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Effects of prolonged drought stress and nitrogen deficiency on the respiratory O_2 uptake of bean and pepper leaves

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Abstract

We analyzed the combined effects of mild drought stress and severe nitrogen (N) deprivation on respiration of acclimated mature leaves of beans (*Phaseolus vulgaris* L. cv. Garrofal) and peppers (*Capsicum annuum* L., pure line B6). Rates of oxygen uptake were measured polarographically, and inhibitors were added to the closed cuvette to compare the effects of environmental stress on the cytochrome (cyt) and alternative pathways of mitochondrial respiration. Dark oxygen uptake was decreased by the water deficit treatment in both plants, and in the case of N limitation leaf respiration rates (R_D) of peppers were also reduced. R_D of leaves of beans and peppers grown under N-limiting conditions did not follow the decrease in leaf N concentration, since R_D expressed per unit of tissue N was considerably higher in the N-stressed leaves. Values obtained with specific inhibitors of the two terminal oxidases of mitochondrial respirations suggested that the cyt pathway of respiration was affected by mild drought and severe N stress. When plants were exposed to both environmental stresses, leaf respiration response was similar to that under N limitation, in this case the most severe stress.

Additional key words: biomass; *Capsicum annuum*; carbon and nitrogen contents; cytochrome pathway; *Phaseolus vulgaris*, salicylhydroxamic acid; water stress.

Introduction

Water and N availability are some of the most common stress factors that inhibit plant growth. Mild drought inhibits whole plant photosynthetic carbon assimilation

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Abbreviations: cyt - cytochrome; R_D - dark respiration rate; SHAM, salicylhydroxamic acid.

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increasing stomatal closure and reducing leaf elongation rate (Hanks and Rasmussen 1982, Peñuelas *et al.* 1993a). Although photosynthesis of higher plants can acclimate to water stress (Matthews and Boyer 1984, Sen Gupta and Berkowitz 1989), information on acclimation of respiration is rather confusing. As the decrease in photosynthesis and plant growth should result in decreased leaf R_D, the maintenance of large amounts of organic solutes in the cells may increase leaf R_D. The balance between growth and maintenance components of respiration results in that R_D either increases, decreases, or remains constant in water stressed plants (Amthor 1989, Lambers 1985).

Limiting N supply also reduces rates of photosynthesis, and plants tend to allocate more biomass to non-photosynthetic organs slowing plant growth and reducing N concentration of tissues. R_D is positively correlated with tissue N concentration (Amthor 1989, Lambers *et al.* 1989) because large investments of reduced N in leaves can increase respiratory costs (Penning de Vries 1975). An important factor of these costs is related to larger maintenance and turnover rates of proteins and other N containing compounds (Bouma *et al.* 1994). Thus, low N supply is often reported to slow respiration activity (Kishitani and Shibles 1986, Makino and Osmond 1991, Byrd *et al.* 1992), to stimulate accumulation of saccharides, and to reduce activity of enzymes involved in respiratory pathways (Paul and Stitt 1993).

Despite the importance of water stress and N deficiency on plant growth, little is understood of their combined effects on the acclimation of plant respiration. In the present study, we analyzed the combined effects of mild drought stress and low N supply on the R_D of leaves of N-fixing beans and non-N-fixing peppers. During the same experiment, water and N stressed plants of beans and peppers had lower biomass production in all their organs according to lower photosynthetic rates (Peñuelas *et al.* 1993a). The negative effect of N deficiency on plant growth was stronger in peppers than in N-fixing beans (Peñuelas *et al.* 1993a). We used respiratory inhibitors (cyanide for cyt *c* oxidase, and salicyl-hydroxamic acid, SHAM, for the alternative pathway) in a liquid phase oxygen electrode to obtain information on the relative response of the cyt and alternative pathways to the different treatments. The cyt pathway of plant respiration is coupled to synthesis of ATP, whereas the alternative pathway is not associated to net synthesis of ATP releasing energy in form of heat. Of course, SHAM-resistant respiration of the cyt pathway (respiration in the presence of SHAM) does not necessarily reflect the activity of the cyt pathway before the inhibitor was added (Millar *et al.* 1995, Ribas-Carbó *et al.* 1995). Ribas-Carbó *et al.* (1995) have demonstrated, using isotope fractionation techniques, that the electrons from the alternative pathway can be redirected to the cyt pathway upon the addition of SHAM. Thus, SHAM-sensitive (formerly, activity of the alternative pathway, v_{alt}) and SHAM-resistant respiration (formerly activity of the cyt pathway, v_{cyt}) should be treated with caution when referenced to the activity of the pathways before the addition of the inhibitors. Inhibitors provide information on whether or not the non-phosphorylating cyanide-resistant respiration was engaged before the addition of the inhibitors. They can be useful to qualitatively compare effects of different treatments on the two electron transport pathways (Day *et al.* 1996).

