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Physiological Response to Drought Stress of Common Bean (*Phaseolus vulgaris* L.) Genotypes Differing in Drought Resistance

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TABLE OF CONTENTS

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LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

ψ	Leaf water potential
ψ_p	Turgor potential (pressure)
ψ_s	Osmotic potential
A	Net photosynthetic rate
ABA	Abscisic acid
AGR	Absolute growth rate
BrSp	cv. Brown Speckled
C_a	Ambient CO_2 concentration
C_i	Leaf intercellular CO_2 concentration
d	day
DW	Dry weight
g_s	Stomatal conductance to water vapour
IWUE	Instantaneous water-use efficiency
LAR	Leaf area ratio
LWR	Leaf weight ratio
NAR	Net assimilation rate
RGR	Relative growth rate
RWC	Leaf relative water content
S.D.	Standard deviation
S.E.	Standard error
SEA 15	CIAT inbred line
SLA	Specific leaf area
SLW	Specific leaf weight
WHC	Water-holding capacity
WUE	Water-use efficiency
WUE_{BY}	Total biomass yield based water-use efficiency
WUE_{SY}	Seed yield-based water-use efficiency

1. INTRODUCTION

As much as 60% of common bean (*Phaseolus vulgaris* L.) production in the developing world occurs under conditions of significant drought stress (Graham and Ranalli, 1997). Consequently, the average global yield of beans remains low (<900 kg ha^{-1}) (Singh, 2001; Thung and Rao, 1999). To date, progress in improving common bean cultivars for dry environments of the tropics has been achieved by yield testing of large collections over several locations and years. Such empirical approaches are, however, slow, laborious, and expensive because of the need to assess the yield of large numbers of lines across several locations and years, and the substantial variation from the effects of environment, error, and genotype-environment interactions (Blum, 1988). Success in developing drought-resistant common bean cultivars has further been limited due to the irregularity of available moisture, lacks of screening techniques and practical selection criteria other than yield (Ramirez-Vallejo and Kelly, 1998; Acosta-Gallegos and Adams, 1991).

In the above context, there is a strong argument that an indirect (or analytical) approach, based on the understanding of crops at morphological, physiological and molecular levels may help to target the key traits that are currently limiting yield (Araus et al., 2002; Bidinger and Witcombe, 1989; Turner, 1986). The identification of main physiological processes determining yield by comparing genotypes differing in drought tolerance has been proposed as the most reliable and soundest approach to identify the potential secondary traits (Araus et al., 2002; Jat et al., 1991; Bohnert and Jensen, 1996). Comparing physiological bases of the differences in yielding capacity among genotypes released during different periods (retrospective studies) may also serve as a complementary approach (Araus et al., 2002). In fact, examples of the successful use of indirect selection criteria (physiological traits) in breeding for better yields under dry conditions for important crop plants including common bean are rarely found (Ober et al., 2005; Slafer et al., 1994; White and Singh, 1991). Nevertheless, few cases such as selection for low carbon-isotope discrimination ($\Delta^{13}C$) (Passioura, 2002), increased

osmotic adjustment (Chimenti et al., 2002; Morgan, 2000), and introgressing QTLs associated with deeper rooting into a high-yielding cultivar (Babu et al., 2003; Shen et al., 2001) have proven the merit of the approach. By the same token, understanding the key adaptive morphological, physiological and biochemical traits/mechanisms linked to growth and yield of common bean under drought stress may contribute to concerted efforts presently under way to develop drought-resistant cultivars.

1.1. Mechanisms and traits related to drought resistance in common bean

1.1.1. Growth, yield and morphological adaptations

Past research works on adaptation of common beans have demonstrated that compared with shoot traits, root characteristics are of primary importance in determining drought response and differences in yield under low moisture stress (Norman et al., 1995; White and Castillo, 1989). Under drought stress, deeply penetrating and dense roots correlate with leaf gas-exchange (stomatal conductance control) in *P. vulgaris* (White et al., 1990) and *P. acutifolius* (Mohamed et al., 2002). At shoot level, beans respond to drought stress by leaf movement (Pastenes et al., 2005; Ehleringer et al., 1991), leaf flagging and shedding (Acosta-Gallegos, 1988; Adams et al., 1985). Loss of leaf area, which could result from reduced size of younger leaves and inhibition of the expansion of developing foliage, is also considered an adaptation mechanism to drought (Acosta-Gallegos, 1988). Early phenology coupled with rapid ground cover and dry matter production in legumes allows greater post-flower water-use leading to greater partitioning of dry matter into seeds (Siddique et al., 2001). Cultivars that show greater phenological adjustment exhibit higher seed yields under drought conditions (Acosta-Gallegos and White, 1995).

Slower growth has been suggested as an adaptive feature for plant survival under stress, because it allows plants to divert assimilates and energy, otherwise used for shoot growth, into protective molecules to fight stress (Zhu, 2002) and/or to maintain root growth, improving water acquisition (Chaves et al., 2003). In most drought studies, a single harvest date has been used to correlate growth with the physiological effects of stress. The

results from such studies can be misleading when comparing different genotypes or drought treatments because the initial size of the plant can influence the size or rate of growth at harvest (Hunt, 1990). The relative growth rate (RGR) takes this factor into account by dividing the absolute growth rate by the initial weight of the plant. This gives a relative basis on which to compare growth rates of plants. The use of formal growth analysis, therefore, has value in discriminating alternative mechanisms of drought stress at the whole plant level.

Shoot biomass accumulation is considered an important trait to attain high seed yield in grain legumes (Saxena et al., 1990). Significant differences have been observed for shoot biomass accumulation among dry bean cultivars grown under moderate to severe drought stress conditions (Rosales-Serna et al., 2002; Ramirez-Vallejo and Kelly, 1998; Acosta-Gallegos and Adams, 1991). Strong positive correlations have often been reported between total plant biomass and seed yield under drought stress and non-stress conditions (Shenkut and Brick 2003; Ramirez-Vallejo and Kelly, 1998). Because plant biomass has moderate to high heritability and exhibits low genotype × environment interactions, it has been suggested that the trait could be used as an indirect selection criterion to improve and stabilize seed yield for low moisture areas (Shenkut and Brick, 2003). According to Chaves et al. (2002), in addition to dry matter accumulation, the ability of genotypes to partition stored vegetative biomass to reproductive organs to a large extent determines sink establishment and economic yield under drought stress.

In general, drought causes considerable reduction in seed yield of common bean although the ranges of reductions are highly variable due to differences in the timing and intensity of the stress imposed and the genotypes used (Frahm et al., 2004; Shenkut and Brick 2003; Ramirez-Vallejo and Kelly, 1998; Foster et al., 1995; Halterlein, 1983). Seed yield-based genotypic differences for drought resistance have been reported for common bean (Terán and Singh, 2002; Abebe et al., 1998). Bean seed yield reduction due to drought stress are attributed to adverse effects of the stress on individual yield components

(number of pods per plant, number of seeds per pod, seed weight and harvest index). The relative importance of individual components as determinants of seed yield varies from experiment to experiment (Shenkut and Brick, 2003; Boutraa and Sanders, 2001; Ramirez-Vallejo and Kelly, 1998; Singh, 1995).

1.1.2. Water-use and water-use efficiency (WUE)

Under moisture-limiting environments, productivity in crop plants may be increased by improving water-use efficiency (WUE) (Ehleringer et al., 1993). To achieve this goal, it is important to identify the factors underlying variations in the WUE since they can either positively or negatively be correlated with productivity, depending on the main processes determining changes in WUE (Udayakumar et al., 1998). Carbon isotope discrimination ($\Delta^{13}C$), specific leaf weight (SLW), and canopy temperature have been proposed as potential surrogate tools for selecting genotypes with higher WUE in several legumes (Saranga et al., 1998; Menendez and Hall, 1995; Johnson and Tieszen, 1994; Ismail and Hall, 1993; Gutschick and Currier, 1992; Hattendorf et al., 1990; Farquhar and Richards, 1984). In cereals, traits such as deeper root systems, early vigor, osmoregulation, smaller photosynthetic surfaces and small erect upper canopy leaves may help crops either to use more water or enhance WUE when subjected to drought stress (Araus et al., 2002). Genotypic variation for WUE has been demonstrated in common beans using carbon isotope discrimination ($\Delta^{13}C$) technique (Ehleringer et al., 1990). Also, positive associations between $\Delta^{13}C$ and bean seed yield have been reported (Ehleringer et al., 1990; White et al., 1990). Nevertheless, key physiological traits that offer a potential to improve WUE in common bean are not thoroughly studied.

1.1.3. Leaf-water relations and gas-exchange

Leaf water potential (ψ) and its two components, osmotic potential (ψ_s) and turgor potential (ψ_p) are useful as selection criteria for improving drought tolerance in crop plants. Leaf water potential evaluates the water stress intensity sensed by leaves (Hsiao,

1973) and is recognized as an index for whole plant water status (Pantuwan et al., 2004; Turner, 1982). It is considered as a reliable parameter for quantifying plant water stress response (Siddique et al., 2000). In general, the maintenance of high ψ determined by the interaction of numerous plant mechanisms at both shoot and root levels is considered to be associated with dehydration avoidance mechanisms (Levitt, 1980). Maintenance of leaf turgor in the face of decreasing soil moisture has been emphasized as an important adaptational trait that contributes to drought tolerance (Hsiao et al., 1976). Jongdee et al. (2002), Pantuwan et al. (2002) and Sibounheuang et al. (2001) found that genotypes with high ψ had less reproductive sterility and produced higher yield than genotypes with lower ψ under drought stress conditions. Other reports suggest that plant metabolic processes are in fact more sensitive to turgor and cell volume than absolute water potential (Jones and Corlett, 1992). Among the physiological mechanisms that act to maintain leaf turgor pressure under lower leaf water potential, decreased osmotic potential resulting either from a decrease in osmotic water fraction or from an osmotic adjustment (net accumulation of solutes in the symplast) has been pointed out (Jones and Turner, 1980).

A satisfactory basis for relating cellular water status to metabolism is relative water content (RWC), an easily measured, robust indicator of water status for comparison of tissues and species, which 'normalizes' water content by expressing it relative to the fully turgid (hydrated) state (Lawlor and Cornic, 2002). Sinclair and Ludlow (1985) proposed that leaf relative water content (RWC) is a better indicator of water status than was water potential (ψ). RWC is a measure of relative change in cell volume; ψ is the resultant of cell turgor (ψ_p) and osmotic potential (ψ_s), and thus depends both on solute concentration and cell wall rigidity and does not relate directly to cell volume (Kramer and Boyer, 1995; Lawlor, 1995; Kaiser, 1987). RWC as an integrative indicator of internal plant water status under drought conditions has successfully been used to identify drought-resistant

cultivars of barley (*Hordeum vulgare*) (Martin et al., 1989) and common bean (Costa França et al., 2000).

Photosynthesis is the main process responsible for dry matter accumulation and consequently affects plant development and growth, which are strongly affected by the environment (McCree, 1986). In common bean, drought stress at its initial phase limits photosynthesis due mainly to stomatal closure (Miyashita et al., 2005, Amede et al., 2003b). However, as the stress progresses over a longer period, non-stomatal inhibition of photosynthesis may become more important (Lawlor and Cornic, 2002; Medrano et al., 2002). Increasing evidence suggests that down-regulation of different photosynthetic processes under drought stress depends more on CO_2 availability in the mesophyll (i.e. stomatal closure) rather than ψ or RWC (Medrano et al., 2002). Stomatal control is one of the main mechanisms for adapting to water stress in common bean (Laffray and Louguet, 1990). In crops such as beans, stomata often close in response to drought before any change in ψ and/or RWC is detectable (Miyashita et al., 2004; Socías et al., 1997). Information on a common pattern of photosynthetic response to drought for common bean is currently meagre.

1.2. Assimilate metabolism in source and sink organs under drought stress

Drought stress decreases photosynthetic rate thereby disrupting carbohydrate metabolism in leaves (Pelleschi et al., 1997; Kim et al., 2000). As a consequence, the amount of assimilates available for export to the sink organs may be reduced leading to an increased rate of reproductive abortion. In drought-stressed maize (*Zea mays* L.) (Schussler and Westgate, 1991, 1995) and wheat (Wardlaw, 2002), smaller/loss of kernel set was correlated with the extent of loss in photosynthesis and the photosynthate influx into kernels. As sucrose is the principal form of photosynthate for long-distance transport to sink organs, its concentration in leaves represents the current availability of assimilate for reproductive development (Westgate and Thomson Grant, 1989). Leaf sucrose concentration is determined by several factors including the rate of photosynthesis, the

partitioning of photosynthetic carbon between starch and sucrose, the rate of sucrose hydrolysis, and the rate of sucrose export (Huber, 1989; Egli et al., 1980). Any effect of drought on these processes would modify leaf sucrose concentration. In sucrose-transporting plants, the sucrose status of a tissue plays a crucial role in the regulation of metabolism, and sucrose export from mature leaves is related to sucrose synthesis (Geiger and Fondy, 1991). In pigeon pea (*Cajanus cajan*), leaf starch and sucrose concentrations decreased rapidly and became close to zero, while the concentrations of glucose and fructose significantly increased in response to drought stress (Keller and Ludlow, 1993). Similar results have been observed in several plant species under drought conditions (Lawlor and Cornic, 2002). Overall, it is suggested that the starch and sucrose pools in plant leaves are depleted under drought conditions; in the meantime, the resulting high concentrations of hexose may be involved in a feedback regulation of photosynthesis (Chaves et al., 2002). Consequently, the total amount of sucrose for export is significantly decreased.

Drought stress can also affect carbohydrate metabolism in plant reproductive organs (Liu et al., 2004). It has been often observed that sucrose concentrations in reproductive structures of drought-stressed plants, i.e., in maize ovaries and rice (*Oryza sativa* L.) anthers, generally are higher or at least similar to those of the well-watered controls (Setter et al., 2001; Zinselmeier et al., 1995; Sheoran and Saini, 1996). The results imply that rather than sucrose concentration *per se*, the capacity for sucrose utilization may be affected by drought stress. In drought-stressed maize, accumulation of sucrose in young ovaries coincided with a cessation of ovary growth, an accumulation of sucrose, and a decrease in the concentration of hexose (Zinselmeier et al., 1999). These results suggest that drought-induced changes in carbohydrate status and metabolism in crop reproductive structures during the early stage of development are crucial for successful fruit set. In addition to photosynthate supply, loss of pod set caused by drought stress during the critical, abortion-sensitive phase of soybean pod development was associated with a

decrease in water potential and with higher ABA accumulation in the reproductive structure (Liu et al., 2004, 2003).

1.3. Protein changes in response to drought stress

In addition to the physiological and biochemical responses of plants to water stress, the information on the molecular mechanisms of drought stress adaptation could be useful for the genetic improvement of drought-resistant crops/genotypes. Proteomics are a recent addition to the molecular tools used to analyze drought-affected plants (Salekdeh et al., 2002), and have been applied to the study of drought response of barley (Neslihan-Ozturk et al. 2002), maritime pine (Costa et al., 1998), maize (Riccardi et al., 1998) and wild watermelon (Kawasaki et al., 2000). Two-dimensional gel electrophoresis (2DE) is known to be a powerful method to resolve qualitative variations (positional shifts, present and absent) and quantitative variations (increase or decrease) of proteins and to follow the modification of gene expression under various conditions (Damerval et al., 1986).

Water deficit induces the expression of proteins that are directly or indirectly related to the stress and some functions have been assigned to some of the sequenced proteins. Among the stress-induced proteins identified are those implicated in the biosynthesis of osmolytes (Bohnert et al., 1995; Ishitani et al., 1995), in the uptake and compartmentation of ions (Lisse et al., 1996; Niu et al., 1995), in hydroxyl-radical scavenging (Ingram and Bartels, 1996; Bohnert et al., 1995; Smirnoff and Cumbes, 1989) and in protein turnover (Kiyosue et al., 1994; Koizumi et al., 1993). Some induced proteins are expressed in order to protect the cellular machinery. These protective proteins include different classes of late embryogenesis abundant (LEA) proteins such as dehydrins (Neslihan-Ozturk et al., 2002; Colmenero-Flores et al., 1997; Lisse et al., 1996). There is a strong circumstantial evidence for the involvement of LEA proteins in the plant adaptation to water deficit through their protective role in maintaining specific cellular structures or ameliorate the effects of drought stress (Lisse et al., 1996). Proteins that show significant down-regulation under drought stress were observed for photosynthesis-related function

(Neslihan-Ozturk et al., 2002). Water deficit may also induce the expression of proteins, which are not specifically related to the stress but rather to reactions against cell damage, and those whose functions are not directly related to the stress (reviewed by Riccardi et al., 1998).

