBP determined by the electron transport rate or Calvin cycle capacity or triose phosphate regeneration capacity (Rajasekaran *et al.* 1997, Liu *et al.* 2002). The P_{Nmax} value represents the acclimation phase of the curve in our model.

The α values observed from our study (Table 1) under normal moisture conditions were lower than those observed by Kyei-Boahen *et al.* (2003) for carrots which ranged from 0.068 to 0.091 µmol m^{-2} s^{-1} and with those observed for *Glycine max* L. by Lauer *et al.* (1989). Slightly lower irradiations (against 450 µmol m^{-2} s^{-1} used by Kyei-Boahen *et al.* 2003) and different growth and experimental conditions may have caused this variation. In response to a mild moisture deficit (–30 kPa), a two fold increase in α compared with unstressed controls (–5 kPa) was observed. However, this effect was not statistically significant (Table 1). Apparently, mild moisture stress or EC had no deleterious effect on the activity or regeneration of RuBP. Mild moisture deficit (–30 kPa) triggered a significant (58 %) reduction in g_s (Table 2), as expected. EC also contributed to the closure of stomata. An inverse relationship between EC and g_s observed in our study (Table 5) provides evidence for this effect. A 40 % reduction in g_s as a result of EC, especially under moisture stress, was reported previously by Li *et al.* (2004). A characteristic consequence of reduced g_s is restricted CO_2 influx and hence a drop in α or P_N is normally expected. Paradoxically, mild water deficit produced

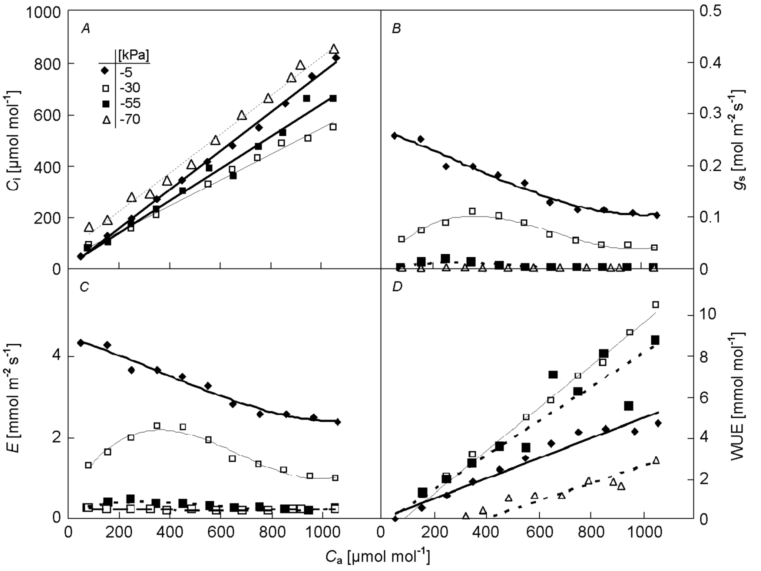

Fig. 1. Intercellular CO_2 concentration, C_i (*A*), stomatal conductance, g_s (*B*), transpiration rate, E (*C*), and water use efficiency, WUE (*D*) responses of *Daucus carota* cv. Red Core Chantaney leaves to various soil moisture potentials and external CO_2 concentrations at 20 °C and 450 µmol m^{-2} s^{-1} irradiance. Means of four replications.

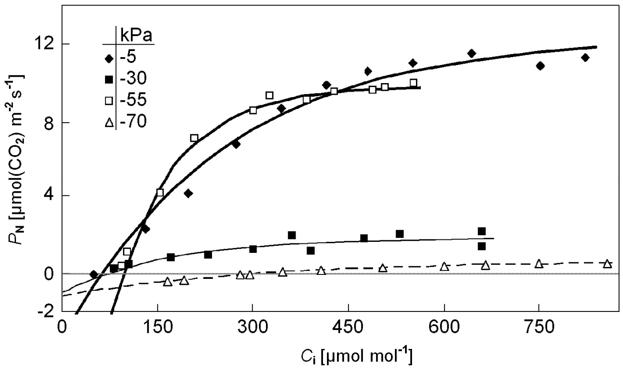

Fig. 2. Photosynthetic response curves (P_N-C_i) of *Daucus carota* cv. Red Core Chantaney leaves at elevated CO$_2$ concentrations under four soil moisture potentials at 20 °C and 400 μmol m^{-2} s^{-1} irradiance.

