Comparison of drought tolerance in nitrogen-fixing and inorganic nitrogen-grown common beans

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Abstract

In this work, we evaluated how the use of alternative N sources affects drought-stress tolerance in common beans. To this end, plants were cultivated employing either N₂ fixation or two levels of inorganic nitrogen: 1 mM NH₄NO₃ (limiting) or 10 mM NH₄NO₃ (sufficient). Drought was imposed by withholding watering at 30 days after planting (DAP) — coinciding with flowering. At 20 DAP, growth and N content were significantly higher in NH₄NO₃-sufficient plants than in N₂-fixing and NH₄NO₃-limited beans. At later times, only N₂-fixing and NH₄NO₃-sufficient plants continued assimilating N and growing, with the NH₄NO₃-sufficient plants being consistently bigger. After 10 days of stress (40 DAP), desiccation was evident, but only NH₄NO₃-sufficient plants suffered drought-induced senescence. After 20 days of stress (50 DAP), N content increased in NH₄NO₃-sufficient but not in N₂-fixing beans, despite the latter’s lesser state of wilt. Pod dry weight dropped 43% in NH₄NO₃-sufficient beans with respect to well-watered plants, while remaining constant in N₂-fixing beans. Under drought conditions, the number of pods limited pod yield regardless of the nitrogen source used; nevertheless, the translocation of soluble matter to pods continued in both NH₄NO₃-sufficient and N₂-fixing beans. We conclude that common beans grown under conditions of N₂ fixation were more drought tolerant than those provided with sufficient levels of NH₄NO₃. The most stress-sensitive traits in these plants were the incorporation of N into their shoots and the number of pods remaining on them.

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1. Introduction

The extent of damage caused by drought stress in plants depends on, among other factors, nitrogen nutrition [1,2]. In crop legumes, atmospheric nitrogen may be fixed via the nitrogenase pathway in the root nodules, and/or inorganic nitrogen in solution may be assimilated from the soil principally through the nitrate-reductase reaction sequence in leaves. Preferential nitrogen assimilation by either one of these routes might lead to modifications in plant architecture, or have diverse influences on drought tolerance because of the differences in the utilisation of energy and reducing power characteristic of those pathways [3,4]. There are thus important practical consequences in ascertaining whether or not drought stress has different impacts on N₂-fixing and inorganic-N-fed crop legumes.

The response to drought conditions shares many properties with ageing [5]. Thus, after prolonged dry periods, an acceleration in senescence-related processes, along with a premature cleavage of proteins and a translocation of the resultant peptides and amino acids, might be expected. Indeed, both in ageing and in drought stress, oxidative damage seems to increase and to be, at least in part, responsible for triggering senescence, in
leaves as well as nodules [6,7]. The plant defence against oxidative damage involves changes in catalase activity and the operation of the glutathione-ascorbate cycle, both of which functions contribute to the detoxification of cells through the scavenging of active oxygen species [6–8].

Depending on the degree of senescence and the tissues affected, the translocation of soluble carbon and nitrogen compounds to developing pods might be limited. Moreover, in N2-fixing plants, the impact of transient-drought damage on grain yield depends on the developmental stage at which the stress takes place, with the effect being more severe if the adverse conditions arise during the reproductive period when the N2-fixation rate is maximal [9].

Since plant growth, N assimilation and the mentioned translocations are affected differently by the means of N nutrition, the influence of drought on these three processes might differ, thus giving rise to dissimilar tolerance mechanisms. This possibility, however, does not apply to all the legumes tested, in that some species adopted different drought-tolerance strategies under conditions of nitrate feeding and N2 fixation, whereas others did not; and this adaptation, in turn, altered the resistance level achieved with each N-assimilation pathway [10–12]. In response to increased salinity, nitrate-fed chick-pea and faba bean plants were more drought tolerant than their corresponding N2 fixers [13,14]. Extrapolations of osmotic-duress responses to drought stress, however, should be made with care, since osmotic and drought-induced effects on nodule O2 permeability and energy metabolism have not proved to be as similar as originally thought (see Ref. [15]).