1.4. Underlying hypotheses and objectives of the study

Past studies have shown that common bean genotypes selected for specific adaptations to drought conditions produce significantly higher seed yield compared with landraces and standard cultivars grown under similar drought conditions (Téran and Singh, 2002). Profound differences have also been reported among old and modern cultivars of other crops in terms of water-use and water-use efficiency when subjected to drought stress (Koç et al., 2003; Siddique et al., 1990). In agreement with these findings, we hypothesized that common bean genotypes selected for specific adaptation to drought stress exhibit significant variation from those developed for wider agro-ecological adaptations in terms of drought resistance and water-use efficiency. Differential responses in growth, yield and biomass partitioning under drought stress of the genotypes may account for such differences.

The differences in drought resistance (determined based on grain yield) among drought-resistant and susceptible genotypes are often related to the ability to partition biomass stored in vegetative biomass to reproductive organs and the subsequent capacity to establish new sink under drought stress conditions (Koç et al., 2003; Siddique et al., 1990). In line with this, drought stress, when initiated during the reproductive phase, may differentially affect the sink strength (i.e. capacity to establish new sink) of common bean genotypes differing in drought resistance. We supposed that genotypic differences in sink strength are due to the differential effect of drought stress on assimilate synthesis and availability at source level and/or availability of assimilates for metabolism in the sink organs of the genotypes. In accordance with the observations of Schulze (1986) and Kubiske and Abrams (1993) plants of a drought-resistant bean genotype may maintain

higher rates of photosynthesis and stomatal conductance than plants of a drought-susceptible bean genotype when subjected to drought stress at different growth stages of the crop. The disparity in gas-exchange rate between the contrasting genotypes may lead to different rates of assimilate synthesis and availability for export to sink organs. Drought stress may also affect the accumulation of seed storage products by limiting the seed sink capacities (i.e. reduces the number and volume of storage organelles).

Drought stress induces changes in proteins, which play a pivotal role in the adaptive response of plants to stress (Riccardi et al., 1998; Bray, 1997; Ingram and Bartels, 1996). Accordingly, relative to non-stressed growth conditions drought stress initiated during the vegetative phase may induce quantitative and qualitative changes in proteins of mature bean leaves.

The objective of this study was to test the hypotheses that I) differences exist in biomass accumulation, yield and water-use efficiency among common bean cultivars developed for wider agro-ecological adaptation and inbred lines selected for specific adaptation to drought situations when subjected to drought stress; II) a drought-resistant genotype has a higher sink strength than a susceptible genotype and the difference between the genotypes is related to the ability to maintain assimilate synthesis and availability of assimilates for metabolism in the reproductive sink organs under drought stress; III) drought stress induces higher accumulation of ABA in sink leaves of a drought-susceptible genotype than in the leaves of a resistant genotype; and IV) relative to non-stressed plants, drought stress alters the protein patterns in a mature bean leaf.

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2. MATERIALS AND METHODS

2.1. Genotypes

Three adapted cultivars and three inbred lines of common bean (*Phaseolus vulgaris*, L.) varying in seed characteristics and growth habits were initially screened to assess seed yield-based drought resistance and water-use efficiency of the genotypes (Table 1). The adapted (old) cultivars were chosen among varieties developed by the national bean research program of Ethiopia for wider adaptations to different agro-ecological conditions of the country. A recent yield-testing study carried out across years and locations representing major bean-growing regions of the country demonstrated that the cultivar Mexican 142 was relatively stable across the range of environments used, whilst the other two cultivars (Roba 1 and Brown Speckled) were more adapted to marginal environments (Mekbib, 2003). The inbred lines were obtained from the bean research program of CIAT. The drought-resistance degrees of these materials have been demonstrated in earlier field studies (CIAT, 2002). A drought-tolerant (SEA 15) and a drought-susceptible genotype (Brown Speckled) selected from the screening experiment were used in subsequent experiments carried out thereafter.

2.2. Plant cultivation

Seeds of the tested genotypes were grown in either Mitscherlich or Ahr pots filled with 6 or 13 kg of Kleinlindener soil, respectively. At the time of planting, the soil was fertilized with Blaukorn (12.0% N, 5.2% P, 14.1% K, 1.2% Mg and 6.0% S). Eight seeds per pot were initially sown and later thinned to three (for Mitscherlich pots) or four (for Ahr pots) plants when the first trifoliate leaves were unfolded. Plants were raised in a vegetation hall. The daily minimum and maximum temperatures (mean±S.D.) during the growth periods of 2003, 2004 and 2005 were (12.2±2.6; 27.3±4.7), (12.6±3.2; 26.2±5.1) and (11.2±3.2; 23.8±4.8) °C, respectively. The respective daily average temperatures during same period were 22.2±3.7, 21.3±4.4 and 19.3±4.1.

Table 1. Some important features of the genotypes used

Genotype	Source/ Background	Seed size	Growth Habit†	Special features
Mexican 142	AC, Ethiopia	Small	III	Popular export-type cultivar
Roba 1	AC, Ethiopia	Medium	II	Popular food-type cultivar
Brown Speckled	AC, Ethiopia	Large	II	Less popular food-type cultivar
SEA 15	IL, CIAT	Medium	II	Combines Middle American races with Mesoamerica and Durango races
SEA 23	IL, CIAT	Medium	II	Combines Middle American race Mesoamerica
BAT 881	IL, CIAT	Medium	I	Combines Middle American and Andean races

Note: AC = adapted cultivar; IL = inbred line

† Bean Growth Type I = determinate bush; Type II = indeterminate bush; Type III = indeterminate prostrate (Singh, 1982)

The pots were weighed daily and watered to restore the appropriate moisture by adding a calculated amount of water. Daily additions of water (equivalent to the amount of water lost) to each pot were recorded to calculate the total water consumed (kg plant^{-1}) by the genotypes under contrasting soil moisture regimes. In order to minimize the variation, which may arise due to differences in the original fresh weights of the genotypes, the amount of water applied in both watering regimes was corrected for the fresh weight per plant determined shortly before the initiation of drought stress.

2.3. Experimental procedure

The descriptions, *vegetative* and *reproductive phase experiment* may be used as required in the forthcoming sections in order to facilitate communication throughout the manuscript. They refer to set(s) of experiments carried out with drought stress initiated at either the vegetative or reproductive growth stage of the crop.

Before the commencement of drought stress treatment at either growth stage of the crop, plants were grown under optimal soil moisture conditions. Drought stress was imposed by withholding the amount of water applied in order to keep the moisture level at about 30% of the maximum water-holding capacity (WHC) of the soil. For control treatments, the soil moisture was maintained at 70% of the maximum WHC until the plants were harvested. The exposure of plants to the indicated intensity of stress for the vegetative phase experiment began at growth stage V6, when plants had six trifoliate leaves. The plants attained this stage 30 d after planting. For experiments in which drought stress was imposed at reproductive phase, the stress treatment began at early pod-filling stage, when plants had at least one pod that had grown to its maximum length. Only for the initial genotype-screening experiment, drought stress was initiated at 100% bloom stage, when the plants had at least one open flower. In all experiments, the treatments were replicated four times and the pots were regularly randomized.

2.4. Sample collection and parameters measured

2.4.1. Biomass and seed yield

Plants were harvested at the end of 5 and 10 d stress (for the vegetative phase experiment) and 5, 10 and 20 d after imposing drought stress for the reproductive growth phase experiments. Above-ground fresh weight was determined by adding up the various plant parts (leaves, stems, pods and seeds) harvested separately. Similarly, above-ground dry weight was obtained by adding up various plant parts dried at 80^{o}C for 48 h. Biomass partitioning ability of the genotypes was evaluated by computing the ratio of reproductive structures (pods/pod walls + seeds) to vegetative biomass (leaf + stem dry weight).

At harvest, pods were categorized into two groups as productive pods (Pr-P) and aborted pods (Ab-P). The classification of pods was based on length attained and whether or not they bore seeds at the time of harvesting. Productive-pods (Pr-P) were defined as pods that were longer than 5 cm (for the harvest made at 5 d stress) and bore at least one seed

per pod (for the harvests made at 10 and 20 d stress). During the course of pod growth and development, it was observed that the underdeveloped pods (whether dropped off the plant or loosely hanging to the reproductive branches) had less than 5 cm length. These pods were considered as aborted pods (Ab-P). Also, pods that grew to a length of more than 5 cm but did not possess typical and healthy seeds (usually found at 10 and 20 d stress) were regarded as aborted pods.

Seed yield (g $plant^{-1}$) was calculated as a product of the yield components (number of productive pods per plant, number of seeds per pod and seed weight). Hundred seed weight (HSW, g) was determined on 100 seeds randomly sampled from all plants harvested per pot. Harvest index (HI) was calculated as the proportion of seed weight to the above-ground dry weight (stem + leaves + pod + seed) at harvest.

2.4.2. Plant water-use and leaf-water relations

Water-use efficiency (WUE, *mg* g^{-1}) of the bean plants at vegetative phase was calculated according to the following formula: $WUE = (w_2 - w_1) / T$, where w_1 and w_2 are the total dry weights at the end of 5 and 10 d stress, respectively, and T is the total amount of water used for transpiration between the first and the second harvest. Seed yield-based water-use efficiency (WUE_{SY}) was estimated as the ratio of seed yield to the amount of water consumed from emergence to physiological maturity of the genotypes. Instantaneous water-use efficiency (IWUE, µmol mol^{-1}) was calculated as the ratio of net photosynthetic rate (*A*) to stomatal conductance (g_s) determined during the reproductive phase.

Leaf growth and water relation parameters were determined on young expanding trifoliate leaves. During the vegetative phase, these leaves were located at the 7^{th} and 8^{th} (5 d stress) and at the 8^{th} and 9^{th} (10 d stress) main stem nodes of Brown Speckled and SEA 15, respectively. The central leaflets of the selected trifoliate leaves were cut and fresh weight (FW) taken immediately. The weighed leaves were then placed in a petri-dish containing

wet filter paper and kept in the dark. After 24 h, the turgid weight (TW) was obtained. For the dry weight (DW), the leaflets were oven-dried for 48 h at 80°C. The second leaflet from the same trifoliate leaf used for fresh weight determination was cut and the leaf area (LA) was measured using a leaf area meter AM200 (ADC BioScientific Ltd., UK). Leaf relative water content (RWC, %) and specific leaf weight (SLW, g m^{-2}) were calculated as follows:

RWC = [(FW-DW) / (TW-DW)] x 100

SLW = DW / LA

where FW, DW, TW and LA are the fresh weight, dry weight, turgid weight and leaf area, respectively.

Leaf water potential and its components were determined at developmental stage R_5 (plants had at least one pod with fully developed seeds) for bean plants subjected to drought stress at flowering stage. Water potential (ψ) was measured with the central leaflet of the youngest expanding leaf using the pressure probe method (Scholander et al., 1965). The second leaflets were cut simultaneously, put in a 5 ml syringe, frozen in liquid nitrogen and kept in a cool box until transfer to a deep freezer where they were kept at –80^{o}C. The solute osmolality was measured using Osmomat 030 (Gonotec, Berlin) in duplicate from the press sap of the frozen leaves after pressing mechanically at room temperature. Readings were converted to pressure units by using the van´t Hoff equation ($\pi = -cRT$), where π is the osmotic pressure, c is the osmolality (mosmol kg^{-1}), R is the gas constant and T the temperature (K). Turgor potential (ψ_p) was calculated as the difference between osmotic and water potentials.

2.4.3. Growth analysis

To investigate the effect of drought stress on plant growth of two distinct common bean genotypes, the relative growth rate (RGR, g g^{-1} d^{-1}), net assimilation rate (NAR, g $m^{-2}d^{-1}$), leaf area ratio (LAR, m^2 g^{-1}), specific leaf area (SLA, m^2 g^{-1}), and leaf weight ratio (LWR, g g^{-1}) were calculated according to Beadle (1993). For the growth analysis, shoot (leaf +

stem) dry weight and estimated total leaf area of bean plants subjected to drought stress for 5 (t_1) and 10 days (t_2) during the vegetative phase were used. Total leaf area per plant was estimated by measuring maximum length (mL) and width (mW) of leaves and multiplying these inputs (mL x mW) by a correction factor of 0.6 derived from the actual leaf area determined with a leaf area meter AM200 (ADC BioScientific Ltd., UK). The estimations were considered accurate because the differences in correction factor between the two genotypes and the leaf age were very small, so that comparisons between the genotypes and the watering regimes were not significantly biased. RGR and its components were calculated between sampling dates as follows:

$RGR = (\ln W_2 - \ln W_1) / t_2 - t_1$

$NAR = [(W_2 - W_1) / (A_2 - A_1)] [(\ln A_2 / A_1) / (t_2 - t_1)]$

$LAR = [(A_2 - A_1) / (W_2 - W_1)] [(\ln W_2 / W_1) / (\ln A_2 / A_2)]$

$SLA = [(A_2 - A_1) / (W_{L2} - W_{L1})] [(\ln W_{L2} / W_{L1}) / (\ln A_2 / A_2)]$

$LWR = [(W_{L2} - W_{L1}) / (W_2 - W_1)] [(\ln W_2 / W_1) / (\ln W_{L2} / W_{L1})]$

where W is the total dry weight, t is the time, A is the total leaf area, W_L is the total dry weight of leaves, and 1 and 2 are 5 and 10 d stress periods, respectively.

Absolute growth rate (AGR, g d^{-1}) and relative growth rate (RGR, g $g^{-1}d^{-1}$) of reproductive structures (pods + seeds) were also calculated to study whether growth rates of the genotypes were similarly affected when subjected to drought stress during different growth phases. Both growth rate parameters were computed using dry weights of reproductive structures obtained from the harvests made at the end of 5 and 10 d stress.

2.4.4. Photosynthetic parameters

Gas-exchange characteristics, net photosynthetic rate (A), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were measured on the central leaflets of fully-matured upper canopy leaves of both stressed and non-stressed treatments using a portable photosynthesis system (Li-COR LI-6200, Li-Cor, Inc., Lincoln, NE) assembled with an infra-red gas analyzer (Li-COR LI-6250) and data logger. Measurements were

made on 5th and 10th d of the stress imposition during the vegetative phase experiment. Five measurements were made during the reproductive phase beginning on day two of the stress imposition and continued on alternate days until 10th d Leaf gas-exchange measurements were initiated (usually between 09.30 and 13.30 h) at ambient relative humidity and temperature, when CO_2 concentration in the 0.25 L leaf chamber approached ambient concentration. When the photosynthetically active radiation (PAR) was below 800 μmol m^2 s^{-1}, leaflets were illuminated by a light source to maintain a saturating irradiation of up to 1200 μmol m^{-2} s^{-1} PAR. Photosynthetically active radiation (PAR), leaf temperature (T_l), and air temperature (T_a) were recorded simultaneously.

For dark respiration analyses, the chamber was covered with black plastic sheath for 2 min so that the leaf was in complete darkness (a PAR value close to zero was displayed on the LI-6200 Console's monitor). Measurements began immediately after an increase in CO_2 concentration in the leaf chamber was detected.

2.4.5. Chemical analysis

For sugar and starch analyses, leaf, stem, pod and seed samples were obtained from the harvests made at 5 and 10 d stress (for the vegetative phase) and at 5, 10 and 20 d stress (for the reproductive phase). On the other hand, plant materials (leaf, stem, pod and seed) for α-amino N and crude protein analyses were collected from intact (growing) plants that were subjected to drought stress for periods of 7, 14 and 21 days. The various plant parts were dried at 80^{o}C for 48 h and finely ground materials were used for the chemical analyses.