Table 1. Gas exchange parameters, convexity factor (θ), maximum net photosynthetic rate (P_{Nmax}) [μmol m^{-2} s^{-1}], initial slope of curve (*α*) [μmol m^{-2} s^{-1}], dark respiration rate (R_D) [μmol m^{-2} s^{-1}], and CO$_2$ compensation concentration (Γ) [μmol mol^{-1}] for the P_N-C_i relationship curves of leaves of carrot cv. Red Core Chantaney exposed to various moisture deficits. The values within a column followed by same letter are not significantly different at the 5 % level.

Soil moisture potential [kPa]	θ	P_{Nmax}	*α*	R_D	Γ
–5	0.996 a	11.23 a	0.030 a	1.53 a	63
–30	0.967 ab	10.24 ab	0.071 ab	1.19 c	93
–55	0.991 b	1.52 b	0.004 b	–0.01 d	71
–70	0.524 c	1.57 c	0.007 c	1.33 b	324

no significant reduction in *α* or P_N. A saturated supply of CO$_2$ to RuBPCO presumably had a compensatory effect against the negative CO$_2$ flux inflicted by stomatal limitations (Chaves and Pereira 1992). Furthermore, EC may have competed against O$_2$ molecules for binding with the active exchange sites at RuBPCO subsequently resulting in better carboxylation rates, reduced photorespiration, and increased P_N (Bowes 1996). Similar alleviative effects of EC on carboxylation efficiency in other C$_3$ crops grown under mild stress were reported by Huxman *et al.* (1998).

In contrast, moderate (–55 kPa) or extreme (–70 kPa) moisture deficits experienced no beneficial effect from elevated C_a. A 75–85% reduction in *α* ($p<0.05$) was noticed under moderate (–55 kPa) and extreme (–70 kPa) moisture deficits when compared with controls (Table 1). Crystallization, inactivation, and/or declined rate of regeneration of RuBPCO enzyme due to severe dehydration may have caused the reduction in *α* (Vu *et al.* 1997). Apart from this primary effect, extremely low g_s and impaired CO$_2$ influx also added to this adverse effect. The EC under extreme moisture deficits showed absolutely no beneficial effect since the enzyme complex involved in CO$_2$ fixation was inactive. The results strongly suggest that EC has no recuperative effect on RuBPCO activity due to consequences imposed by non-stomatal limitations such as enzyme dysfunction.

The P_{Nmax} values observed in the present study were comparable to those observed by Kyei-Boahen *et al.* (2003) from a similar study. The P_{Nmax} values did not differ between –5 kPa (11.23 μmol m^{-2} s^{-1}) and –30 kPa (10.24 μmol m^{-2} s^{-1}) moisture deficits (Table 1). Although a similar trend was observed with –30 kPa moisture deficit, a slightly lower (350 μmol mol^{-1}) C_i was able to cause the saturation of P_N. Soaring *α* rates accelerated by mild moisture deficit and EC presumably depleted the P$_i$ (inorganic phosphate) more rapidly than the unstressed controls. The converged nature of P_N/C_i response curve at –30 kPa clearly depicted this quick down-regulation. Such a characteristic phenomenon demonstrated that downward acclimation is independent of C_a and is rather dictated by P$_i$ regenerative capacity in combination with other non-stomatal factors of the photosynthetic apparatus.

Table 2. Effect of moisture stress on net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (*E*), and water use efficiency (WUE) of carrot cv. Red Core Chantaney leaves. Least square mean values followed by same letter are not significantly different at $p<0.05$.