Drought conditions limit the yield of the common bean (Phaseolus vulgaris) in northwest Argentina owing to the combined effects of high temperature and periods of scant rainfall during the growing season. These crops are grown under conditions of either N2 fixation or inorganic-N fertilisation without regard to the possible differential impact of these modes of cultivation on drought tolerance. Moreover, plant breeding, including selection for drought tolerance, is performed mainly with nitrate-fertilised plants as opposed to N2-fixing ones, under the assumption that the same drought-tolerance phenotypes will be expressed. Since, as already described, the use of different N-assimilation pathways might lead to dissimilar levels of drought tolerance, we sought to evaluate whether or not the manipulation of the N source during plant growth might have a favorable influence on drought resistance. We also wished to examine whether drought limits plant yield by affecting the same or different parameters with each N source. To this end, in the work reported here, we have compared the sensitivity of the common bean to severe drought stress during its reproductive stage under conditions requiring the utilisation of different N-assimilation pathways for growth.

2. Materials and methods

2.1. Plant growth and conditions for imposing drought stress

We cultivated Dor 500 black beans (provided by EERA-INTA, Cerrillos, Argentina) at 28/20°C day/night temperature in a greenhouse having uncontrolled relative humidity that varied by around 60/90% day/night. Seeds were surface sterilised with 20% (v/v) commercial bleach followed by six sterile-distilled water washes before placing them for germination on 1.5% (w/v) aqueous agar [16]. We planted 3-day-old uncontaminated seedlings (length around 3–4 cm) at two seedlings per 2.5-l pot filled with sterile vermiculite, and immediately watered the pots with a sterile N-free plant-nutrient solution that contained, in g/l distilled water: CaCl2·2H2O, 0.10; MgSO4·7H2O, 0.12; KH2PO4·3H2O, 0.17; K2HPO4, 0.20; NaCl, 0.06; ferric citrate, 0.005; and micronutrients from Fåhraeus solution [17] (pH 7.0). NH4NO3 was included as indicated later.

We irrigated with sterile distilled water every 2 days and provided one watering with 1:5-diluted nitrogen-free plant-nutrient solution at 15 days after planting (DAP). With one-half of the
plants provided with each nitrogen source, we then stopped the watering at 30 DAP. Finally, we sampled four plants per time interval (for each nitrogen source, either with or without continued watering) at 20, 30, 35, 40, and 50 DAP.

Relative water content (RWC) was determined in leaves as previously described [19]. For each time interval, at 3 h after the onset of the photophase, we excised 2.5 cm diameter discs from the middle of leaflets from the uppermost fully expanded leaves and recorded the fresh, turgid (discs soaked in double-distilled water up to constancy of weight), and dry (discs heated at 80°C down to constancy of weight) weights of each disc. We then calculated the RWC as: 100 \times \left(\frac{\text{fresh weight} - \text{dry weight}}{\text{turgid weight} - \text{dry weight}}\right)^{-1}$. Disc weight divided by the disc area gave the leaf weight per unit area.

2.2. Biochemical and analytical determinations

Total organic-N content of oven-dried shoots and pods were measured by the Kjeldahl method [20].

Soluble proteins from leaves and nodules frozen at $-135°C$ were extracted by first grinding the samples with a pestle in a precooled mortar after the addition of insoluble polyvinylpolypyrrolidone powder, and then placing the ground tissue for thawing in 25 mM phosphate buffer (pH 7.0), containing 1 mM CaCl$_2$·2H$_2$O. After clarification of the resulting suspension by centrifuging at 12,000 $\times$ g for 10 min at 4°C, followed by cloth filtration of the supernatant, we dispensed 1 ml aliquots for storage at $-80°C$ until further processing. We estimated the concentration of total proteins in the extracts by the method of Bradford [21].

We determined tissue-protease content using 12.5 mg ml$^{-1}$ azocasein as the substrate after Malik et al. [22], except that one unit of protease activity was defined as the amount of enzyme producing an increase in absorbance at 440 nm of 0.1 h$^{-1}$ at 37°C.

Catalase activity was measured by following the decomposition of 20 mM H$_2$O$_2$ at 240 nm [23]. One unit of catalase activity was defined as the amount of enzyme that degraded 1 μmol H$_2$O$_2$ min$^{-1}$ at 30°C.

We assayed ascorbate peroxidase with 0.1 mM ascorbate plus 1 mM H$_2$O$_2$ by following the decomposition of ascorbate at 265 nm and 30°C, and correcting those values for the autooxidation of ascorbate in the absence of H$_2$O$_2$ [8]. One unit of ascorbate-peroxidase activity was defined as the amount of enzyme that degraded one nanomole of ascorbate per minute.

Finally, tissue-polypeptide composition was analysed by discontinuous sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [24] followed by silver staining [25]. Carbonic anhydrase from bovine erythrocytes (29 kDa), egg albumin (45 kDa) and bovine serum albumin (66 kDa) were used as molecular-weight standards.