Sugars: Three-hundred mg ground plant material was weighed into a 50 ml volumetric flask and 30 ml of double-demineralized water was added. The material was then extracted by incubating in a shaking water bath at 60^{o}C for 30 min. The flask was quickly cooled on ice, and filled up to the mark with double-demineralized water followed by filtration with (blue-band) filter paper (Faltenfilter 595$^{1/2}$, Scheicher and Schüll Co.,

Dassel, Germany). Sugars (sucrose, glucose and fructose) were determined by using enzymatic test kits and absorbances of the solutions were read at 340 nm.

Principles of the determination of sucrose, D-glucose and D-fructose using Enzymatic BioAnalysis:

The D-glucose concentration is determined before and after the enzymatic hydrolysis of sucrose; D-fructose is determined subsequent to the determination of D-glucose:

Determination of D-glucose before inversion:

At pH 7.6, the enzyme hexokinase (HK) catalyzes the phosphorylation of D-glucose by adenosine-5′-triphosphate (ATP) with the simultaneous formation of adenosine-5′-diphosphate (ADP) (1).

(1) D-Glucose + ATP $\xrightarrow{HK}$ G-6-P + ADP

In the presence of glucose-6-phosphate dehydrogenase (G6P-DH), the D-glucose-6-phosphate (G-6-P) formed is specifically oxidized by nicotinamide-adenine dinucleotide phosphate ($NADP^+$) to D-gluconate-6-phosphate with the formation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) (2).

(2) G-6-P + $NADP^+$ $\xrightarrow{G6P\text{-}DH}$ D-gluconate-6-phosphate + NADPH + H^+

The NADPH formed in this reaction is stoichiometric to the amount of D-glucose and is measured by means of its light absorbance at 340 nm.

Determination of D-fructose:

Hexokinase also catalyzes the phosphorylation of D-fructose to D-fructose-6-phosphate (F-6-P) with the aid of ATP (3).

(3) D-Fructose + ATP $\xrightarrow{HK}$ F-6-P + ADP

On the completion of the reaction (3) F-6-P is converted by phosphoglucose isomerase (PGI) to G-6-P (4)

F-6-P $\xrightarrow{PGI}$ G-6-P

G-6-P reacts again with $NADP^+$ with the formation of D-gluconate-6-phosphate and NADPH (2). The amount of NADPH formed now is stoichiometric to the amount of D-fructose.

Enzymatic inversion:

At pH 4.6, sucrose is hydrolyzed by the enzyme β-fructosidase (invertase) to D-glucose and D-fructose (5).

(5) Sucrose + $H_2O \xrightarrow{\beta\text{-fructosidase}}$ D-glucose + D-fructose

The determination of D-glucose after inversion (total D-glucose) was carried out according to the principle outlined above. The sucrose concentration is calculated from the difference of the D-glucose concentration before and after enzymatic inversion.

Amino acids: Free amino acid concentrations were determined by quantifying α-amino N using the ninhydrin method. Ground dry materials (100 mg) of both leaves and pods were extracted with 20 ml phosphate buffer in a 100 ml poly flask with an end-over-end shaker for 1 h. After filtration of the extract (Faltenfilter $595^{1/2}$, Scheicher and Schüll Co., Dassel, Germany), 0.4 ml of the sample solution was mixed with 4 ml citrate buffer and 4 ml ninhydrin solution in a reagent glass and incubated for 15 min in a water bath at 100^{o}C. After cooling down the reagent glass in water for 5 min, the solution was added into a micro cuvette and α-amino N concentration was determined by means of a spectrophotometer at a wavelength of 570 nm. A calibration curve was made with L-glutamine, which was prepared in the same way with the sample solution, and data were expressed in mmol α-amino-N kg^{-1} dry weight (DW).

The reaction between alpha-amino acid and ninhydrin involved in the development of color is described by the following five mechanistic steps:

alpha-amino acid + ninhydrin → reduced ninhydrin + alpha-imino acid + H_2O

alpha-amino acid + H_2O → alpha-keto acid +NH_3

alpha-keto acid + NH_3 → aldehyde + CO_2

Step (1) is an oxidative deamination reaction that removes two hydrogen from the alpha-amino acid to yield an alpha-imino acid. Simultaneously, the original ninhydrin is reduced and loses an oxygen atom with the formation of a water molecule. In Step (2), the NH group in the alpha-imino acid is rapidly hydrolyzed to form an alpha-keto acid with the production of an ammonia molecule. This alpha-keto acid further undergoes

decarboxylation reaction of Step (3) under a heated condition to form an aldehyde that has one less carbon atom than the original amino acid. A carbon dioxide molecule is produced here. These first three steps produce the reduced ninhydrin and ammonia that are required for the production of color in the last two Steps (4) and (5). The overall reaction for the above reactions is simply (slightly inaccurately) expressed in Reaction (6) as follows:

alpha-amino acid + 2 ninhydrin → CO_2 + aldehyde + final complex (blue) + 3 H_2O

In summary, ninhydrin, which is originally yellow, reacts with amino acid and turns deep purple. It is this purple color that is detected in this method.

Ninhydrin will react with a free alpha-amino group, NH_2-C-COOH. This group is contained in all amino acids, peptides, or proteins. Whereas the decarboxylation reaction will proceed for a free amino acid, it will not happen for peptides and proteins. Thus, theoretically only amino acids will lead to the color development.

Starch: Starch determination was performed following enzymatic assay procedure using the starch determination kit from Boehringer (Mannheim, Germany). Homogenized ground seed and leaf samples of 300 mg were weighed into Erlenmeyer flasks, and 20 ml of dimethylsulfoxide and 5 ml HCl (8 mol/l) were added. The sealed flask was then incubated for 30 min at 60^{o}C in a shaking water bath. The sample solutions were cooled quickly to room temperature and approximately 50 ml water were added. The pH was adjusted to 4 – 5 with sodium hydroxide (5 M) under vigorous shaking. The solution was then transferred to a 100 ml volumetric flask, rinsed with water, filled up to the mark with water and filtered using Faltenfilter 595$^{1/2}$ (Scheicher and Schüll Co., Dassel, Germany).

Principle of starch determination using the Boehringer method: In the presence of the enzyme amyloglucosidase, starch is hydrolyzed to glucose. The content of glucose is determined with hexokinase and glucose-6-phosphate dehydrogenase.

1. Starch + (n-1)H_2O ⟶ n D-glucose

The glucose is phosphorylated to glucose-6-phosphate by ATP in the presence of hexokinase with formation of ADP.

2. D-glucose + ATP ⟶ G-6-P + ADP

The glucose-6-phosphate is oxidized by ($NADP^+$) to gluconate-6-phosphate with formation of (NADPH).

3. G-6-P + $NADP^+$ ⟶ D-gluconate-6-phosphate + NADPH + H^+

The amount of NADPH formed in the above reaction is stoichiometric to the amount of D-Glucose formed by hydrolysis of starch. NADPH is determined by means of light absorbance by means of spectrophotometer at the absorption maximum of 340 nm.

Protein: The nitrogen concentration was determined by means of sulphuric acid digestion in a Büchi K-324 (BÜCHI Labortechnik AG, Switzerland). Ground leaf samples of 500 mg were digested by adding 20 ml of H_2SO_4 and a Kjeldhal Cu–Se catalytic pill. The digestion process was left to run its course until the samples were clarified. The samples were then diluted to 50 ml with distilled water. In order to determine the nitrogen concentration, 5 ml of ionic strength adjuster was added to 5 ml of measuring solution. Measurements were performed with an ammonia-selective electrode using 0.1 m*M* of ammonium chloride as a standard. The nitrogen content quantified by this method was multiplied by an approximate factor of 6.25 to estimate the crude protein content of the bean seed samples.

ABA: Freeze-dried sink leaf tissue samples of two common bean genotypes were homogenized and extracted in 80% aqueous methanol solution. Extracts were passed through a Sep Pak C18-cartridge. Methanol was removed under reduced pressure and the aqueous residue was partitioned three times against ethyl acetate at pH 3.0. The ethyl acetate of the combined organic fractions was removed under reduced pressure. The newly obtained residue was taken up in TBS-buffer (Tris buffered saline; 150 mmol/L NaCl 1 mmol/L $MgCl_2$ and 50 mmol/L Tris at pH 7.8) and subjected to an immunological ABA assay (ELISA) as described earlier (Mertens et al., 1985). The accuracy of the ELISA has been verified in earlier investigations (Hartung et al., 1994).

2.5. Determination of the numbers and sizes of cotyledonary cells and amyloplasts

The numbers and sizes of cotyledonary cells and amyloplasts were determined on 10 randomly selected seeds per replication obtained from the last harvest (20 d stress). Seed volume was obtained using Archimedes principle (Wessel-Beaver et al., 1984). Dried bean seed weight was determined immediately before measurement of seed volume.

Seeds were softened by soaking in distilled water for one night and then separated into seed coat, cotyledons, and embryonic axes. The cotyledons were cut into small pieces, dried at 104 °C for 24 h, and dry weights were determined. The cotyledon samples were then immersed in an enzymatic solution (sorbitol 0.45M; $MgCl_2$ 10 mM; KH_2PO4 1 mM; MES 20 mM; Macerozyme® R-10 1%; pH 5.6) under vacuum conditions for few minutes. The samples were then placed in an oven at 37 °C for 72 h and then macerated gently with mortar and pestle. Macerated cells were separated on a 300 µm nylon mesh to obtain a homogeneous 100 ml cotyledonary cell suspension. Parts of the cell suspensions were transferred to a 20 ml tube and vortexed before transferring 2 µl aliquots with a micropipette to the middle of a counting grid on a hemacytometer (Medicihaus, Berlin). A cover slip was applied and moved in a circular motion to evenly distribute the cells. Cells were counted under a microscope (Leitz, Wetzlar, Germany) at 25× magnification, and the counts were taken from the four outer squares of the counting chamber (each $1mm^2$) for four aliquots for each cell suspension.

Counts from the four aliquots were averaged to compute the number of cells per unit volume (cells ml^{-1}) in the cell suspension. This average of cells ml^{-1} was multiplied by the volume of the total suspension to give an estimate of cell number per cotyledon. The estimate of cell number per cotyledon was divided by cotyledonary mass to yield an estimate of cotyledon cells per unit mass according to the following equations:

$Cells_{ss}$ = (cell $count_h$/$volume_h$) (suspension volume)

Cells per unit mass = $cell_{ss}$/$mass_{ss}$

Cotyledon cells per seed = (cotyledon mass) ($cell_{ss}/mass_{ss}$)

Mass per cell = $cell_{ss}/mass_{ss}$

Volume per cell = (mass per cell) (seed volume/seed mass); where $cells_{ss}$ = total number of cells in the cotyledon; cell $count_h$ = the average of the cell counts observed in the hemacytometer grid; $volume_h$ = the volume of the grid space used for cell counts within the hemacytometer; suspension volume = total volume of the cotyledon cell suspension; and $mass_{ss}$ = oven-dry mass of the cotyledon sample. Cotyledon cell number per seed was estimated as the product of cotyledon cells per unit mass and the measured cotyledon mass per seed (as calculated above). Individual cotyledon cell volume was estimated by dividing mass per cell by the seed density previously determined from seed weight and seed volume as described earlier. This assumed that seed density and cotyledon density were similar. Because the seeds were about 90% cotyledon, this appeared to be an acceptable assumption.

From same aliquot used for cell counts, 1 ml was removed from the suspension and diluted with an equal volume of an iodine solution (3.3 g/l I_2 + 6.7 g/l KI) to stain the starch granules. Stained starch granules (amyloplasts) were counted on a hemacytometer (Medicihaus, Berlin) at 40× magnification. The counts were then multiplied by the number of cotyledonary cells to determine number of amyloplasts per seed. The same solution used for the determination of number of amyloplasts was also used for measuring the size of the granules. Approximately 3 to 5 µl of the solution was transferred to the middle of a slide on a microscope (LEICA DM IRB, LUDL electronics, NY) equipped with a digital camera (CoolsnapCF, Photometrics). Pictures of 15 to 20 randomly selected cells observed under microscope were acquired to the computer and the sizes of 3 to 15 granules per cell with distinct boundaries from the neighboring amyloplasts were measured using Meta Vue Software (Universal Imaging Corporation). The distance for measurement was calibrated at 40× magnification (0.11625 µm / pixel).

2.6. Proteomic analysis

2.6.1. Protein preparation

Proteins were prepared for isoelectric focusing using a DTT–TCA–acetone precipitation method adopted from Zörb et al. (2004). Plants of the genotype Brown Speckled grown under non-stress and drought stress imposed at vegetative growth stage were used for the analysis. Mature leaf material was disrupted by grinding the tissue under liquid nitrogen in a mortar. Ground powder was stored at -80 °C. Protease activity was inhibited by lowering the temperatures of the cell material (4 °C) and the use of strong denaturants, such as urea and TCA, in the protein sample buffer supplemented by the use of the protease inhibitor Pefablock. 1.6 ml lysis buffer (10% TCA in acetone) was added to ¾-filled ground tissue in a 2 ml Eppendorf tube. After vortexing, samples were incubated for 15 min in an ice-cold ultrasonic bath and incubated at -20 °C for 1 h or overnight before centrifugation (20000 g, 15 min, 4 °C). The precipitant was resuspended in 1 ml 4 °C cold buffer A (50 mM DTT; 2 mM EDTA, in acetone). Samples were incubated for 10 min in an ice-cold ultrasonic bath. This procedure was repeated twice. Pellets were lyophylized under N_2. The collected pellets were resuspended in 1 ml protein sample buffer (8 M urea, 2 M thiourea, 0.5% pharmalyte buffer (v/v, pH 3–10); 4% CHAPS; 30 mM DTT; 20 mM Tris–base, pH 8.8; 5 mM Pefablock). For solubilization of proteins, samples were incubated for 2 h at 33 °C and for 15 min in an ice-cold ultrasonic bath. After vortexing, samples were centrifuged (18000 g, 30 min) and the supernatant was subjected to isoelectric focusing (IEF). Protein concentration was determined in 1:50 dilutions of the samples according to the 2D QUANT protein determination kit from Amersham Biosciences.

2.6.2. 2D gel electrophoresis

Two-dimensional gel electrophoresis was done following the method described by Zörb et al. (2004). IPG strips (11 cm, pH 3–10, Amersham Biosciences) were placed in the trays and 200 µl of the protein solution (150 µg protein) were applied. Strips were covered with paraffin oil. IEF was carried out in a IPGphor chamber (Amersham

Biosciences) applying the following conditions: 10 h rehydration; 100 V, 2 h; 500 V, 1 h; 1000 V, 2 h; 8000 V, 2 h. Temperature was 20 °C and current was 45 μA per strip. After running the first dimension, the strips were placed in equilibration buffer (50 mM Tris–HCl, pH 8.8; 6 M urea; 30% glycerol; 2% (w/v) SDS; bromophenol blue, 0.001% (w/v) containing 1% DTT (w/v)) and carefully shaken for 15 min. Thereafter, the strips were incubated for additional 15 min in equilibration buffer with 4% (w/v) iodoacetamide without DTT under slow agitation. The strips were then rinsed with SDS-PAGE running buffer (25 mM Tris–base; 192 mM glycine; 0.1% (w/v) SDS) for 15 min.

The second dimension SDS gels contained 12.5% (v/v) acrylamide. Molecular weight standards in a range from 10 to 220 kDa were obtained from Invitrogen. The marker lane was positioned at the acidic side (pH 3) of the gel. Strips and marker dyes were mounted onto the gel surface and sealed with 1% (w/v) agarose containing 0.001% (w/v) bromophenol blue. The second dimension was run at 20 °C and with a constant current of 45 mA per gel in a Hoefer (20 cm × 20 cm) vertical gel electrophoresis chamber. Electrophoresis was stopped when the bromophenol blue left the gel and thereafter the gels were fixed with 50% ethanol and 12% acetic acid.

2.6.3. Staining and computer analysis

Coomassie staining was done according to a hot-staining protocol with Coomassie R350 tablets (Westermeier and Naven, 2002). Gels were digitized by scanning on an image scanner (Amersham Biosciences) with 300 dpi and 16 bits per pixel. The Coomassie-stained gel replicates for each of the drought-stressed and non-stressed treatments were fused and subsequent spot quantification was performed using Delta2D software (version 3.3) (Decodon, Greifswald, Germany). Matching of protein/peptide spots was performed manually. The most interesting spots in terms of expression levels (up- or down-regulated by at least the factor of 2 or newly appearing or disappearing) were displayed using the statistical tools option of the software.