Soil moisture potential [kPa]	P_N [μmol m^{-2} s^{-1}]	*E* [mmol m^{-2} s^{-1}]	g_s [mol m^{-2} s^{-1}]	WUE [mmol mol^{-1}]	C_i/C_a
–5	6.21 a	3.25 a	0.165 a	2.57 ab	0.772 a
–30	4.89 a	1.59 b	0.069 b	4.39 a	0.626 ab
–55	1.06 b	0.32 bc	0.003 c	3.58 a	0.665 b
–70	0.05 b	0.24 c	0.000 c	0.35 b	0.943 a

With –55 and –70 kPa of moisture deficits, an 85 % reduction in P_{Nmax} (Table 1) ($p<0.05$) was noticed. Extreme moisture deficit reduces leaf water potential and stomatal closure is the primary defence against turgor loss in leaves (Flexas *et al.* 1998). As a result, obstruction in CO$_2$ uptake under these extreme moisture regimes was the principal cause for the acute drop in P_{Nmax} values. Additionally, lower *α* values (reduced RuBPCO activity) negatively contributed to low values of P_{Nmax} under extreme moisture deficits. In a CO$_2$ perspective, C_i of 450 μmol mol^{-1} saturated P_{Nmax} values for controls. Rajasekaran *et al.* (1997) suggested that limitation in P$_i$ regeneration is the principle reason for down-regulation of P_N in response to increasing C_a. Under extreme moisture deficits the down-regulation occurred at C_i of 400 μmol mol^{-1}. The dramatic effect of dehydration superseded the diminutive beneficial effect of EC under extreme moisture deficits (–55 and –70 kPa). Near zero g_s, little RuBPCO functionality, limitation in supply of P_i in combination with impaired enzyme functionalities caused by dehydration may probably be the reason for this non-responsiveness towards EC. Any reduction in photosynthetic capacity is normally accompanied by an increase in C_i (Farquhar *et al.* 2001). The larger C_i/C_a values observed under extreme moisture deficits (Table 7) when compared with unstressed controls or mild moisture deficits also provide substantiation for this suggested mechanism. In general, down-regulation of P_N occurred with no reference to the magnitude of P_{Nmax}. Therefore, no feedback inhibition was noticed in our study.

Table 3. Effect of CO_2 concentrations on net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (E), and water use efficiency (WUE) of carrot cv. Red Core Chantaney leaves. Least square mean values followed by the same letter are not significantly different at $p<0.05$.

C_a [μmol mol^{-1}]	P_N [μmol m^{-2} s^{-1}]	E [mmol m^{-2} s^{-1}]	g_s [mol m^{-2} s^{-1}]	WUE [mmol mol^{-1}]	C_i/C_a
50	0.76 d	1.56 ab	0.078 a	–0.63 g	1.31 a
150	1.33 c	1.65 a	0.084 a	0.50 gf	0.86 b
250	2.34 c	1.60 a	0.076 ab	1.06 ef	0.80 bc
350	2.74 b	1.64 a	0.080 a	2.05 de	0.74 cd
450	2.96 ab	1.59 a	0.071 ab	2.62 de	0.74 cd
550	3.30 ab	1.44 abc	0.063 abc	3.16 d	0.73 cd
650	3.30 ab	1.20 bcd	0.048 bc	4.41 bc	0.69 d
750	3.40 ab	1.11 cd	0.042 c	4.81 b	0.70 d
850	3.58 ab	1.07 cd	0.039 c	5.49 ab	0.70 d
950	3.26 ab	1.01 d	0.038 c	5.51 ab	0.72 cd
1 050	3.99 a	0.97 d	0.035 c	6.95 a	0.69 d

Table 4. The interactive effect of CO_2 concentrations (C_a) and moisture deficits on net photosynthetic rate (P_N) [μmol m^{-2} s^{-1}] of carrot cv. Red Core Chantaney leaves. Least square mean values followed by the same letter are not significantly different at $p<0.05$.