2.3. Statistics

We carried out two independent experiments that yielded essentially the same results. We thus present here the mean values ± the standard deviations (S.D.) from one of these, as being representative of both. In addition, an analysis of variance (ANOVA) and the Tukey test [26] were performed to determine the statistical significance of differences among mean values.

3. Results and discussion

3.1. Growth determined by nitrogen availability

Dor 500 black bean is a cultivar of indeterminate growth which, under our present greenhouse conditions, completed the full expansion of the first trifoliate leaf at 20 DAP, started flowering at 30 DAP, and was in pod development and grain filling at between 40 and 50 DAP. These physiological states were reached at the same times by all the plants, independent of the N treatment received (except for pod development in 1 mM NH$_4$NO$_3$-fed plants; see later). Plant size and growth rate, however, varied according to the nitrogen source. At 20 DAP, the appearance as well as the fresh and dry weights of the N$_2$-fixing and the 1 mM NH$_4$NO$_3$-fed plants were similar, whereas the 10 mM NH$_4$NO$_3$-fed plants were larger (Table 1). The greater green mass of the 10 mM NH$_4$NO$_3$-fed plants resulted, at least in part, from increased leaf area rather than from thicker leaves, since the leaf fresh and dry weights per unit area did not vary significantly throughout the experiment (not shown). This smaller size of both
the N₂-fixing and the 1 mM NH₄NO₃-fed plants indicated that they were N-limited at this growth stage in comparison with the 10 mM NH₄NO₃-fed beans. The subsequent rate of increase in shoot fresh weight at 30, 40, and 50 DAP was highest for the 10 mM NH₄NO₃-fed plants, intermediate for the N₂-fixing beans (Table 1; see also our preliminary communication [27]), and lowest at virtually 0 mg day⁻¹ for the 1 mM NH₄NO₃-grown plants. Likewise, the increase in shoot dry weight also ceased in these latter plants in contrast to the progressively greater values seen with those of the other two experimental groups.

As these results suggested, after 30 DAP, the nitrate available to plants fed NH₄NO₃ at 1 mM had apparently dropped to levels sufficient to produce nitrogen starvation, thus precluding further assimilation of nitrogen; whereas the plants of the other two groups were able to continue this process, although at a lower rate in beans fixing atmospheric N₂ than in the plants fed 10 mM NH₄NO₃ (Fig. 1, shaded bars). This latter difference is in agreement with the low efficiency of the nitrogenase pathway in the common bean [28,29].

To underscore these contrasting features, we will hereafter refer to plants fed 1 and 10 mM NH₄NO₃ as NH₄NO₃-limited and NH₄NO₃-sufficient beans, respectively.

3.2. Effects of nitrogen content and growth on desiccation

We evaluated the progress of drought stress through calculation of the RWC of leaves at 20, 30, 35, 40, and 50 DAP (Fig. 2). By the first 5 days after withdrawal of watering (30–35 DAP), the RWC values remained essentially unchanged, suggesting that the plants had not yet suffered dehydration stress. By 40 DAP, however, the RWC of both the unwatered NH₄NO₃-sufficient and N₂-

Fig. 1. Shoot nitrogen content per plant at 20, 30, 40, and 50 DAP. Shaded bars, well-watered plants; open bars, drought-stressed plants. N1 = 1 mM NH4NO3-fed beans; N10 = 10 mM NH4NO3-fed plants; F = N2-fixing plants. The striped portion of the bars represents the nitrogen content of pods. The full height of the bars represents the total N content in shoots (leaves + stems + pods). Error bars indicate the S.D.

fixing beans dropped, with the values for NH4NO3-sufficient plants suggesting the onset of senescence. This pattern continued to 50 DAP, with the NH4NO3-sufficient beans being now at the point of death. By contrast, the RWC of NH4NO3-limited plants stayed constant throughout this same period.

In drought-stressed beans, shoot fresh weight remained constant in the N2-fixing and the NH4NO3-limited beans, but diminished in the NH4NO3-sufficient plants (Table 1). In the N2-fixing beans, the rate of increase in shoot dry matter was slightly slower than with well-watered plants, whereas in the NH4NO3-sufficient group, this parameter was markedly decreased by drought conditions relative to the corresponding value for the watered beans (Table 1; see also Ref. [27]). The root mass of this latter group increased between 40 and 50 DAP in response to drought, while that of N2-fixing plants actually diminished (Table 1). The shoot:root ratio diminished significantly at 50 DAP only in the unwatered NH4NO3-sufficient beans, being about one-half of the value for their well-watered counterparts. By contrast, the limiting factor in the growth of NH4NO3-limited plants would appear to be the NH4NO3 supply rather than the availability of water (Table 1).