2.7. Data analysis

Data were analyzed using the statistical package MSTAT-C, developed by Michigan State University (MSTAT-C, 1989). Data were subjected to analysis of variance (ANOVA) to determine significant differences among treatments for various parameters. Means of the treatments that exhibited significant differences were separated using the least significant difference (LSD) test. The differences of means between control and drought-stressed treatments were tested for statistical significance using the t-test. Relationships between selected parameters were determined using the Pearson's simple correlation test. For all analyses, a P-value of less than 0.05 was interpreted as statistically significant.

3. RESULTS

3.1. Effects on seed yield and yield components

During the initial screening experiment in which six common bean genotypes were subjected to drought at early flowering stage, the stress caused significant reduction in seed yield that ranged from 30% (in BAT 881) to 72% (in Brown Speckled) (Table 2). With only about 33% decrease due to drought relative to the non-stressed treatment, SEA 15 produced the highest seed yield under both growth conditions. Seed yield under drought stress and non-stress conditions were highly and positively correlated ($r = 0.96$, $p<0.01$). The old adapted cultivars generally suffered higher yield losses due to drought stress compared with the inbred lines. Severe yield losses encountered by the old adapted cultivars under drought conditions were a consequence of reductions in individual yield components (data not presented).

Table 2. The effect of drought stress imposed at early flowering stage on seed yield and harvest index of six common bean genotypes. Data are means±S.E. of four replications.

Genotype	Seed yield (g plant $^{-1}$)		% Reduction	Harvest index (%)		% Reduction
	Control	Stress		Control	Stress	
Mex.142	9.0±0.8	5.2±0.6**	42	37.8±2.5	27.3±2.9*	28
Roba 1	13.1±0. 5	6.9±0.7**	47	57.6±1.7	45.7±4.3	21
Br.Speckl.	8.4±0.9	2.4±0.5**	72	28.4±2.4	13.5±2.2*	52
SEA 15	20.5±0.6	13.8±1.4**	33	64.0±1.2	63.4±4.2	1
SEA 23	17.7±0.9	11.2±1.4*	37	62.1±0.7	55.4±7.3	11
BAT 881	13.5±0.2	9.5±0.4**	30	45.8±0.8	42.8±1.4	6

**, *The difference between drought stressed and control treatments are significant at 1 and 5% levels of probability, respectively, according to t-test.

For both Brown Speckled and SEA 15, the degree of seed yield reduction due to drought stress imposed at pod-filling stage was comparable with drought stress initiated at early

flowering stage. Drought stress commenced at early pod-filling stage and lasted for 20 d resulted in 53 and 30% seed yield reductions for Brown Speckled and SEA 15, respectively (Table 3). The effect of drought on seed yield was primarily due to the significant reduction in number of seeds per plant (Table 3). The smaller numbers of seeds per plant under stress for Brown Speckled (20 under drought vs. 41 under control) were ascribed to the significant decrease by about 26% in the numbers of pods per plant and ca. 28% reduction in numbers of seeds per pod. For SEA 15, however, the reduction in the number of seeds per plant owing to drought was due mainly to ca. 25% less number of productive pods retained per plant. The seed weight of both genotypes remained relatively stable under the contrasting soil moisture regimes (Table 3).

Table 3. Seed yield and yield components of two common bean genotypes 20 d after the commencement of drought stress at pod-filling stage. Data are the means±S.E. of four replications.

Treatment		No. Seeds (pod $^{-1}$)	No. Seeds (plant $^{-1}$)	100-seed wt. (g)	Seed yield (g plant $^{-1}$)	Harvest Index (%)
Br Sp	Control	3.18±0.16 ^{b}	40.8±2.5 ^{c}	21.0±1.7 ^{bc}	8.5±0.3 ^{c}	23.9±0.9 ^{b}
	Stress	2.29±0.20 ^{c}	21.4±1.1 ^{d}	18.4±0.9 ^{c}	3.9±0.2 ^{d}	16.9±1.5 ^{c}
SEA15	Control	4.10±0.14 ^{a}	62.1±2.5 ^{a}	24.0±0.9 ^{b}	14.9±0.6 ^{a}	61.1±2.1 ^{a}
	Stress	3.59±0.14 ^{b}	40.4±2.2 ^{b}	25.7±0.8 ^{a}	10.4±0.5 ^{b}	58.2±1.1 ^{a}

Means in the same column having same letters in common are not significantly different according to LSD test at 5% level of probability.

As reported earlier, the number of pods per plant destined for final harvest to a large extent determined the differences in yielding levels of the tested genotypes under drought conditions. Five days after the commencement of drought stress, Brown Speckled had higher pod numbers per plant than SEA 15 under both soil moisture supply regimes (Fig. 1). Nevertheless, relative to the initial pod number, the number of productive pods retained per plant at 20 d stress was considerably more reduced for Brown Speckled

(32%) than for SEA 15 (49%) (Fig. 1). In fact, the drought-susceptible genotype had a higher rate of pod abortion than the resistant one under control conditions, too. For the susceptible genotype, the absolute number of aborted pods per plant increased significantly due to drought stress, whilst the plants of the resistant genotype maintained a similar number of aborted pods under the contrasting soil moisture supply regimes (data not shown).

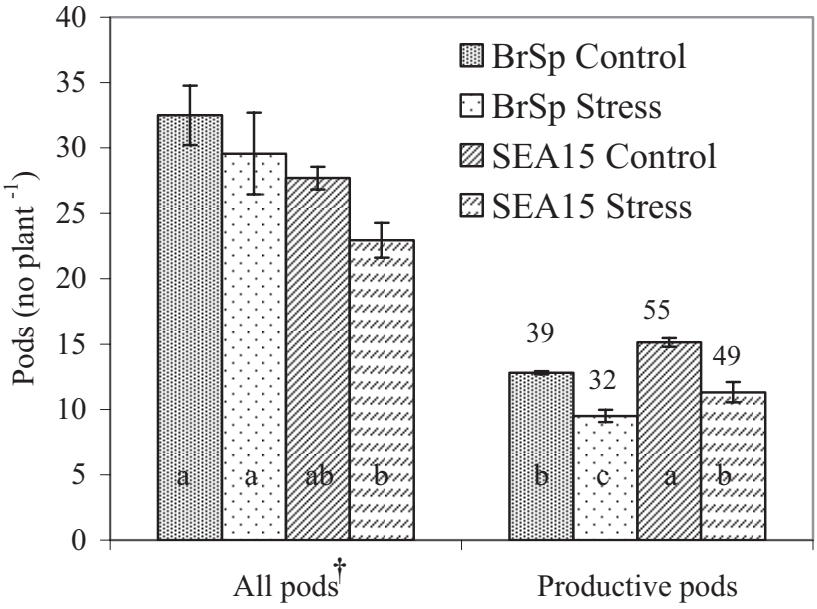

Fig. 1 The effect of drought stress imposed at early pod-filling stage on pod set (total number of pods counted at 5 d stress) and number of productive pods (pods possessing at least one seed at the end of 20 d stress) per plant of two common bean genotypes. Mean-values for each pod category having same letters in common are not significantly different according to LSD test at 5% level of probability. Vertical bars show ±S.E. of four replications. Numbers above bars are percentages of productive pods (Pr-P) relative to total pod set counted at 5 d stress. † All pods = productive pods + aborted pods.

During the initial genotype screening experiment, drought stress reduced the harvest indices of the adapted cultivars (Roba 1, SEA 15 and BAT 881) between 21 and 52% (Table 1). No such effect was found for the inbred lines. When the two selected genotypes

(Brown Speckled and SEA 15) were compared for the same parameter determined 20 d after the imposition of drought stress at pod-filling stage, the reduction owing to drought for Brown Speckled was about 29% relative to the control treatment (Table 3). SEA 15, on the other hand, maintained comparable harvest indices under the contrasting soil moisture regimes (Table 3). In fact, the inherent difference in the harvest indices of the two bean genotypes was substantial. Under both growth conditions, biomass remobilization ability assessed using harvest index values was much lower for Brown Speckled compared with SEA 15.

3.2. Effects of drought stress on growth and biomass production

The effects of genotype and soil moisture regimes were highly significant for above-ground fresh and dry weights determined 5 and 10 d after the imposition of drought stress during the vegetative phase. Compared with Brown Speckled, SEA 15 accumulated significantly higher above-ground biomass under both growth conditions (Table 4). Relative to non-stressed treatments, drought stress caused significant reductions in above-ground biomass yield in the range of 36 - 40% for SEA 15 and 16 - 29% for Brown Speckled. Likewise, drought stress imposed at early pod-filling stage of the crop significantly reduced above-ground fresh and dry weights (leaves + stems + pods + seeds) of both genotypes at all durations of stress considered (Table 4). Drought-induced reductions in above-ground fresh weight (30 - 36% for Brown Speckled vs. 21 - 37% for SEA 15) and dry weight (24 - 33% for Brown Speckled vs. 17 - 29% for SEA 15) were comparable between the genotypes.

Leaf biomass (with up to 40 and 50% reduction for Brown Speckled and SEA 15, respectively) was the most affected fraction of above-ground dry biomass yield due to drought stress (Fig. 2). On the other hand, stem dry weight was relatively unaffected due to drought stress, in general, and for the drought-resistant cultivar, in particular. Unlike the other components of biomass, the difference in seed yield biomass ratio of drought-

stressed to control treatments between SEA 15 (0.70) and Brown Speckled (0.47) was significant (Fig. 2).

Table 4. The effect of drought stress imposed at vegetative and pod-filling stages on above-ground fresh and dry biomass weights (g plant $^{-1}$) of two common bean genotypes. Data are the means±S.E. of four replication.

Duration of stress (d)	Treatment		Vegetative phase		Reproductive phase	
			Fresh wt.	Dry wt	Fresh wt.	Dry wt
5	Br Sp	Control	44.3±2.3 ^{b}	6.1±0.2 ^{c}	162.1±8.5 ^{a}	27.3±0.5 ^{a}
		Stress	33.6±0.9 ^{c}	5.1±0.2 ^{c}	113.9±5.6 ^{c}	20.9±0.9 ^{b}
	SEA 15	Control	72.7±5.9 ^{a}	11.6±0.8 ^{a}	133.3±2.3 ^{b}	24.9±1.0 ^{a}
		Stress	41.4±0.8^{bc}	7.4±0.2 ^{b}	105.5±8.7 ^{c}	20.7±1.4 ^{b}
10	Br Sp	Control	67.1±2.6 ^{b}	9.9±0.4 ^{b}	153.2±2.7 ^{a}	28.5±0.6 ^{a}
		Stress	44.8±3.3 ^{c}	7.0±0.5 ^{c}	99.9±3.9 ^{b}	20.3±0.8 ^{b}
	SEA 15	Control	93.7±5.1 ^{a}	17.8±0.8 ^{a}	144.5±8.1^{a}	30.7±1.8 ^{a}
		Stress	54.7±0.5 ^{c}	10.7±0.1 ^{b}	93.8±0.7 ^{b}	21.7±0.6 ^{b}
20	Br Sp	Control	-	-	134.4±3.3 ^{a}	35.4±0.7 ^{a}
		Stress	-	-	85.5±4.3 ^{c}	23.7±1.3 ^{b}
	SEA 15	Control	-	-	102.8±7.3 ^{b}	24.3±0.6 ^{b}
		Stress	-	-	65.2±3.0 ^{d}	17.8±0.5 ^{c}

Mean values within the same column and for the same duration of stress having similar letters in common are not significantly different according to LSD test at 5% level of probability.

Under drought as well as non-stress growth conditions, SEA 15 maintained higher reproductive (pods + seeds) to vegetative (leaves + stems) mass ratio than Brown Speckled (Fig. 3). Soil moisture supply regime, however, did not affect the partitioning of

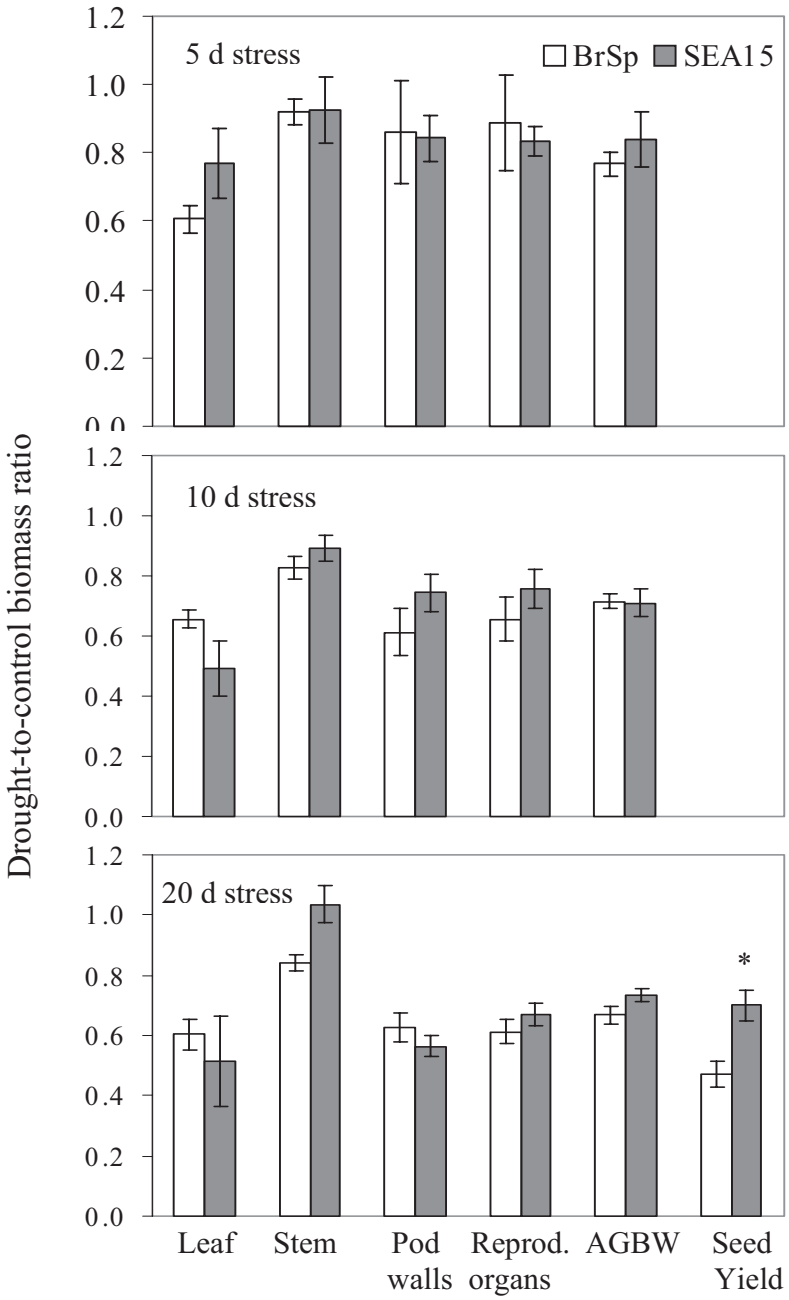

Fig. 2. Biomass ratio (drought tissue / control tissue) of leaves, stems, pods, reproductive organs (pod wall + seeds), above-ground biomass weight (AGBW) (leaves + stems + reproductive materials) and seed yield of two common bean genotypes at different durations of stress. For the calculation of the biomass ratio, g of dry weight was the unit used. Vertical bars show ±S.E. of four replications. * Difference between the two genotypes is significant at 5% level of probability according to t-test.

