Atmospheric CO_2 increase benefits symbiotic N_2 fixation by legumes under drought

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Leguminous plants are considered to have a competitive advantage under global climate change because of increased rates of symbiotic nitrogen (N_2) fixation in response to increased atmospheric CO_2. However, this hypothetical advantage may not be realized under actual climate change due to the associated increase in frequency and duration of drought, as N_2 fixation in legume species such as soybean is sensitive to soil drying. Yet, it has been discovered that N_2 fixation in soybean becomes drought-tolerant under increased CO_2 concentration. The reduced susceptibility of N_2 fixation to drought was associated with an increase in total nonstructural carbohydrates and a decrease in ureides in leaves. These results empirically indicate that legumes will have substantial comparative advantage over cereals under climate change.

THE increase in atmospheric CO_2 concentration associated with global climate change is well-documented[1,2], and these increases are expected to be even more dramatic in the future[3]. Plant photosynthesis rates are stimulated by increased CO_2 concentration[2,4], but increases in overall plant growth in the natural environment are less certain. Plant growth is often limited by factors other than potential photosynthetic rate, of which water and nitrogen availability are the two most commonly restrictive resources[5,6]. It has been hypothesized that legumes might particularly benefit from increased atmospheric CO_2 because their capability of establishing symbioses with N_2-fixing bacteria allows them to minimize natural nitrogen limitations to growth[7,8]. Legumes have been shown to be highly responsive to increased CO_2 under well-watered conditions[4,8–10].

An important consideration, however, is that N_2 fixation in some legumes is highly sensitive to soil drying[11]. In fact, N_2 fixation activity has been shown in soybean (*Glycine max* L. Merr.) to decrease well in advance with soil drying compared to any other plant process[12,13]. Because global environment changes associated with increased atmospheric CO_2 are likely to include weather variability, including more frequent and severe episodes of drought[14,15], there is a possibility that the important N_2 fixation advantage of legumes might be neutralized or completely lost under climate change.

It is, therefore, critical to determine if there is any inherent change in the response to soil drying of key physiological processes that contribute to legume productivity. While it is well-recognized that increased CO_2 may slowdown the loss rate of soil-water[1,9], it is unknown if the basic response of plant processes to soil drying are changed when compared at equivalent soil-water contents. Experiments were undertaken to measure plant responses, including N_2 fixation to a drying cycle under atmospheres of ambient CO_2 and 700 μmol CO_2 mol^{-1}. Soybean (cv. Braxton) plants were grown in 2.3 l pots for four weeks following germination in a common greenhouse subjected to ambient CO_2 concentrations in order to avoid confounding factors during the drying cycle of differing plant sizes as a result of early growth under different CO_2 concentrations. For the CO_2 and drying treatments, the plants were divided into two groups and transferred to identical greenhouses; one was maintained at ambient atmospheric CO_2 (approximately 360 μmol mol^{-1}) and the other was maintained at 700 μmol CO_2 mol^{-1}. Half the plants in each greenhouse were well-watered and half were subjected to soil drying over 17 days[16,17].

The net daily decrease in water per pot in simulated drought treatments was maintained at 70 g. As a result, differences in soil drying between the two CO_2 treatments were negated. On each day, measurements were made of plant transpiration, N_2 fixation by acetylene reduction activity, and leaf area development. Leaf photosynthesis rates were made on days with at least 1000 μmol m^{-2} s^{-1} photosynthetically active radiation. These physiological data from each individual drying pot were expressed relative to the mean of the values obtained for the control pots for each CO_2 treatment. To facilitate comparisons at equivalent soil moisture levels, the fraction of transpirable soil-water for each pot was calculated each day based on the measured pot weight[16,17].

The second objective was to investigate the mechanism of N_2 fixation sensitivity to soil drying by measuring ureide and total nonstructural carbohydrate in the shoots (exp. 1) or leaves (exp. 2). Accumulation of ureides in the shoot of soybean has been closely linked to the depression in N_2-fixation activity in response to soil drying[12,18]. An important aspect of this study was to determine if the CO_2 treatments resulted in altered levels of ureides.

Increased CO_2 resulted in water conservation under both well-watered and drought treatments, as expected (Table 1). The daily re-watering of plants, however, minimized any influence of water conservation in this study on plant response. Nevertheless, under drought conditions, plants subjected to 700 μmol CO_2 mol^{-1} had significantly more growth than those under ambient CO_2 (Table 1). The basis of this growth difference was not associated with relative response of plant gas exchange and leaf-area development to drought (Figure 1). Each of these processes showed similar responses between the CO_2 treatments, with a decline in rate occurring between fraction of transpirable soil water of 0.3 and 0.4. The responses for the two experiments were virtually identical

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and similar to previous reports[19,20]. These results indicated that no special change in the response to drought of these processes occurred when subjected to increased atmospheric CO_2.