N content increased under drought stress in the NH4NO3-sufficient but not in the N2-fixing beans (Fig. 1, open bars), although the latter did not become as withered as the former (Fig. 2). This difference in response to water withdrawal agrees with the high sensitivity of nodule energy metabolism [30] and the nitrogenase system [31] to hydration state, both functions being abruptly inhibited at the onset of drought.

The greater availability of N to the NH4NO3-sufficient beans from the time of their early development led to larger plants with more extensive leaf expansion; this difference made them more sensitive to desiccation when confronted with an abrupt reduction in the water supply. The NH4NO3-limited beans, however, grew less rapidly and were also less sensitive to drought conditions, while N2-fixing plants manifested an intermediate response. With these latter plants, in contrast to those fertilised with nitrate, atmospheric N2 (their sole nitrogen source) did not become available immediately after planting because the N2-assimilating system requires the inoculation, root invasion, and formation of root nodules by the

Fig. 2. Relative water content as a function of time (DAP). Filled symbols, well-watered plants, open symbols, drought-stressed plants from the time of 30 DAP. Triangles, 10 mM NH4NO3-fed beans; circles, N2-fixing plants; squares, 1 mM NH4NO3-fed plants. Error bars indicate the S.D. Where not represented, the S.D. is smaller than the symbol.
Rhizobium bacteria followed by the differentiation of the nodule rhizobia to bacteroids, at which stage the nitrogenase pathway becomes functional [32]. This entire process takes at least 10 days from the time of inoculation. This lag period was reflected in the fact that at 20 DAP, the N$_2$-fixing plants were still rather similar to the NH$_4$NO$_3$-limited beans with respect to all the parameters tested, although these same N$_2$-fixing plants later became much less nitrogen limited at subsequent stages in their growth.

Apart from their greater size, the continuous N assimilation by the NH$_4$NO$_3$-sufficient beans even under drought stress might have contributed to their lower desiccation tolerance. Low levels of external nitrate reduce stomatal conductance and transpiration within 2–5 days after a reduction in N level [33]. Plants with low levels of N supply, moreover, have a smaller leaf area [34–36] and retain more water under drought stress, since the sensitivity of the stomatal response to water deficit is increased by N deficiency [1]. This enhancement could result in part from an increased release of abscisic acid from the symplast into the apoplast in response to a drop in N availability [2]. In soybean, shoot:root ratios and root-growth responses under drought conditions were similar to those reported here: in inorganic-N-fed soybeans, but not in N$_2$-fixing plants, root mass increased during drought stress [10]. Moreover, the threshold value of leaf-water potential for stomatal closure was higher for N$_2$-fixing than for inorganic-N-fed plants [10]. This difference could imply that in the N$_2$-fixing plants, photosynthetic capability could be diminished earlier on after the cessation of watering. Drought stress inhibits nitrogenase activity in soybean nodules through a decrease in respiratory capacity [37]; which reduction could, in turn, be caused by a prompt and pronounced downregulation of sucrose synthase, thereby preventing sucrose cleavage within the nodule [30].

Alternatively, nascent oxygen along with the oxygen-active species that arises during drought stress can cause irreversible damage to the nitrogenase protein.

3.3. Senescence

To evaluate the importance of oxidative damage and other senescence-related effects in the common bean as a consequence of drought stress, we measured catalase, ascorbate peroxidase, and protease activities, enzymes which are indicators of these processes [8,38,39]. Since NH$_4$NO$_3$-limited plants were quite insensitive to water withdrawal and produced no pods, thus becoming irrelevant to the issue of grain yield (see below); we restricted our analyses to the NH$_4$NO$_3$-sufficient plants as compared with the N$_2$-fixing beans.