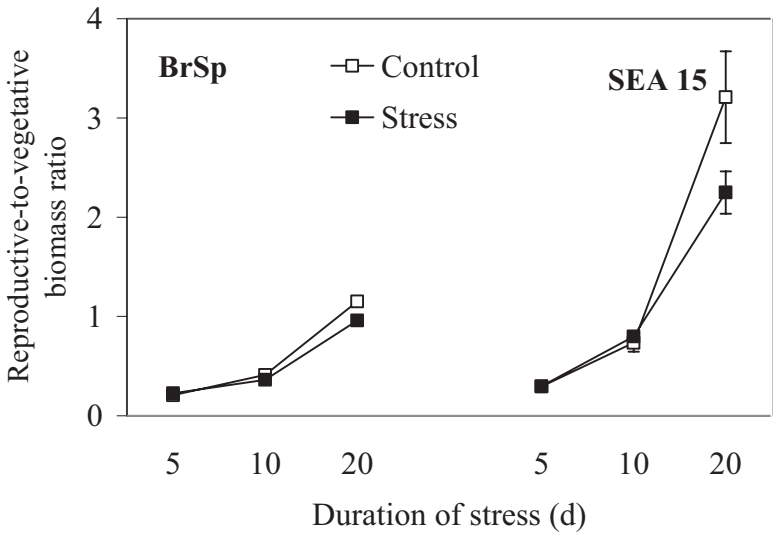

Fig. 3. The effect of drought stress initiated at pod-filling stage on reproductive to vegetative biomass ratio of two common bean genotypes. Vertical bars show ±S.E. of four replications.

biomass between the vegetative and reproductive parts of both genotypes. During the final harvest (20 d stress), the biomass weight of reproductive organs was two and three-fold larger than the vegetative parts for drought-stressed and non-stressed SEA 15 plants, respectively (Fig. 3). On the contrary, the dry weights of reproductive and vegetative structures of Brown Speckled remained fairly proportional under both growth conditions (Fig. 3).

Main effects due to genotype and soil moisture regime were significant for leaf area and specific leaf weight (SLW) determined during the vegetative growth phase of the crop. SEA 15 reacted to the stress imposed with an enormous leaf area reduction (by about 65% relative to the control treatment at both sampling times) compared with Brown Speckled, which encountered only ca. 40 % reduction (Fig. 4A). A drought stress period of 10 d during the vegetative phase significantly increased (by ca. 16%) the specific leaf weight (SLW) of SEA 15 relative to the control treatment (Fig. 4B). On the other hand, drought

stress did not significantly alter the SLW of Brown Speckled at both sampling times. SLW exhibited significant correlation with leaf dry matter content (LDMC, ratio of leaf fresh weight to dry weight) ($r = 0.98$, $p<0.01$) but not with leaf area ($r = - 0.27$, $p>0.05$).

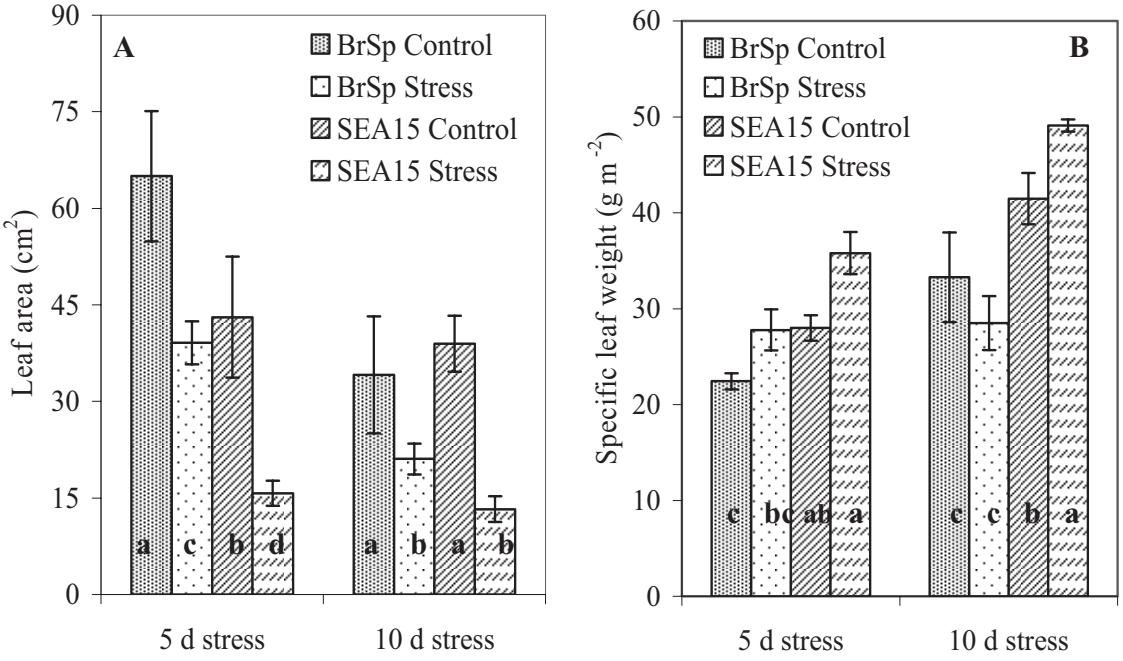

Fig. 4. The effect of drought stress imposed at vegetative stage on leaf area (A) and specific leaf weight (B) of two common bean genotypes. Means followed by the same letter during the same duration of stress are not significantly different according to LSD test at 5% level of probability. Vertical bars are ± S.E. of four replications.

3.3. Effects on vegetative and reproductive growth rates

Relative to the control treatments, drought stress significantly reduced the vegetative absolute growth rate (AGR) computed on shoot dry weight basis by about 38 and 47% for Brown Speckled and SEA 15, respectively (Fig. 5). Relative growth rates (RGR) of both genotypes were also negatively affected by drought stress (Table 5). Drought stress imposed during vegetative growth phase reduced the net assimilation rate (NAR) of Brown Speckled and SEA 15 by ca. 18 and 28%, respectively (Table 5). Leaf area ratio (LAR) is a composite parameter, determined partly by allocation (leaf weight

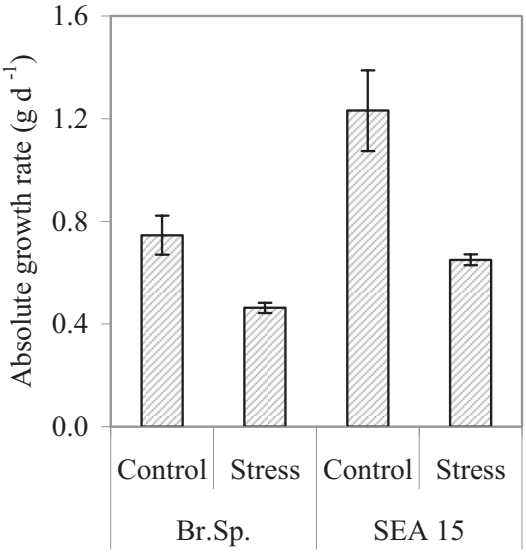

Fig. 5. The effect of drought stress imposed at vegetative phase on the absolute growth rate of two common bean genotypes. Vertical bars show ±S.E. of four replications.

Table 5. The effect of drought stress initiated during the vegetative growth phase on relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA) and leaf weight ratio (LWR) of two common bean genotypes. Data are means ($\times 10^{-2}$) ± S.E. of four replications.

Treatment		RGR ($g\ g^{-1}\ d^{-1}$)	NAR ($g\ m^{-2}\ d^{-1}$)	LAR ($m^2\ g^{-1}$)	SLA ($m^2\ g^{-1}$)	LWR ($g\ g^{-1}$)
BrSp	Control	9.46±0.78 [a]	450.4±49.6 [a]	2.12±0.09 [a]	3.05±0.16 [a]	69.6±0.6 [a]
	Stress	7.17±0.26 [b]	366.8±11.9 [b]	1.95±0.04 [ab]	2.89±1.00 [ab]	67.7±1.3 [a]
SEA15	Control	10.56±0.03 [a]	594.8±21.1 [a]	1.78±0.06 [bc]	2.68±0.06 [b]	66.5±1.8 [a]
	Stress	7.27±0.33 [b]	424.4±29.7 [b]	1.72±0.04 [c]	2.92±0.07 [ab]	59.0±0.5 [b]

Means having similar letter within the same column are not significantly different according to LSD test at 5% level of probability.

ratio, LWR) and partly by leaf morphology (specific leaf area, SLA). Brown Speckled maintained higher LAR than SEA 15 under drought as well as control conditions (Table

5). Whereas drought stress did not have a significant impact on specific leaf area (SLA) of both genotypes, the leaf weight ratio (LWR) component of LAR was significantly reduced for SEA 15 due to drought stress (Table 5). RGR correlated significantly with NAR ($R^2 = 0.81$, $p<0.05$) but not with leaf area ratio (LAR) ($R^2 = 0.18$, $p>0.05$).

Absolute growth rate (AGR) and relative growth rate (RGR) of reproductive structures (dry weights of aborted pods + pod walls + seeds) were also computed to examine whether the bean genotypes maintain similar growth rates when subjected to drought stress at different growth phases. AGR of the reproductive structures due to drought stress was significantly reduced by 49 and 32% for Brown Speckled and SEA 15, respectively (Fig. 6). On the other hand, the decrease in RGR caused by drought was significant only for Brown Speckled. Compared with Brown Speckled, SEA 15 generally maintained higher AGR and RGR of reproductive structures under both soil moisture supply regimes.

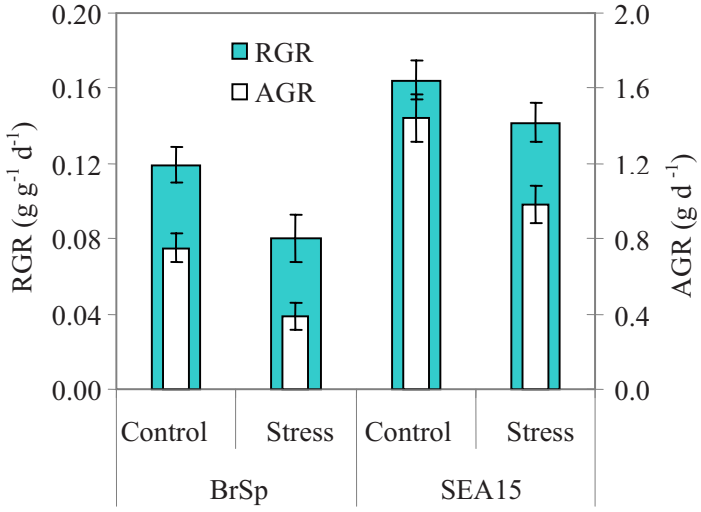

Fig. 6. The effect of drought stress imposed at pod-filling stage on absolute growth rate (AGR) and relative growth rate (RGR) of reproductive structures (pod walls + aborted pods + seeds) in two common bean genotypes. Vertical bars show ±S.E. of four replications.

3.4. Effects on plant-water relations

3.4.1. Relative water content

The leaf relative water content (RWC) of Brown Speckled was influenced by drought stress at neither the vegetative nor the reproductive growth stages (Fig. 7A, B). On the contrary, SEA 15 maintained significantly lower RWC under drought stress relative to control treatments at 5 d stress during the vegetative phase and during most sampling times of the reproductive phase (Fig. 7A, B). RWC as a key reference parameter of leaf water status, exhibited a positive and significant correlation with net photosynthetic rate ($r = 0.54$, $p < 0.05$) and stomatal conductance ($r = 0.57$, $p < 0.01$) during the reproductive phase. However, the degree of relationship between RWC and net photosynthetic rate (A) was smaller ($R^2 = 0.33$, $p<0.01$) as compared with the relationship between A and stomatal conductance (g_s) ($R^2 = 0.89$, $p < 0.01$) (Fig. 8A, B).

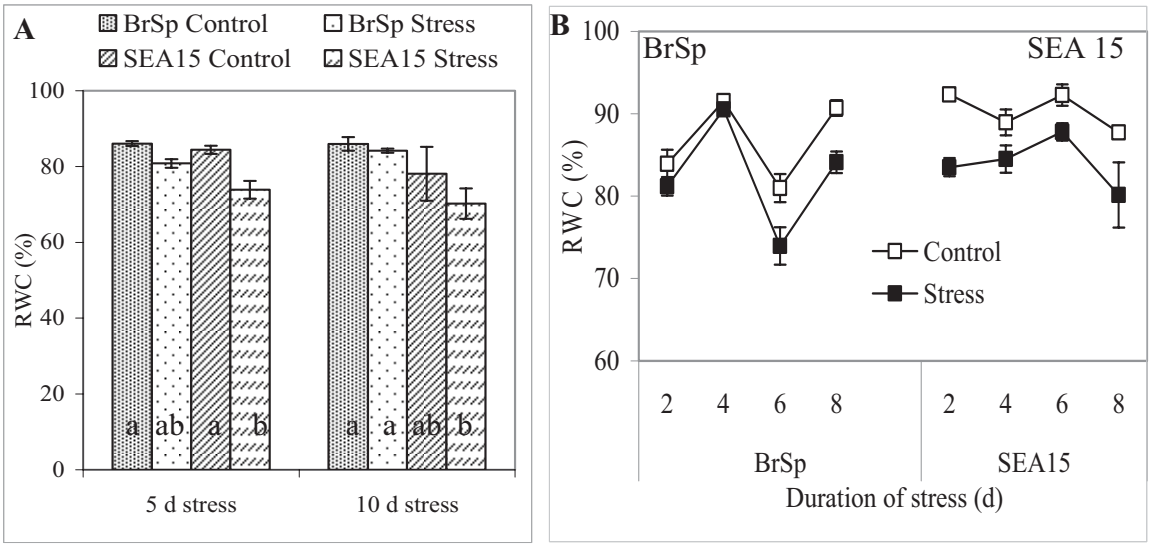

Fig. 7. Leaf relative water content (RWC) of two common bean genotypes under drought stress and non-stress growth conditions during vegetative (A) and reproductive (B) growth phases. Means followed by the same letter during the same duration of stress are not significantly different according to LSD test at 5% level of probability. Vertical bars are ± S.E. of four replications.

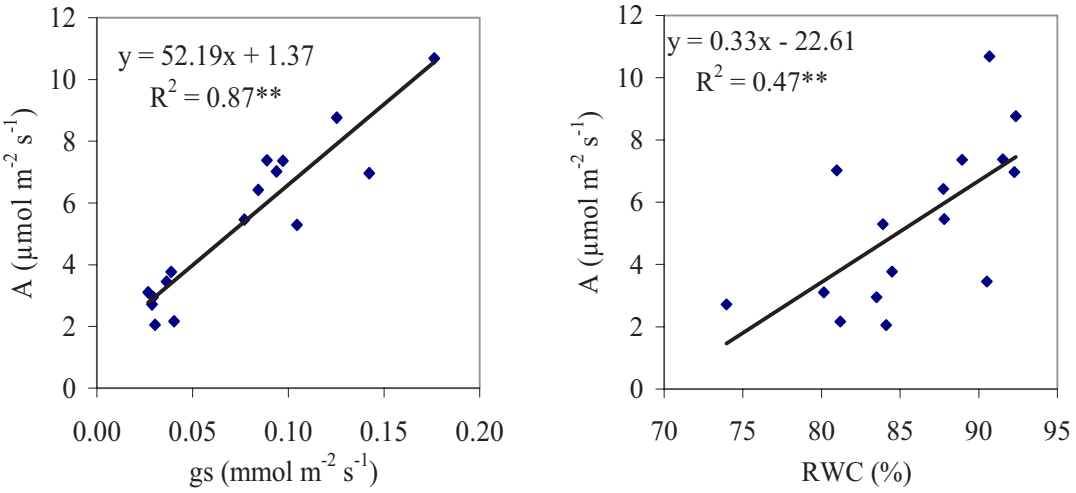

Fig. 8. The relationship of stomatal conductance (g_s) and leaf relative water content (RWC) with net photosynthetic rate (A) of two common bean genotypes grown under drought stress (imposed at reproductive phase) and non-stress growth conditions. * Significant at 1 % level of probability.

A marked difference was found between the two genotypes for the maintenance of pod relative water content under drought stress (Fig. 9). Drought-induced reduction in pod water concentration of Brown Speckled was higher than that of SEA 15 (Fig. 9). Twenty days after drought stress was initiated at early pod-filling stage, pod water concentration dropped from ca. 86% (determined at 5 d stress for both genotypes) to 54 and 74% for Brown Speckled and SEA 15, respectively (Fig. 9).