C_a	Soil moisture potential [kPa]			
[μmol mol^{-1}]	–5	–30	–55	–70
50	–0.086 k-o	–0.530 mn	–0.044 j-o	–0.524 mn
150	2.247 c-i	1.809 d-k	0.497 g-m	–0.300 lm
250	3.689 b-h	3.928 a-g	0.874 g-l	–0.317 lm
350	6.775 a-f	6.670 a-f	0.982 g-l	0.052 i-m
450	8.621 a-d	7.222 a-f	1.119 g-l	0.172 i-n
550	9.824 ab	8.596 abc	1.014 g-l	0.177 i-n
650	10.497 a	7.610 a-f	1.755 e-k	0.250 i-n
750	10.841 a	8.502 a-e	1.591 f-k	0.287 i-n
850	11.394 a	8.245 a-e	1.757 e-k	0.393 h-n
950	10.763 a	8.793 abc	1.077 g-k	0.375 h-n
1 050	11.204 a	9.207 abc	2.127 c-k	0.663 g-k

R_D varied significantly among all moisture regimes. The value decreased by 22 % ($p<0.05$) at –30 kPa when compared with controls (Table 1). This was accompanied by an increase in α and sustained P_N. R_D reached its bare minimum at –55 kPa and surprisingly, the model predicted an increase in R_D at –70 kPa. An inverse relationship between P_N and R_D was found at –5 and –30 kPa, but was not noticed with other stress levels. The CO_2 compensation concentration (Γ) ranged from 63 to 91 μmol mol^{-1} of CO_2 up to a moisture deficit of –55 kPa, however, increased tremendously to 324 μmol mol^{-1} at–70 kPa. Low g_s found at the extremely high moisture deficit likely increased the Γ.

Interaction effects between moisture and C_a were noticed in all physiological parameters except WUE. Increasing C_a from 250 to 450 μmol mol^{-1} doubled P_N, however, beyond 650 μmol mol^{-1} C_a, only a 19 % increase was observed under all moisture regimes (Table 4). Similar regulated P_N increases up to 40 % were reported in other C_3 plants such as *Phaseolus vulgaris* L. (Radoglou *et al.* 1992) and carrots (Kyei-Boahen *et al.* 2003) in response to approximately 600 μmol mol^{-1} of C_a grown under limited nutrient supply or moisture deficit conditions. Kirschbaum (1994) attributed this decrease to feedback inhibition whereas Sharkey (1990) associated this effect with loss in sucrose synthesis capacity. However, research by Sage *et al.* (2002) and Rajasekaran *et al.* (1997) suggested limitation in P_i regenerative capacity as the principal cause. From our model parameter analysis, we hypothesize a strong possibility against feedback inhibition theory.

Table 5. The interactive effect of CO_2 concentrations (C_a) and moisture deficits on stomatal conductance (g_s) [mol m^{-2} s^{-1}] of carrot cv. Red Core Chantaney leaves. Least square mean values followed by the same letter are not significantly different at $p<0.05$.

C_a [µmol mol^{-1}]	Soil moisture potential [kPa] –5	–30	–55	–70
50	0.258 a	0.055 fghi	0.000 i	0.000 i
150	0.251 ab	0.072 e-i	0.011 ghi	0.000 i
250	0.198 abc	0.086 d-i	0.017 ghi	0.000 i
350	0.198 abc	0.109 c-g	0.011 ghi	0.000 i
450	0.180 bcd	0.100 c-i	0.004 hi	0.000 i
550	0.165 cde	0.087 f-i	0.000 i	0.000 i
650	0.128 c-f	0.064 d-i	0.000 i	0.000 i
750	0.113 d-g	0.054 e-i	0.000 i	0.000 i
850	0.113 d-g	0.044 f-i	0.000 i	0.000 i
950	0.107 e-h	0.044 f-i	0.000 i	0.000 i
1 050	0.103 e-i	0.038 f-i	0.000 i	0.000 i

Table 6. The interactive effect of CO_2 concentrations (C_a) and moisture deficits on transpiration rate (E) [mmol m^{-2} s^{-1}] of carrot cv. Red Core Chantaney leaves. Least square mean values followed by the same letter are not significantly different at $p<0.05$.