In contrast, it was discovered that the N_2-fixation activity response to soil drying was greatly altered by increased CO_2. Consistent with previous observations, N_2 fixation under ambient CO_2 was sensitive to soil drying and decreased in response to soil drying compared to the other measured processes[11–13]. In sharp contrast, N_2 fixation became highly tolerant to soil drying under the 700 μmol CO_2 mol^{-1} treatment (Figure 1). Only in the final stage of soil drying, when the drought stress was quite severe, did N_2 fixation under 700 μmol CO_2 mol^{-1} finally decrease. These results indicate that the advantage of legumes under global climate change is even greater than anticipated, because of the induced increase in N_2-fixation tolerance to drought.

The association between the induced N_2-fixation sensitivity to drought, and shoot or leaf ureide and total non-structural carbohydrate (TNC) levels was also observed in this study. Increased CO_2 resulted in dramatically decreased levels of ureide (Table 1). Particularly important was the fact that at the end of drought stress, ureide levels under increased CO_2 had risen only slightly greater compared to the levels for ambient CO_2 under well-watered conditions. Low ureide levels have been previously shown in comparison among legume species[13] and among soybean cultivars[21] to be associated with N_2-fixation drought tolerance. Not surprisingly, TNC increased in response to increased CO_2 (Table 1). Under drought, there was a large decrease in TNC in both CO_2 treatments, but TNC under the increased CO_2 decreased only to the level of the well-watered, ambient CO_2 treatment. These data indicate the possibility that increased TNC resulting from elevated CO_2 might result in decreased ureide levels in the shoots. Decreased ureide levels under elevated CO_2 are associated with N_2-fixation drought tolerance[22].

Table 1. Interaction of drought stress and atmospheric CO_2 concentration on soybean biomass, water use, and total nonstructural carbohydrates and ureide levels. Data are based on five plant replicates. Means followed by the same letter within a row are not significantly different as determined by LSD ($P \leq 0.5$)

	Well-watered		Drought-stressed	
$[CO_2]$ concentration	360	700	360	700
Total dry weight, DW (g $plant^{-1}$)	19.1[a]	21.1[a]	10.4[c]	13.4[b]
Total water use (g $plant^{-1}$)	2586[a]	1915[b]	1434[c]	1282[d]
TNC (μmol glucose g^{-1} DW)				
Leaf	103.1[b]	224.2[a]	22.8[d]	46.5[c]
Nodule	11.7[c]	18.4[c]	36.1[b]	66.6[a]
Ureide (μmol g^{-1} DW)				
Leaf	4.5[bc]	2.8[c]	11.7[a]	6.8[b]
Nodule	21.0[c]	22.4[c]	52.7[a]	37.8[b]

Soybean plants were grown in a sandy loam soil in 2.3 l pots for four weeks following germination in a greenhouse subjected to ambient $[CO_2]$. Treatments were imposed by dividing the plants into two groups and transferring them to identical greenhouses; one was maintained at ambient atmospheric $[CO_2]$ (approximately 360 μmol mol^{-1}) and the other was maintained at 700 μmol CO_2 mol^{-1}.