In the nodules of the N$_2$-fixing plants, fresh weight decreased sharply both with age and as a result of drought, although the total dry mass remained constant throughout (not shown). The total soluble protein content also remained constant as well as the protease, catalase, and ascorbate-peroxidase specific activities (expressed per milligram of tissue protein; cf. Table 2). Qualitative analysis of the polypeptide profile by SDS-PAGE (Fig. 3) corroborated that general polypeptide integrity was maintained. This observation, in agreement with a previous study in nodules from soybean plants under moderate drought stress [30], indicates that nodule proteins did not undergo massive degradation and translocation under conditions of either ageing or drought stress. In addition, the lack of detectable changes in catalase and ascorbate peroxidase could indicate that oxygen-active species have not risen to dangerous levels. Our results are not consistent with those obtained in nodules from pea plants subjected to a similar drought-stress treatment [7], where total soluble-protein content along with the levels of catalase, ascorbate peroxidase, and other antioxidant enzymes decreased under drought stress. Therefore, the determinate nodules in common bean and soybean would appear to be more protected against drought-induced senescence and oxidative damage than the indeterminate nodules in pea.

In leaves, the total protein decreased, while the specific protease activity increased with ageing and with water deprivation as well, except for the nitrogen-fixing plants at 40 DAP. As already mentioned, NH$_4$NO$_3$-sufficient drought-stressed beans were completely dried up by 50 DAP, with only few necrosed leaves remaining on the plants. We therefore did not carry out any measurements on these samples. Specific protease activity varied similarly with age for both the N$_2$-fixing and the NH$_4$NO$_3$-sufficient plants, ranging from 4.63 and 3.74 U mg$^{-1}$ at 40 DAP to 9.38 and 9.21 U mg$^{-1}$
Table 2  
Nodule and leaf protease, catalase, and ascorbate-peroxidase specific activities in N\textsubscript{2}-fixing or 10 mM NH\textsubscript{4}NO\textsubscript{3}-fed beans at progressive ages and in different hydration states\textsuperscript{a}

<table>
<thead>
<tr>
<th>Organ</th>
<th>Drought</th>
<th>Age (DAP)\textsuperscript{b}</th>
<th>Protein (mg plant\textsuperscript{−1})</th>
<th>Protease activity\textsuperscript{c} (U mg protein\textsuperscript{−1})</th>
<th>Catalase activity\textsuperscript{d} (U mg protein\textsuperscript{−1})</th>
<th>Ascorbate peroxidase activity\textsuperscript{e} (U mg protein\textsuperscript{−1})</th>
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<tr>
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<td>11.82 A</td>
<td>2.02 A</td>
<td>7.86 A</td>
<td>85.2 A</td>
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<td>2.72 A</td>
<td>6.90 A</td>
<td>51.3 A</td>
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\textsuperscript{a} Values followed by different letters (within each plant organ) differ significantly at \( P<0.01 \) according to the ANOVA and Tukey test.

\textsuperscript{b} DAP, days after planting.

\textsuperscript{c} One unit of protease activity is expressed as the amount of enzyme that produces a \( \Delta A_{440} \) value of 0.1 h\textsuperscript{−1} at 37\textdegree C.

\textsuperscript{d} One unit of catalase activity is expressed as the amount of enzyme that decomposes 1 \( \mu \)mol H\textsubscript{2}O\textsubscript{2} min\textsuperscript{−1} at 30\textdegree C.

\textsuperscript{e} One unit of ascorbate peroxidase activity is expressed as the amount of enzyme that oxidizes 1 nmol ascorbate min\textsuperscript{−1} at 30\textdegree C.

\textsuperscript{f} ND, Not determined.

Fig. 3. SDS-PAGE polypeptide profiles from plant tissues. Approximately 20 \( \mu \)g protein were loaded per lane. Panel a, leaves (lanes 1–4) or nodules (lanes 5 and 6) at 40 DAP (lanes 1, 3 and 5) or 50 DAP (lanes 2, 4 and 6) from well-watered plants grown on the indicated nitrogen source. Panel b, leaves (lanes 1–4) or nodules (lanes 5 and 6) of well-watered plants (lanes 1, 3 and 5) or drought-stressed plants (lanes 2, 4 and 6), all at 40 DAP, grown on the indicated nitrogen source. Migration of molecular weight standards is denoted by lines at the right, while the positions of the polypeptides of molecular weight 30, 55, 66 and 78 kDa referred to in the text are marked by arrows at the left.
at 50 DAP, respectively, representing corresponding 2.0- and 2.5-fold increases (Table 2). These increases correlated with a visible degradation of tissue proteins at both of these time intervals, especially in PAGE bands of approximate molecular weight 66 and 55 kDa, the last presumably being the large subunit of Rubisco [40] (Fig. 3a; compare the relative intensities of these bands with that of the 78 kDa species for each lane).