C_a [µmol mol^{-1}]	Soil moisture potential [kPa] –5	–30	–55	–70
50	4.371 a	1.139 a-e	0.280 k-n	0.260 k-n
150	4.294 a	0.654 f-l	0.406 i-l	0.244 k-n
250	3.674 ab	0.619 h-l	0.495 i-l	0.245 k-n
350	3.684 ab	0.595 ij	0.379 j-m	0.230 k-n
450	3.521 abc	0.654 f-l	0.369 j-m	0.230 k-n
550	3.285 bcd	0.585 ij	0.321 k-	0.231 k-n
650	2.835 b-h	0.588 ij	0.261 k-n	0.208 k-n
750	2.588 d-h	0.568 ij	0.288 k-n	0.230 k-n
850	2.580 d-h	0.572 ij	0.249 k-n	0.237 k-n
950	2.515 d-h	0.533 j	0.228 k-n	0.242 k-n
1 050	0.986 h-n	0.526 j	0.257 k-n	0.247 k-n

The g_s was maximum (0.25 mol m^{-2} s^{-1}) at the lowest moisture stress level (–5 kPa) and at the lowest C_a (50 µmol mol^{-1}) (Tables 2 and 3). Stomatal closure for conservation of moisture in leaves is a common strategy of drought tolerance in many crops (Anyia and Herzog 2004). Our results confirm the negative relationship of moisture stress with g_s and E, a well established phenomenon (Leuning *et al.* 1998). The g_s declined gradually with increasing C_a at –5 and –30 kPa moisture levels, but the differences were significant only beyond 450 C_a (Table 5). At a –55 kPa, g_s reached its bare minimum of 0 mol m^{-2} s^{-1} at a CO_2 concentration of 550 µmol mol^{-1} and remained the same thereafter (Table 5). At –70 kPa, g_s remained at 0 mol m^{-2} s^{-1} despite C_a increased. These results are in acceptance with observations made by Rodriguez *et al.* (2001). They have reported a decrease in g_s in wheat plants under EC growth conditions. E closely followed g_s as expected. Moisture stress negatively influenced E and it followed the same trend observed with g_s (Table 6). E remained unaffected when the CO_2 concentrations were raised from 50 to 650 µmol mol^{-1}, however, the concentrations above 650 µmol mol^{-1} showed a significant negative impact. Furthermore, 1 050 µmol mol^{-1} C_a resulted in the lowest E (0.99 mmol m^{-2} s^{-1}) (Table 6).

Table 7. The interactive effect of CO_2 concentrations (C_a) and moisture deficits on C_i/C_a ratio of carrot cv. Red Core Chantaney leaves. Least square mean values followed by the same letter are not significantly different at $p<0.05$.

C_a [µmol mol^{-1}]	Soil moisture potential [kPa] –5	–30	–55	–70
50	0.946 b-i	1.139 a-e	1.028 a-h	2.000 a
150	0.837 b-j	0.654 f-l	0.662 e-l	1.194 bc
250	0.773 b-j	0.619 h-l	0.808 e-l	1.086 b-g
350	0.767 b-j	0.595 ij	0.650 g-l	0.886 b-j
450	0.753 b-j	0.654 f-l	0.642 g-l	0.822 d-k
550	0.743 b-j	0.585 ij	0.526 c-k	0.866 bj
650	0.726 b-j	0.588 ij	0.549 j	0.846 d-k
750	0.719 b-j	0.568 ij	0.616 ij	0.848 d-k
850	0.732 b-j	0.572 ij	0.608 ij	0.830 d-k
950	0.762 b-j	0.533 j	0.673 e-l	0.852 d-k
1 050	0.763 b-j	0.526 j	0.618 ij	0.798 e-k