Materials and methods

Plants: Seeds of *Phaseolus vulgaris* L. cv. Garrofal and *Capsicum annuum* L., pure line B6 were grown in growth cabinets at 25/20 °C (day/night), 14/10 h (light/dark) photoperiod cycle at 300-500 μmol m^{-2} s^{-1}. Bean plants were inoculated with *Rhizobium sp.* to favor N fixation. At the time of harvest, nodulation of roots was evident across all the treatments. The 2 500 cm^3 pots contained a 1:1 sand and peat mixture. For the water stressed plants, pots were fertirrigated when soil water potential reached -0.04 MPa, and never reached values over -0.01 MPa. The two levels of N supply were established by irrigating with high N solution containing 4.9 mM NO_3^-, 1.4 mM NH_4^+, 0.6 mM H_2PO_4, 2.2 mM SO_4^{2-}, 2.8 mM K^+, 4.4 mM Ca^{2+}, 1 mM Mg^{2+}, 8.9 μM Fe^{2+}, and 9.1 μM Mn, 1.8 μM Zn, 7.9 μM Cu, 1.0 μM Mo, 4.6 μM B, 25 μM Mg. The limited N supply solution had only 1.2 mM NO_3^- as N source.

Respiratory measurements: After three months of growth, fully developed leaves were sampled for measurements of R_D. Leaf disc samples were kept in the dark in 0.2 mM $CaCl_2$ for 30 min before measurements. Oxygen uptake rates were measured in the dark at 25 °C, using a Clark-type oxygen electrode (*Rank Brothers*, Cambridge, UK), in rapidly stirred, ambient air saturated 20 mM MES buffer (pH 6.0), containing also 0.2 mM $CaCl_2$. Inhibitors (KCN and SHAM) were added to the cuvette from stock aqueous solutions with a microsyringe. 1 mM KCN was used to assess the cyanide-resistant respiration, and 4 mM SHAM was used to assess the SHAM-resistant respiration for both species. Residual respiration (O_2 uptake in the presence of KCN and SHAM) was less than 15 % of the uninhibited rate, assuring that both mitochondrial terminal oxidases were inhibited in the leaf discs.

Same leaf discs samples used for respiration were oven-dried at 65 °C for several days, and ground for total C and N measurements conducted using a gas chromatograph (*Perkin-Elmer*, USA) which allowed for the expression of the results on a dry mass and N bases.

Results

Carbon and N concentration of leaves: Mild drought stress decreased the dry mass per unit area in leaves of beans but not in leaves of peppers (Table 1). The low N supply treatment had little effect on the dry mass per unit area in beans and peppers, although leaves of non-N-fixing peppers decreased the dry mass to leaf area ratio in the low water and low N treatment (Table 1). Low water and N treatments had little effect on carbon concentration of mature leaves of beans. However, leaf carbon concentration of peppers was increased in the low water treatment and decreased in the low water and low N treatment (Table 1). Leaf N concentration was clearly reduced in the N deficient treatments in both crop plants (Table 1). Similar results were previously found for beans and peppers (Peñuelas *et al.* 1993b, Peñuelas and Matamala 1994).