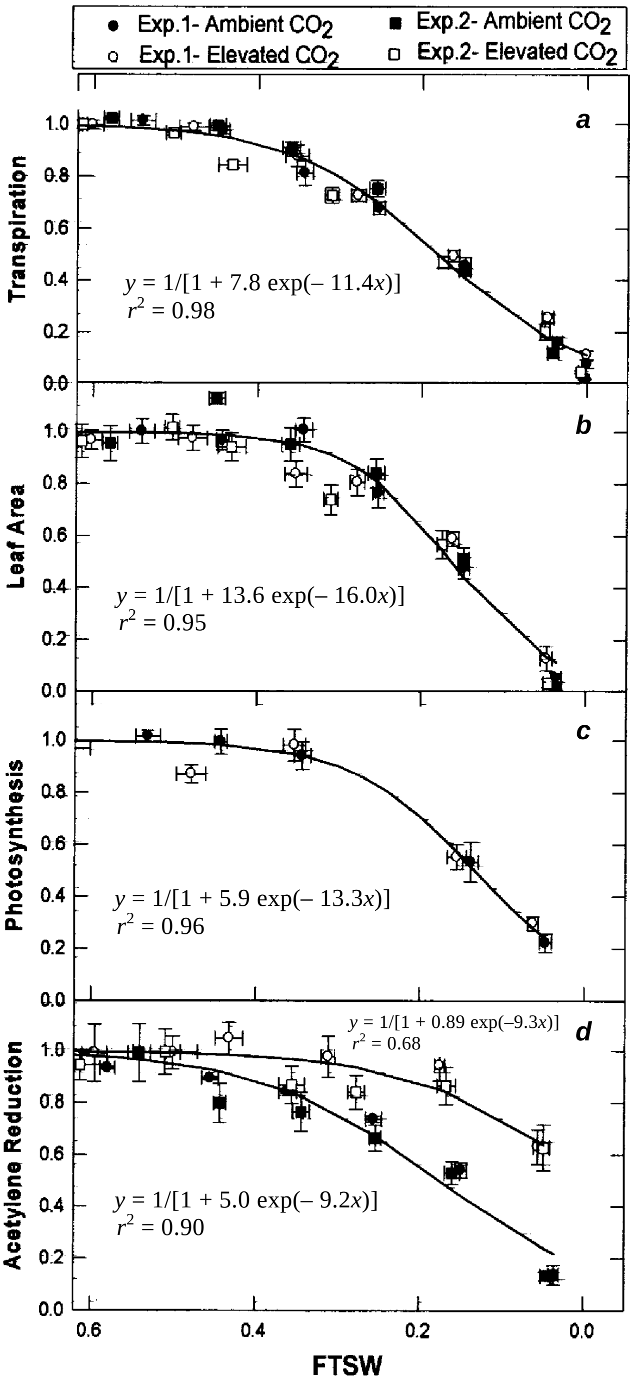

Figure 1 ***a*–*d*.** Mean daily measures of soybean plant transpiration, leaf area development, photosynthesis and N_2 fixation (acetylene reduction) rate, respectively plotted against fraction of transpirable soil water (FTSW)[17,18]. Data are the responses of plants subjected to drying soil normalized on a daily basis against well-watered plants subjected to the same CO_2 treatment. Since there was no response to soil drying at FTSW < 0.6, these graphs present data only at FTSW < 0.6. In all cases, data are means (± standard error) of five replicates. Equations represented by solid lines were obtained from nonlinear regression.

In summary, the discovery that the sensitivity of N_2 fixation to soil drying is ameliorated at increased CO_2 has substantial significance for both natural and agricultural ecosystems. The anticipated advantage under global climate change of legumes is likely to be greater than originally presumed. Certainly, in natural ecosystems, it appears that legumes could be at a substantial competitive advantage over non-nitrogen-fixing species, and this could alter species distribution in favour of legumes in many ecosystems. In agricultural systems, amelioration of the sensitivity of N_2 fixation to soil drying under increased CO_2, combined with the inherent stimulation of growth by CO_2, is likely to result in substantial increases in the yielding capability of legumes, especially soybean.

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Enhanced transformation of plant cells following co-bombardment of VirE2 protein of *Agrobacterium tumefaciens* with DNA substrate

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***Agrobacterium tumefaciens* transfers a segment of its Ti plasmid (T-DNA) to the nucleus of the host plant cell in single-stranded form. Of the two specific proteins, VirE2, that contains a nuclear localization signal plays a major role to mediate transport of target DNA into the nucleus of the plant cell. Here we report a DNA delivery method termed 'Vir-biolistic' that utilizes this property of the VirE2 protein for delivery of DNA target into the plant cell nucleus through microprojectile bombardment. Transient expression assay using *gus* reporter gene in immature wheat and maize embryos indicated higher number of transformants with Vir-biolistic compared to the popular 'biolistic' procedure of DNA delivery used for the production of transgenic plants. We also found that in wheat calluses co-bombarded with VirE2 protein, the stable integration frequencies of *gus* genes were threefold higher compared to the biolistic method. Thus, the Vir-biolistic method has the potential to increase the transformation efficiency in plants that are not amenable to *Agrobacterium*-mediated transformation.**

AGROBACTERIUM prepares and transfers DNA complex into the plant cell. The process is triggered by the activation of a series of *vir* genes residing on the Ti plasmid, by signals obtained from the host plant cell[1]. The activation of *vir* genes results in the generation of site-specific nicks within the T-DNA borders and production of linear single-stranded DNA (ssDNA) molecules (T-strands) which arrive in the plant cell as a single-stranded intermediate[2,3]. During transit, the T-strand is not naked, but is associated (T-complex) with two Vir proteins, VirD2 and VirE2. Both VirD2 and VirE2 possess functional nuclear localization signals that guide the T-DNA to the plant cell nucleus. The VirE2 is a ssDNA-binding protein that binds tightly and cooperatively without sequence specificity, coating the entire length of the T-strand[4–6]. After entry into the plant cell, the T-complex is targetted to the nucleus by a mechanism not fully understood as yet. It has been demonstrated that the VirE2 protein mediates nuclear uptake of ssDNA in plant cells[7].

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