3.4.2. Leaf water potential

The tested genotypes exhibited significant differences for water potential and its components under drought stress imposed at early flowering stage (Table 6). Drought-induced reductions in leaf water potential (ψ) determined at early pod-filling stage (ca. 15 d after drought stress was commenced) were significant only for Brown Speckled and BAT 881 (Table 6). Under drought stress, SEA 15 (–1.17 MPa) and Mexican 142 (–1.03 MPa) maintained the highest and lowest leaf water potentials, respectively. Higher solute accumulation due to drought stress enabled the tested genotypes (except Brown Speckled and SEA 15) to maintain significantly lower osmotic potentials (ψ_s), which ranged from

–2.05 to –1.86 MPa (Table 6). Except for Mexican 142 and BAT 881, drought stress did not significantly affect the turgor pressures (ψ_p) of the bean genotypes.

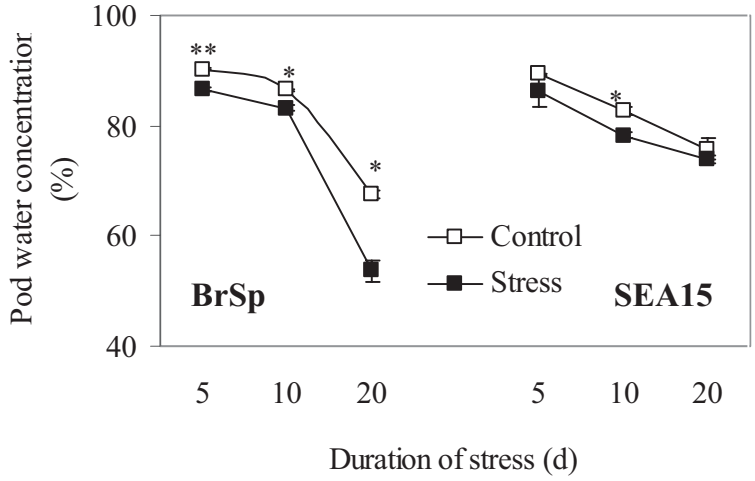

Fig. 9. Pod water concentration of two common bean genotypes grown under drought stress and non-stress growth conditions. *, ** The differences between the drought-stressed and non-stressed treatments are significant at 5 and 1% levels of probability, respectively. Vertical bars show ± S.E. of four replications.

Table 6. The effect of drought stress imposed at early flowering stage on leaf water potential (ψ), osmotic potential (ψ_s) and turgor pressure (ψ_p) of six common bean genotypes.

	ψ (MPa)		ψ_s (MPa)		ψ_p (MPa)	
Genotype	Control	Stress	Control	Stress	Control	Stress
Mex.142	-0.70	-0.83	-1.67	-2.05**	0.98	1.22**
Roba 1	-0.78	-0.88	-1.67	-1.89**	0.90	1.01
Br.Speckl.	-0.76	-0.94*	-1.78	-1.93	1.03	0.99
SEA 15	-1.03	-1.17	-1.62	-1.86*	0.59	0.69
SEA 23	-0.85	-0.99	-1.91	-2.12	1.06	1.13
BAT 881	-0.99	-1.19*	-1.96	-2.41**	0.98	1.22*

*, ** The differences between drought-stressed and non-stressed treatments are significant at 5 and 1% levels of probability, respectively.

3.5. Effects on water-use and water-use efficiency (WUE)

Under non-stress growth conditions, SEA 15 consumed ca. 36% more water than Brown Speckled during a ten-day period of the vegetative phase (Fig. 10A). Nonetheless, the amount of water used by the two genotypes was more or less comparable under drought stress (Fig. 10A). The effects of genotype and soil moisture supply regimes were highly significant for water-use efficiency (WUE, mg dry matter produced per g water used) determined at vegetative growth stage of the crop. Drought stress imposed during the same period increased WUE by about 35 and 37% for Brown Speckled and SEA 15, respectively (Fig. 10B). Nevertheless, the increase in WUE owing to drought stress during the vegetative phase was significantly higher for SEA 15 (3.12 mg g^{-1}) compared with Brown Speckled (2.45 mg g^{-1}).

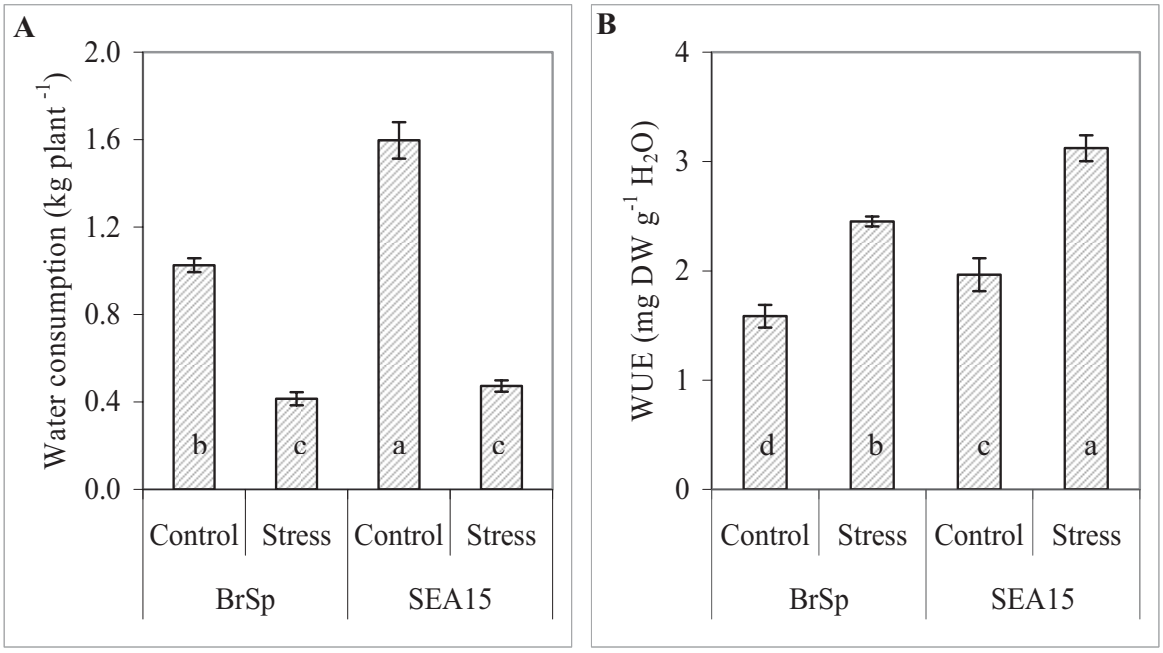

Fig. 10. Water consumption (A) and water-use efficiency (B) of two common bean genotypes under 10 d drought stress and non-stress growth conditions during the vegetative phase. Mean values for each parameter having same letter in common are not significantly different according to LSD test at 5% level of probability. Vertical bars are ± S.E. of four replications.

The amount of water consumed (from emergence to maturity) by the inbred lines SEA 15 and SEA 23 was significantly less than the old adapted cultivars under drought stress as well as non-stress growth conditions (Table 7). Also, the two inbred lines used more than two-third and one-half of the total water supplied for the maintenance of reproductive growth under drought stress and non-stress growth conditions, respectively (Table 7). On the contrary, the adapted cultivars used the highest proportion of water supplied to support vegetative growth. Whether total biological yield (shoot biomass + seed yield) or seed yield alone was used to generate the index, the inbred lines had remarkably higher water-use efficiencies than the old adapted cultivars under both soil moisture regimes (Table 8).

Table 7. Quantity of water consumed from emergency to maturity and share of the reproductive phase in six common bean genotypes grown under drought stress (initiated at flowering stage) and non-stress growth conditions. Data are the means±S.E. of four replications.

Genotype	Water consumption† (kg plant^{-1})		Share of reproductive phase (%)	
	Control	Stress	Control	Stress
Mex.142	11.4±0.21 ^{b}	8.1±0.14 ^{b}	50	36
Roba 1	10.4±0.01 ^{c}	7.4±0.05 ^{c}	60	44
Br.Speckl.	12.1±0.16 ^{a}	8.6±0.11 ^{a}	55	39
SEA 15	9.9±0.07 ^{d}	7.0±0.09 ^{d}	69	57
SEA 23	9.6±0.12 ^{e}	6.4±0.14 ^{e}	70	55
BAT 881	10.8±0.11 ^{b}	7.7±0.22 ^{c}	56	40

†The difference between drought-stressed and non-stressed treatments for all genotypes was significant at 1% level of probability according to t-test. Means having similar letter within the same column are not significantly different according to LSD test at 5% level of probability.

Under drought stress, the highest (1.81 mg g^{-1}) and the lowest (0.33 mg g^{-1}) seed yield-based WUE (WUE_{SY}) were found for SEA 15 and Brown Speckled, respectively (Table 8). Higher WUE_{SY} achieved by the two genetically related inbred lines (SEA 15 and SEA 23) was not only due to higher seed yield produced (Table 1) but also due to less water consumed to realize the yield level attained (Table 7). The correlation of WUE_{SY} with seed yield was highly significant under drought stress ($r = 0.87$, $p<0.01$) as well as non-stress ($r = 0.86$, $p<0.01$) conditions.

Table 8. Water-use efficiency based on above-ground biomass yield (WUE_{BY}) and seed yield (WUE_{SY}) of six common bean genotypes grown under drought stress (imposed at flowering stage) and non-stress growth conditions. Data are the means±S.E. of four replications.

	WUE_{BY} (mg DM g^{-1} H_2O)		WUE_{SY} (mg DM g^{-1} H_2O)	
Genotype	Control	Stress	Control	Stress
Mex.142	2.20±0.11 ^{d}	2.35±0.04 ^{b}	0.84±0.08 ^{d}	0.68±0.07 ^{de}
Roba 1	2.21±0.08 ^{d}	2.15±0.11 ^{b}	1.24±0.04 ^{c}	0.98±0.10 ^{cd}
Br.Speckl.	2.47±0.07 ^{cd}	2.10±0.08 ^{b}	0.74±0.07 ^{d}	0.33±0.06 ^{e}
SEA 15	3.19±0.04 ^{a}	3.02±0.13 ^{a}	2.01±0.06 ^{a}	1.81±0.19 ^{a}
SEA 23	2.87±0.12 ^{b}	3.21±0.08 ^{a}	1.77±0.08 ^{b}	1.61±0.20 ^{ab}
BAT 881	2.73±0.07 ^{bc}	2.90±0.08 ^{a}	1.25±0.02 ^{c}	1.23±0.06 ^{bc}

Means having similar letter within the same column are not significantly different according to LSD test at 5% level of probability.

The main effects due to genotype and watering regime were highly significant for photosynthetic (instantaneous) water-use efficiency (IWUE, ratio of net photosynthetic rate to stomatal conductance) determined during the reproductive phase. Relative to control treatments, the IWUE of SEA 15 increased under drought stress by about 29% (average of five sampling dates) (Fig. 11). On the other hand, drought-induced increase in

IWUE of Brown Speckled (by about 12%) was not significantly different from the control treatment. It is also worth mentioning that IWUE was associated more closely with stomatal conductance (g_s) ($R^2 = 0.42$, $p < 0.01$) than with net photosynthetic rate (A) ($R^2 = 0.20$, $p < 0.05$) (Fig. 12).

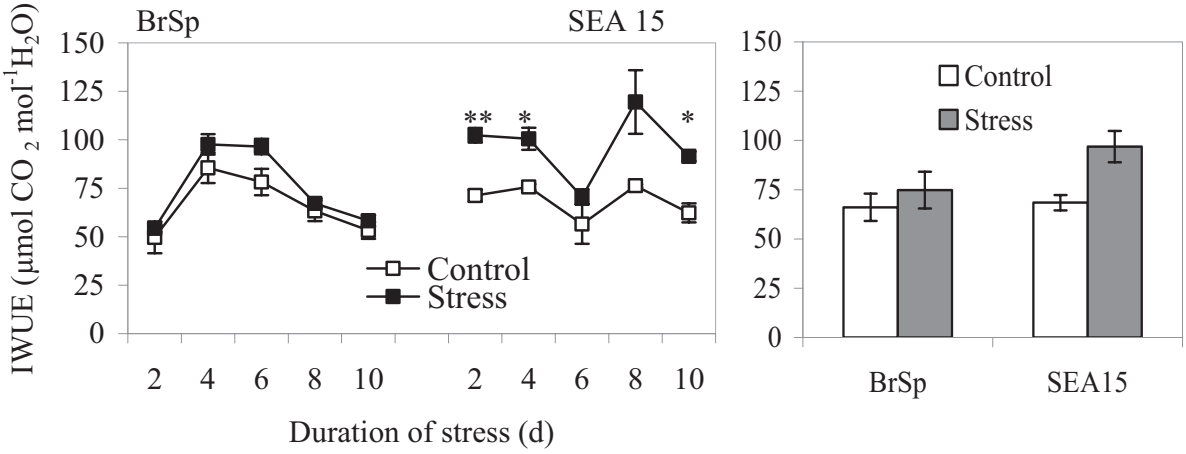

Fig. 11. Instantaneous water-use efficiency (IWUE) of two common bean genotypes grown under drought stress imposed at reproductive stage and non-stress growth conditions. Bar graphs on the right side are average measurements of five sampling dates. Vertical bars are ± S.E. of four replications. *, ** The differences between drought-stressed and non-stressed treatments are significant at 5 and 1% levels of probability, respectively, according to t-test.

3.6. Effects on sink leaf ABA concentration

Five days after the initiation of drought during the vegetative phase, both genotypes accumulated significantly larger amounts of ABA in sink (expanding) leaves in response to the imposed stress (Fig. 13). Relative to the corresponding non-stressed treatments, drought stress increased sink leaf ABA concentration of Brown Speckled by ca. six-fold compared with only about two-fold increase found for the drought-resistant genotype, SEA 15 (Fig. 13).

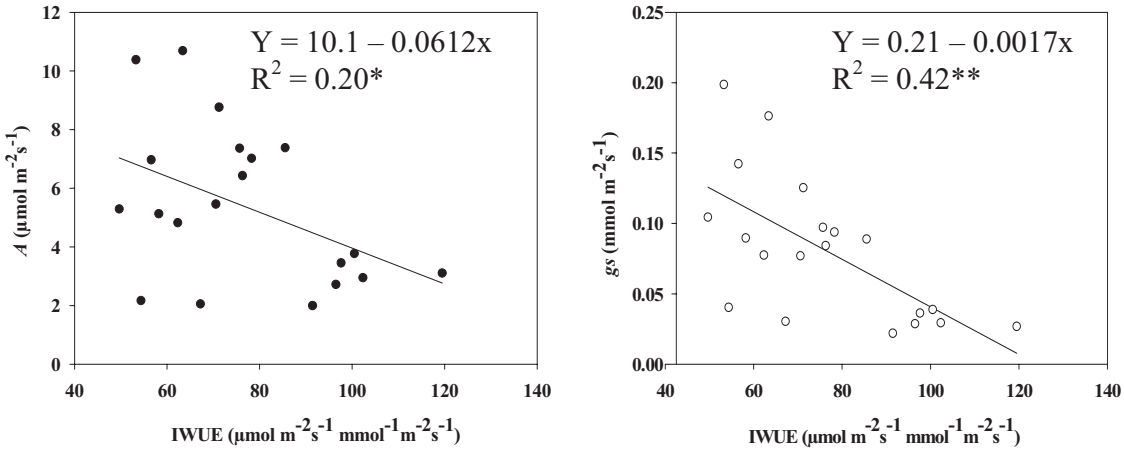

Fig. 12. The relationship between instantaneous water-use efficiency (IWUE) and gas-exchange parameters (*A* and g_s) of two common bean genotypes grown under drought stress (imposed at reproductive phase) and non-stress growth conditions.
*, ** Significant at 5 and 1% levels of probability, respectively.