Respiratory O_2 uptake: Mild drought stress resulted in inhibition of mature leaf respiration of beans especially when expressed on a leaf area basis (Fig. 1). However, N limitation showed little effect on the R_D (on either an area or a dry mass basis) of N-fixing bean plants (Fig. 1). In non-N fixing plants of pepper, both N and water stress resulted in moderate reduction of R_D (Fig. 1). The stressed plants of beans and peppers showed severe N limitations and moderate water stress by decreasing growth and changing allocation and partitioning of biomass, although N stress was smaller in beans (Peñuelas *et al.* 1993a). When respiration was expressed on a tissue N basis, water stress resulted in lower R_D in peppers but not in beans (Fig. 1). However, when N supply was limited, leaf R_D per unit of tissue N of beans and peppers was increased (Fig. 1) according to the lower N concentration of N-stressed leaves (Table 1).

Table 1. Dry mass per unit area and carbon and nitrogen concentration of mature leaves of beans and peppers grown at different water and nitrogen regimes. Values are means of 6-8 replicates ± SE. Letters indicate statistically different groups ($p<0.05$).

Plant	Treatment	Dry mass [g m^{-2}]	Carbon content [g m^{-2}]	[% dry mass]	Nitrogen content [g m^{-2}]	[% dry mass]
Phaseolus	Control	24.5 ± 0.8^{a}	9.9 ± 0.6^{a}	36 ± 2	0.83 ± 0.06^{a}	3.4 ± 0.2^{a}
	Low water	18.5 ± 1.4^{b}	6.7 ± 0.4^{b}	36 ± 3	0.64 ± 0.05^{b}	3.5 ± 0.1^{a}
	Low N	20.2 ± 1.9^{ab}	7.1 ± 0.5^{b}	35 ± 4	0.53 ± 0.06^{bc}	2.7 ± 0.2^{b}
	Low water and N	19.9 ± 1.9^{b}	6.9 ± 0.3^{b}	35 ± 3	0.48 ± 0.04^{c}	2.4 ± 0.2^{b}
Capsicum	Control	19.1 ± 0.7^{a}	6.8 ± 0.5^{a}	34 ± 2	0.60 ± 0.05^{a}	3.5 ± 0.2^{a}
	Low water	19.2 ± 1.6^{a}	7.3 ± 0.6^{a}	38 ± 3	0.71 ± 0.05^{a}	3.7 ± 0.3^{a}
	Low N	18.7 ± 1.0^{a}	6.7 ± 0.3^{a}	36 ± 2	0.27 ± 0.02^{b}	$A.5 \pm 0.1^{b}$
	Low water and N	15.2 ± 1.1^{b}	5.1 ± 0.4^{b}	32 ± 2	0.22 ± 0.03^{b}	1.4 ± 0.2^{b}

The use of specific inhibitors of the two terminal oxidases of mitochondrial electron transport showed that the SHAM-resistant respiration of the cyt pathway was very much reduced in leaves of bean and peppers under water and N stress (Table 2) but the stresses had no effect on the cyanide-resistant respiration of the alternative pathway. The inhibition of O_2 uptake by SHAM suggested that the non-phosphorylating alternative pathway of respiration was engaged especially in N-stressed leaves (SHAM-sensitive respiration; Table 2).

Discussion

Stressed leaves of peppers had lower R_D than control ones, and there was little change in R_D in water and N stressed leaves of beans. However, when R_D was expressed per unit of tissue N, mild drought stress resulted in slight decrease of R_D in peppers but N deprivation resulted in higher R_D in both plants mainly due to the decrease in N concentration in N-stressed leaves. SHAM-resistant respiration was reduced, and the alternative pathway appeared to be engaged in water and N stressed leaves. Respiration responses to the combination of mild drought and N limitation stresses were similar to those for the N deprivation experiment, the most severe stress affecting growth in this experiment (Peñuelas *et al.* 1993a).