The C_i/C_a ratio is an indicator of stomatal limitation of photosynthesis (Farquhar and Sharkey 1982) and it is inversely related to WUE (Brodribb 1996). A high value normally reflects lower P_N values indicating high conversion rates. Minimum C_i/C_a ratio (0.53) or maximum utilization efficiency was observed at –30 kPa level and at C_a of 1 050 µmol mol^{-1} (Table 7). Similar ratios closer to 0.7 under EC and ambient C_a were reported by Drake *et al.* (1997). Irrespective of moisture regimes, increasing C_a resulted in decreasing C_i/C_a ratios, however, no significant reductions were noticed beyond C_a concentrations of 650 µmol mol^{-1} (Table 7). A downward acclimation towards increasing C_a may have happened at this C_a. This corroborates well with P_{Nmax} values observed in this study. In moisture perspective, mild moisture deficit registered a minimum C_i/C_a ratio following –5, –55, and –70 kPa.

Moisture deficit and EC controlled WUE independently. WUE increased in response to mild (–30 kPa) and moderate (–55 kPa) water deficit when compared with unstressed controls (Table 2). However, extreme moisture stress (–70 kPa) reduced WUE by 40 %. Abscisic acid transport from roots to leaves *via* xylem vessels for signalling partial shut-down of stomata during water deficits was suggested to be the most common moisture conservation strategy of plants (Liu *et al.* 2005). This resulted in reduced transpiration losses sustaining P_N thus achieving better WUE (Liu *et al.* 2005). Severity of stress dictates the degree of WUE benefits caused by drought (Chen *et al.* 1993). In our study, acute dehydration presumably affected the photosynthetic apparatus, closed stomatal apertures may have resulted in extremely low WUE. WUE increased with increase in C_a and was at its maximum at 1 050 μmol mol^{-1} (Table 3). EC decreased the g_s and resulted in better WUE due to reduction in E (Magliluo *et al.* 2003). Morison (1985) indicated that the need to maintain a constant C_i/C_a ratio evokes the partial closure of stomatal apertures. It is widely documented that every change in g_s brings about the change in WUE as it directly governs the magnitude of E (Hogan *et al.* 1991). Furthermore, a decreased water consumption under EC growth conditions was reported for a wide range of crops by Lutze and Gifford (1995), Baker *et al.* (1997), and Kyei-Boahen *et al.* (2003). A similar stimulating effect on WUE, especially through high P_N/g_s ratio under mild drought to EC and little or no benefit under severe water stress has been documented in *Quercus robur* (Picon *et al.* 1997). Such responses relate well with the maximum WUE registered at a –30 kPa moisture level providing evidence for better utilization of C_i at EC (Chaves and Pereira 1992). Conversely, moisture stress at –70 kPa registered the maximum C_i/C_a ratio at 50 μmol mol^{-1} C_a. Although there is enough supply of CO_2, a severe water stress has hampered the photosynthetic apparatus rendering low P_N at this moisture level.

In summary, the carboxylation efficiency (α) doubled at –30 kPa moisture level (0.7 μmol m^{-2} s^{-1}) in combination with EC, and declined by more than 3 times at –70 kPa (0.01 μmol m^{-2} s^{-1}) when compared with –5 kPa (0.03 μmol m^{-2} s^{-1}) suggesting inactivation of RuBPCO under extreme moisture stress. P_{Nmax} was positively influenced by EC only under mild moisture deficit. R_D declined with increasing P_N up to –30 kPa moisture stress, however, this relationship was not evident at –55 and –70 kPa. The acclimation of P_N occurred at around 600 and 450 μmol mol^{-1} for moisture deficits of –5 and –30 kPa, respectively. Decline in P_i regenerative capacity presumably caused this down-regulation. P_N remained unaltered under mild water stress (–30 kPa) but declined by 98 % at –70 kPa when compared with –5 kPa. The g_s was 0.25 μmol mol^{-1}, at its maximum at the lowest moisture level and minimum C_a establishing an inverse relationship. E followed a similar trend as g_s. WUE did not differ between –5 and –55 kPa moisture deficits. WUE increased linearly with C_a. Minimum C_i/C_a (0.51) was registered with –30 kPa. Results clearly indicate that EC had no substantial effects on sustaining P_N at moderate and extreme moisture levels, however, showed a positive influence under mild moisture stress.

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