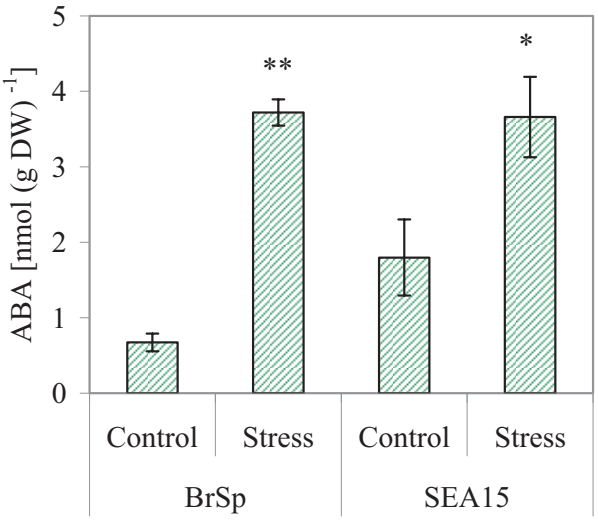

Fig. 13. The effect of drought stress imposed during the vegetative phase on sink leaf abscisic acid (ABA) concentrations of two common bean genotypes. Vertical bars are ± S.E. of four replications. *, ** The differences between drought-stressed and non-stressed treatments are significant at 5 and 1% level of probability, respectively, according to t-test

3.7. Effects on leaf gas-exchange

Drought stress significantly reduced the net photosynthetic rate (*A*) and stomatal conductance (g_s) of the bean genotypes both at the vegetative (Fig. 14A, B) and reproductive (Fig. 15A, B) growth phases. The decreases in both parameters (*A* and g_s) owing to drought stress were observed at all sampling times during the course of the stress. Although significant differences were not found between the genotypes, the average reduction of *A* across the stress period during the reproductive phase was higher for the drought susceptible genotype Brown Speckled (ca. 62%) as compared with SEA 15 (only ca. 50% reduction relative to control plants) (Fig. 15A).

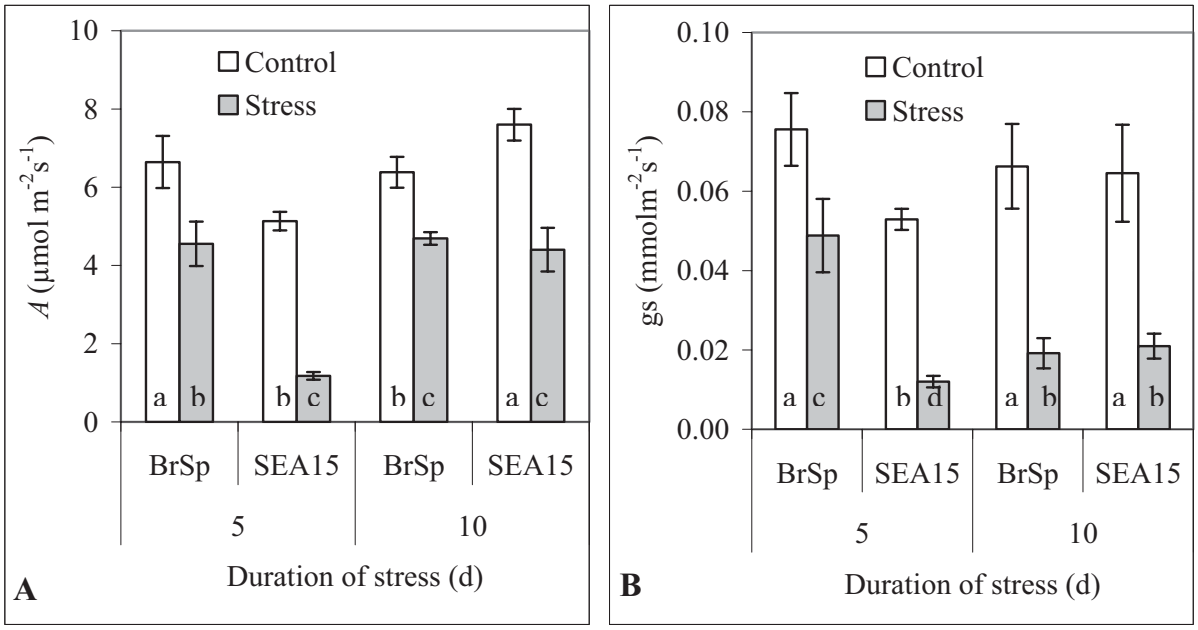

Fig. 14. The effect of drought stress imposed during the vegetative phase on net photosynthetic rate (A) and stomatal conductance (B) of two common bean genotypes differing in drought resistance. Means followed by the same letter during the same duration of stress are not significantly different according to LSD test at 5% level of probability. Vertical bars are ± S.E. of four replications.

Irrespective of the growth stage at which drought stress was imposed, g_s of drought-stressed plants of the bean genotypes decreased by about 40% relative to the

corresponding control plants (Fig. 14B and Fig. 15B). The correlation of the two gas-exchange parameters, *A* and g_s, was high and significant at vegetative (r = 0.84, p<0.01) as well as reproductive (r = 0.95, p<0.01) growth stages of the crop.

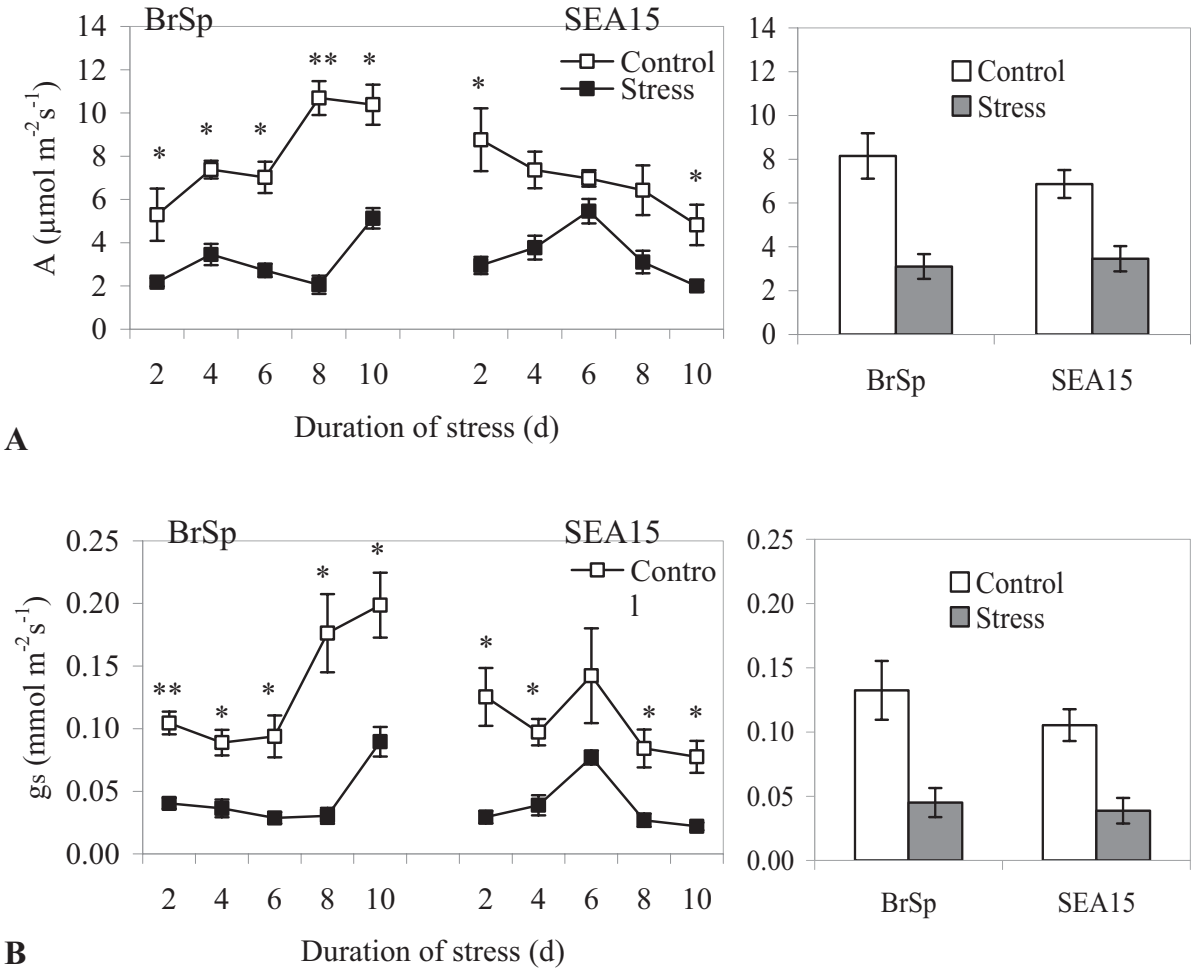

Fig. 15. The effect of drought stress imposed at early pod-filling stage on net photosynthetic rate (A) and stomatal conductance (B) of two common bean genotypes differing in drought resistance. Bar graphs on the right side are average measurements of five sampling dates. Vertical bars are ± S.E. of four replications. *, ** The differences between drought-stressed and non-stressed treatments are significant at 5 and 1% level of probability, respectively, according to t-test.

Despite comparable effects of drought stress on g_s of the two bean genotypes, significant decrease in leaf intercellular CO_2 concentrations (C_i) due to the stress was apparent only for SEA 15 (data not shown). Because the ambient CO_2 concentration (C_a) during measurements change, C_i/C_a ratios instead of C_i are presented (Fig. 16). Corresponding with the C_i levels, drought-induced reduction of the ratio C_i/C_a was higher and more consistent for SEA 15 than it was for Brown Speckled (Fig. 16). Drought stress initiated at the pod-filling stage also reduced the carboxylation efficiency of the bean genotypes, which was estimated by the ratio, A/C_i. Compared with the corresponding control plants, the decrease in A/C_i due to drought was higher for Brown Speckled (average of five sampling dates ca. 63%) than it was for SEA 15 (ca. 46%) (Fig. 17).

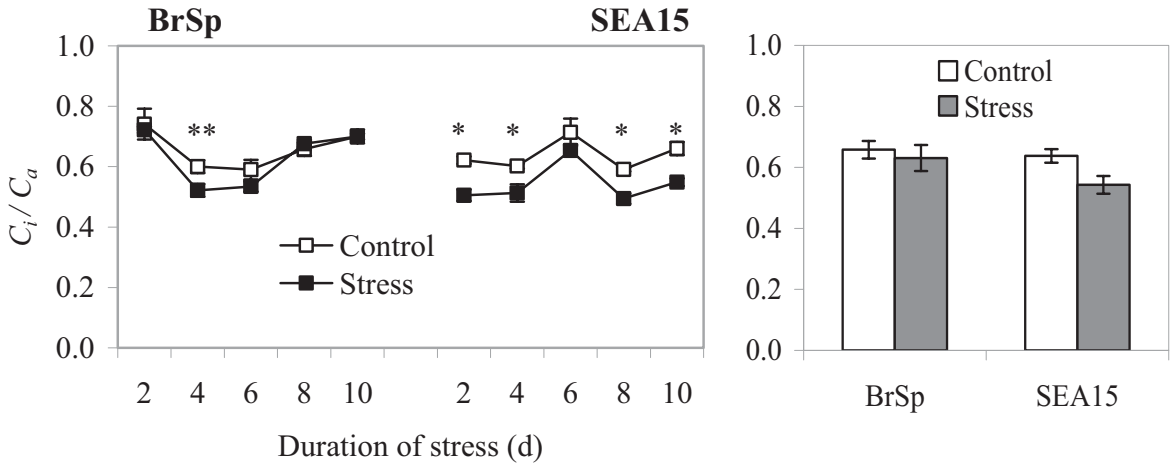

Fig. 16. The effect of drought stress imposed at early pod-filling stage on the ratio of leaf intercellular to ambient CO_2 concentration (C_i/C_a) of two common bean genotypes differing in drought resistance. Bar graphs on the right side are average measurements of five sampling dates. Vertical bars are ± S.E. of four replications. *, ** The differences between drought-stressed and non-stressed treatments are significant at 5 and 1% level of probability, respectively, according to t-test.

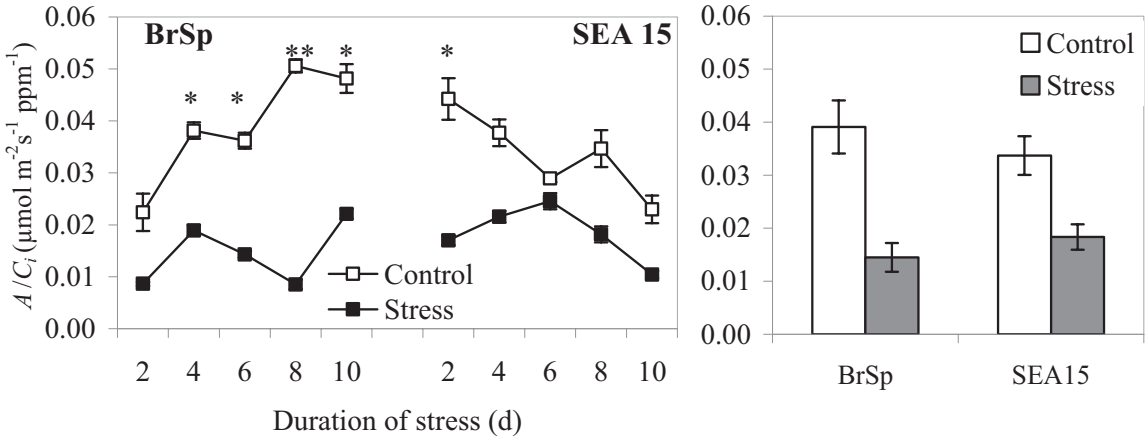

Fig. 17. The effect of drought stress imposed at pod-filling stage on the ratio of net photosynthetic rate to leaf intercellular CO_2 concentration (A/C_i) of two common bean genotypes differing in drought resistance. Bar graphs on the right side are average measurements of five sampling dates. Vertical bars are ± S.E. of four replications. *, ** The differences between drought-stressed and non-stressed treatments are significant at 5 and 1% level of probability, respectively, according to t-test.

The rate of dark respiration (determined as the net release of CO_2 by the leaves into the leaf chamber) measured for plants grown under non-stress conditions was remarkably higher for Brown Speckled relative to SEA 15 (Fig. 18). On the contrary, plants of both bean genotypes subjected to drought during the same period (pod-filling stage) responded to the stress with a comparable increase in the rate of dark respiration relative to the corresponding control plants (Fig. 18).

3.8. Effects of drought stress on assimilate metabolism in the source and sink organs

3.8.1. Assimilate synthesis and availability in leaves

Drought stress imposed during the vegetative phase decreased leaf sucrose concentrations of both genotypes, though the rate of reduction was markedly higher at 5 d than 10 d stress (Fig. 19A). In contrast, assimilate concentrations in the leaves of the two genotypes was differentially affected when drought was initiated during the reproductive phase of the crop (Fig. 20A). For the drought-resistant genotype, SEA 15, drought stress imposed

during the latter stage did not affect the leaf sucrose concentration except at 10 d stress (Fig. 20A). The concentration in the leaves of drought-stressed Brown Speckled during the same period, however, showed a consistent decline by ca. 18 - 30% relative to the non-stressed plants (Fig. 19A).

Concentrations of leaf hexose sugars (glucose + fructose) of the drought-susceptible genotype (Brown Speckled) were not altered due to drought stress imposed at either growth stages of the crop (Fig. 19B, Fig. 20B). Conversely, the concentration of hexoses in the leaves of SEA 15 decreased by over 40% (Fig. 19B) but showed an increment by up to 38% (Fig. 20B) in response to drought stress induced during the vegetative and reproductive phases, respectively. Leaf sucrose to hexose ratio (sucrolytic index) was not significantly altered due to drought during both growth phases of the bean genotypes (data not presented). However, Brown Speckled generally maintained higher leaf sucrolytic index than SEA 15 under drought as well as non-stress growth conditions (not shown).