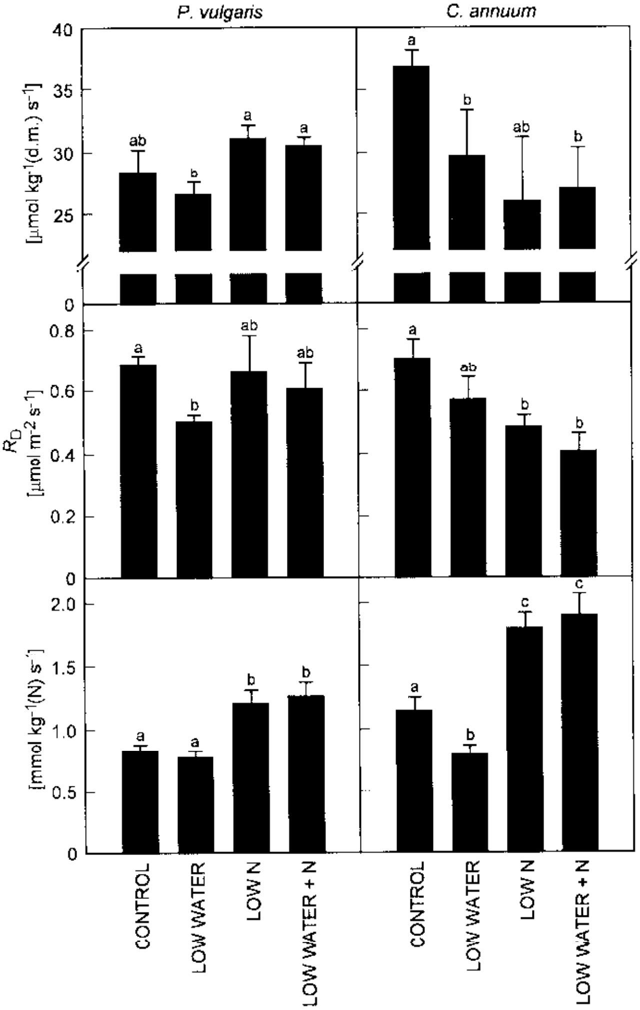

Fig. 1. Mature leaf respiration rates (R_D) of *Phaseolus vulgaris* or *Capsicum annuum* plants grown in normal conditions of water and N (Control), under water stress (Low-water), under N deficiency (Low N), or under simultaneous water and N stress (Low water&N). Values are means of 6-8 replicates ± SE. Letters indicate statistically different groups (p<0.05).

Mild drought stress resulted in slight reduction of R_D in both plants. In bean leaves, R_D was significantly lower only when expressed on a leaf area basis but not on a leaf dry mass basis (Fig. 1) due to a decrease in the dry mass to leaf area ratio (Table 1). Brown and Thomas (1980) also observed that leaf R_D of beans under water stress was reduced when expressed per unit of leaf area but not on a dry mass basis. McCree (1986) suggests that prolonged water stress usually acclimates plants to maintain lower leaf water potentials than non-acclimated stressed plants, resulting in little change in R_D due to a constant photosynthetic carbon gain and growth. When R_D was expressed per unit of total leaf N, rates of O_2 uptake were still lower in leaves of pepper exposed to prolonged mild drought (Fig. 1). Despite the similar leaf N concentration of leaves of pepper under water stress (Table 1), R_D was not maintained at the level of the unstressed plants (Fig. 1) according to the positive correlation between respiration and tissue N (Amthor 1989). Hence the N concentration of water stressed leaves did not maintain the respiratory energy demand proportionally. Wilson *et al.* (1980) showed that prolonged water stress reduced leaf R_D but the proportion of total respiration accounted for maintenance processes increased as water stress developed in the leaves. However, water stress resulted in higher R_D in wheat leaves due to a slight increase in energy demand for protein synthesis and turnover and transport of metabolites (Zagdańska 1995), processes supported by maintenance respiration. Amthor (1989) concludes that whole-plant maintenance respiration decreases and the proportion of specific respiration invested in the maintenance fraction increases with decreased leaf water potential. Thus, accumulation of organic compounds in leaves of peppers to avoid water losses could explain the slight increase in carbon and N content per unit leaf area, and the decrease in leaf respiration expressed per unit N under water stress.