A comparison of the activities and polypeptide profiles at 40 DAP for the stressed and non-stressed plants (i.e. at the middle of the drought period) reveals a small increase in protease specific activity in the NH4NO3-sufficient beans (Table 2), whereas degradation of the 66 and 55 kDa polypeptides is not yet detectable by SDS-PAGE (Fig. 3b). Nevertheless, an increase in the intensity of the ca. 30 kDa band could be observed for the drought-stressed inorganic-nitrogen-fed plants (Fig. 3b; compare the relative intensity of this band with that of 55 kDa for each lane), indicating a stress-induced change in protein degradation in these plants. No change in the specific activity of either catalase or ascorbate peroxidase was observed as a result of drought conditions; only an increase in the specific activity of ascorbate peroxidase was found to occur with age, at between 40 and 50 DAP, in the NH4NO3-sufficient beans (Table 2). Common bean leaves thus appeared to be sensitive to drought stress-induced senescence with respect to protease activity and total protein maintenance, but not as regards antioxidant-enzyme activities; an overall response similar in certain respects to N2-fixing pea plants, the leaves of which showed significant decreases in both total soluble protein and antioxidant activities, under a similar drought stress [6].

According to these criteria, NH4NO3-sufficient were more sensitive to drought-induced senescence with respect to protease activity and total protein maintenance, but not as regards antioxidant-enzyme activities; an overall response similar in certain respects to N2-fixing pea plants, the leaves of which showed significant decreases in both total soluble protein and antioxidant activities, under a similar drought stress [6].

According to these criteria, NH4NO3-sufficient were more sensitive to drought-induced senescence than N2-fixing plants, in agreement with the aforementioned RWC values (Fig. 2).

3.4. Pod development and nitrogen translocation

Pod fresh and dry weights were the highest for the NH4NO3-sufficient well-watered plants, but similar for the NH4NO3-sufficient and the N2-fixing beans under drought stress (Table 1 and Fig. 4), whereas in NH4NO3-limited plants, no pods developed either with or without watering. Therefore, while pod yield dropped to 25% measured as fresh weight or to 57% according to dry weight in the NH4NO3-sufficient beans, this parameter declined only to 51% by fresh weight and even remained constant by dry weight in the N2-fixing plants (Table 1). The distribution of pod dry weights shows that pod number was equally restricted by drought with both sources of N (Fig. 4). This observation suggests that some part of the process between flowering and pod establishment had been interrupted early in the drought period in both the NH4NO3-fed plants and the N2-fixing beans, although grain filling in already established pods continued despite the drought stress in both types of plants. This effect actually compensated for the drop in pod number in the N2-fixing plants (Fig. 4). We conclude that the effect of drought stress on pod number is probably unrelated to N nutrition; rather, it appears to be a very early effect of drought, perhaps involving an imbalance between ABA and gibberellic acid [1,2,41].

N translocation to pods between 40 and 50 DAP was quite efficient even in the drought-stressed NH4NO3-sufficient plants; however, less N, was accumulated in pods of unwatered N2-fixing beans (Fig. 1, open bars, striped portion).

4. Conclusions

N2-Fixing common beans survived better and sustained a higher proportion of pod yield under conditions of drought stress than did the NH4NO3-sufficient plants. The pods of the former, however, contained less N than did those of the NH4NO3-grown beans, perhaps because of a higher drought sensitivity on the part of their nitrogenase system. Drought tolerance in N2-fixing plants depended at least in part on the lack of external N during their early development, the absence of which led to a smaller leaf-transpiration area.

In both N2-fixing and NH4NO3-sufficient plants, pod number, but not grain filling, was limiting for yield under drought stress, despite the aforementioned differences in the plant’s state of desiccation.

These results would point to the convenience in the use of N2 fixation to increase the survival of common bean crops during water deficits, and would furthermore suggest the focusing of plant breeding for drought tolerance on the following traits: (i) an enhanced tolerance to water limitation.
Fig. 4. Distribution of individual pod dry weights from four plants at 50 DAP. The pod weights indicated on the abscissa are represented vertically along the ordinate in the same order as they were present at different heights on the plant stem. Each pod is denoted by an arbitrary number shown on the ordinate. Panel a, well watered N₂-fixing beans; panel b, drought-stressed N₂-fixing beans; panel c, well-watered 10 mM NH₄NO₃-fed plants; panel d, drought-stressed 10 mM NH₄NO₃-fed beans.

in nitrogenase activity; and (ii) a diminished sensitivity in flowering and pod establishment at early stages of desiccation. In such plants, enhanced yields could be obtained since in our experiments, nodule senescence and grain filling in established pods appeared to be less sensitive to stress.

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