Table 2. Estimates of respiratory electron transport pathways [μmol(O_2) m^{-2} s^{-1}] in leaf discs of beans and peppers under two different water and nitrogen regimes. Values are means of 4 replicates ± SE. Letters indicate statistically different groups ($p<0.05$).

Plant	Treatment	SHAM-resistant	SHAM-sensitive	Cyanide-resistant
Phaseolus	Control	0.66 ± 0.01^a	0	0.61 ± 0.03
	Low water	0.43 ± 0.04^b	0.10 ± 0.06	0.67 ± 0.10
	Low N	0.31 ± 0.02^c	0.29 ± 0.04	0.71 ± 0.04
	Low water and N	0.41 ± 0.04^{bc}	0.22 ± 0.03	0.72 ± 0.02
Capsicum	Control	0.70 ± 0.03^a	0	0.47 ± 0.05
	Low water	0.49 ± 0.06^b	0.05 ± 0.080	0.49 ± 0.02
	Low N	0.35 ± 0.03^b	0.13 ± 0.02	0.48 ± 0.01
	Low water and N	0.33 ± 0.04^b	0.11 ± 0.03	0.50 ± 0.06

Leaves of pepper grown under limiting N supply showed a slight decrease in R_D specially when expressed per unit leaf area (Fig. 1). Limiting N supply often results in reduction of leaf R_D (Boot *et al.* 1992), because of reduced energy requirements for biosynthesis and ion and saccharide transports as a result of inhibited N assimilation. At whole plant level, it is observed that despite the proportion of photosynthetic products that are respired increases with decreasing N supply (Van der Werf *et al.* 1992), the specific respiration activity of roots and leaves often decreases with reduced N supply (Bingham and Farrar 1989, Boot *et al.* 1992). In our experiment, both crop plants increased leaf R_D when expressed per unit of total N (Fig. 1) due to a marked decrease in N concentration of the leaves (Table 1). When plants were grown under low water and low N supplies, R_D mirrored the pattern observed for N-deficient, well watered plants (Fig. 1), because severe N deficiency caused more penalties for growth than mild drought stress (Peñuelas *et al.* 1993a). Severe N stress seemed to overcome the effects of mild water stress on leaf R_D.

The inhibitor of the alternative oxidase, SHAM, reduced the O_2 uptake of the alternative pathway specially in the N-deficient plants, suggesting that the non-phosphorylating alternative pathway was engaged in stressed leaves (Table 2). The SHAM-resistant respiration through the cyt pathway was more reduced in N deficient plants than in water stressed plants, specially in peppers (Table 2). Thus the N deficiency limited the substrate supply for respiration or perhaps the activity of enzymes needed for carbon metabolism, as described by Paul and Stitt (1993), including those involved in mitochondrial respiration. Makino and Osmond (1991) observed that total mitochondrial protein-N content decreased as N supply was increasingly limiting. The maximum activity of the cyt *c* oxidase of photosynthetic organs of *Scirpus olneyi* was inhibited when plants were grown under N-limitation and low temperature (González-Meler 1995). However, soluble mitochondrial enzyme activity of pea leaves increased as leaf N concentration decreased due to soil N limitation (Makino and Osmond 1991), although the authors related such an increase in soluble mitochondrial enzymes to changes in photorespiration. N deficiency seemed to have bigger effect on the SHAM-resistant respiration than the water stress in the low water and low N treatment in peppers (Table 2), perhaps because plant growth was more inhibited under deprivation of N than under low water supply (Peñuelas *et al.* 1993a). The results suggest that acclimations of respiration to water and N stress more likely take place at the level of the cyt pathway.

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