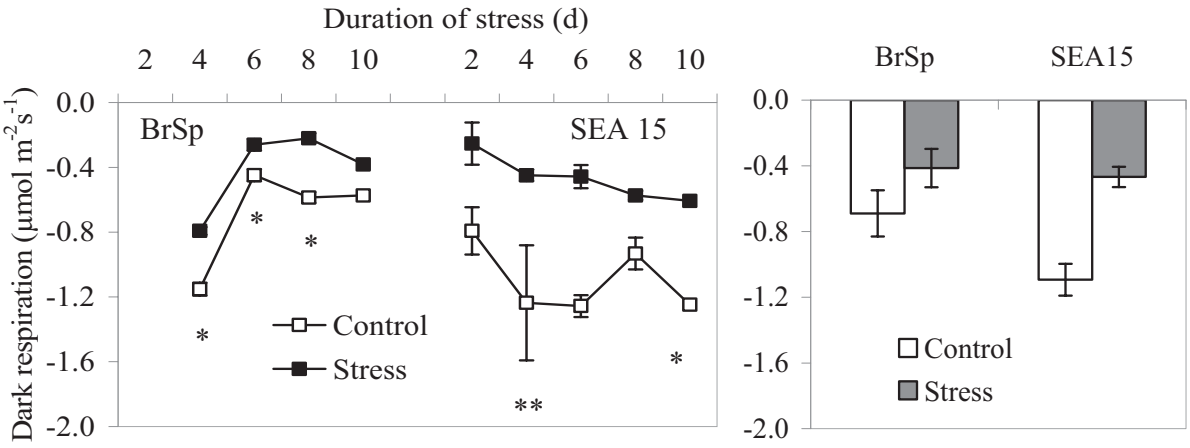

Fig. 18. The effect of drought stress imposed at early pod-filling stage on dark respiration of two common bean genotypes differing in drought resistance. Bar graphs on the right side are average measurements of five sampling dates. Vertical bars are ± S.E. of four replications. *, ** The differences between control and stress treatments are significant at 5 and 1% level of probability, respectively, according to t-test.

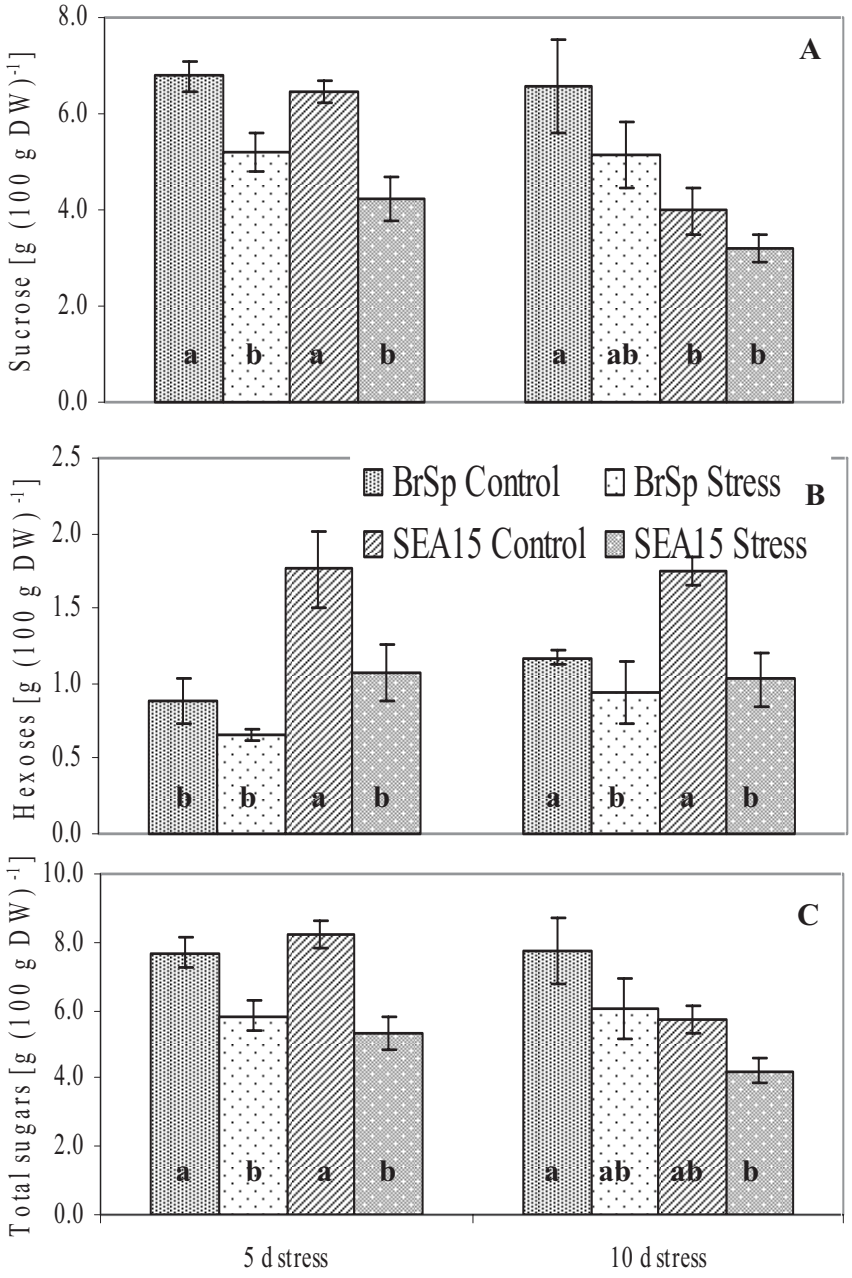

Fig. 19. Leaf sucrose (A), hexose sugars (B) and total sugar (C) concentrations of two common bean genotypes under drought stress and non-stress growth conditions during the vegetative phase. Mean values within the same duration of stress having similar letter in common are not significantly different according to LSD test at 5% level of probability. Vertical bars are ±S.E. of four replications.

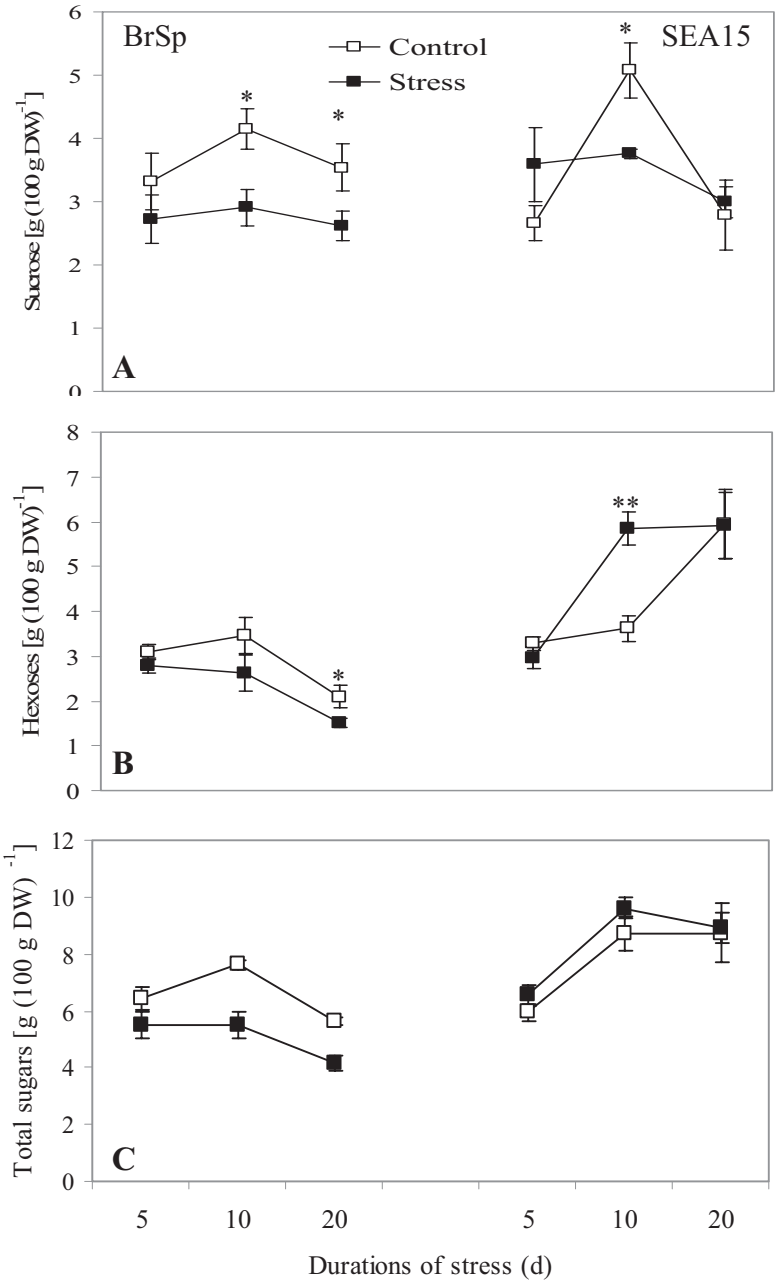

Fig. 20. The effect of drought stress imposed at early pod-filling stage on leaf sucrose (A) hexose sugars (B) and total sugars (C) concentrations of two common bean genotypes. Vertical bars show ±S.E. of four replications. *, ** The differences between drought-stressed and non-stressed treatments are significant at 5 and 1% levels of probability, respectively.

Relative to non-stressed treatments, plants of both genotypes subjected to drought stress during the vegetative phase had significantly lower leaf total sugar (sucrose + hexoses) concentrations (Fig. 19C). When the stress was initiated at early pod-filling stage, leaf total sugar concentrations for SEA 15 remained unaffected, whilst it resulted in 14 - 28% reduction for Brown Speckled (Fig. 20C). With regard to leaf nitrogenous compounds, the bean genotypes reacted to drought stress imposed during the reproductive phase in a similar manner. Leaf free amino acid (α-amino N) concentrations were generally higher under drought stress compared with the non-stressed treatments for both genotypes (Fig. 21).

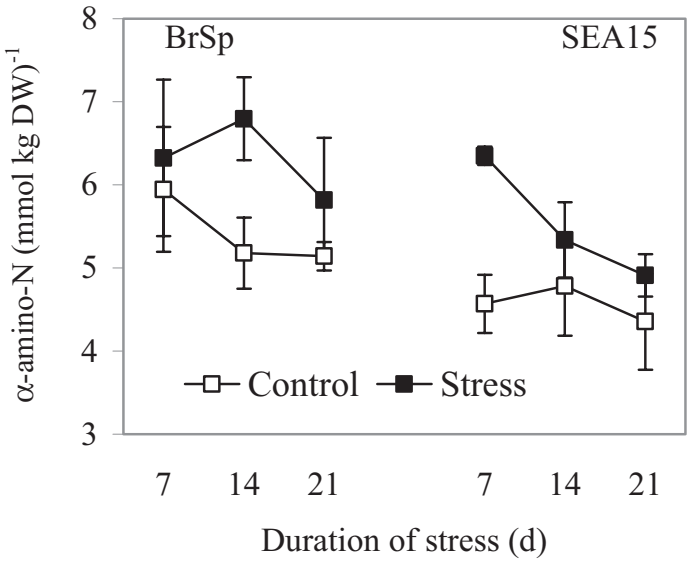

Fig. 21. The effect of drought stress imposed at pod-filling stage on leaf α-amino-N concentrations of two common bean genotypes. Vertical bars show ±S.E. of four replications.

Leaf starch concentrations of the bean genotypes decreased by as much as 63% and 40% for Brown Speckled and SEA 15, respectively, due to drought stress imposed at early pod-filling stage (Fig. 22A). Under both soil moisture supply regimes, starch concentration in the leaves of the drought-resistant genotype, SEA 15, was markedly higher than Brown Speckled. Leaf total non-structural carbohydrate (TNC) (total sugars +

starch) concentrations followed same trend (Fig. 22B). Drought-induced decrease of TNC was more pronounced for Brown Speckled (up to 49% reductions) compared with SEA 15, which experienced only up to 26% reduction (Fig. 22B). Relative to the control plants, leaf sucrose to starch ratio increased significantly under drought stress for Brown Speckled, whereas the stress did not significantly alter the proportion of the two carbohydrates at all sampling times for SEA 15 (Fig. 23). Also, compared with SEA 15 the leaf sucrose-to-starch ratio was considerably higher for Brown Speckled under both growth conditions.

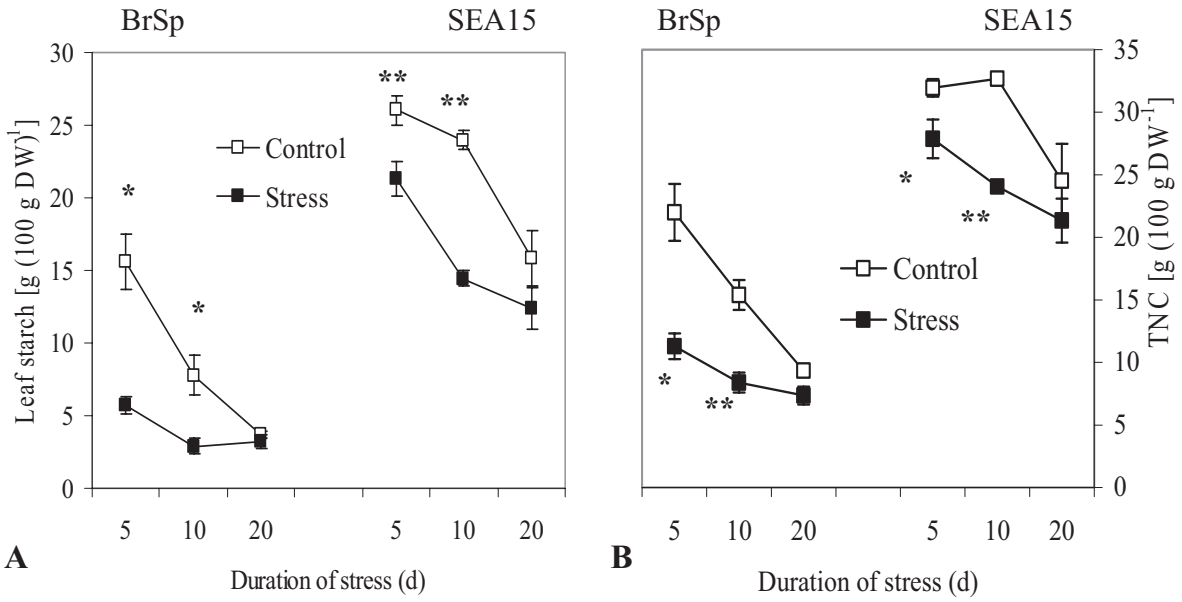

Fig. 22. The effect of drought stress imposed at early pod-filling stage on leaf starch (A) and total non-structural carbohydrate (TNC) (B) concentrations of two common bean genotypes. Vertical bars show ±S.E. of four replications. *, ** The differences between drought-stressed and non-stressed treatments are significant at 5 and 1% levels of probability, respectively.

3.8.2. Assimilate import and availability in sink organs

No effect of drought stress was found for the sucrose concentration in stems of both genotypes (Fig. 24). Nevertheless, the difference between the genotypes was significant under both growth conditions. SEA 15 maintained higher stem sucrose concentration than

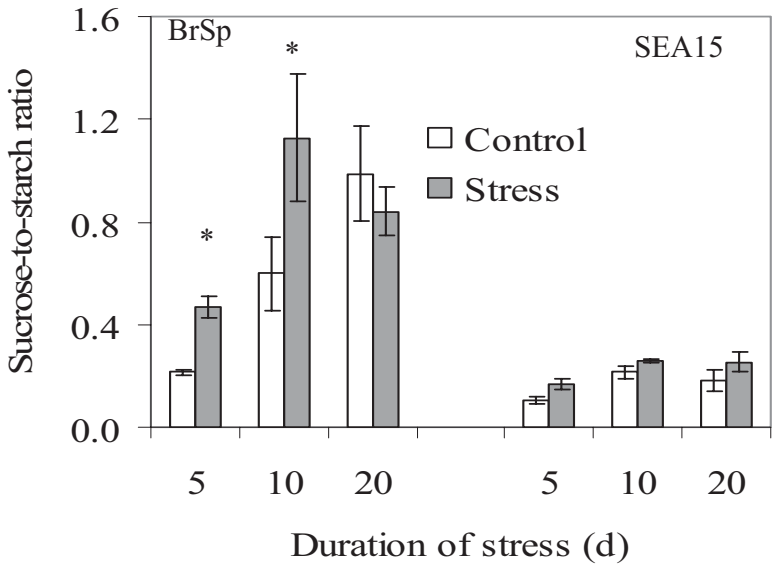

Fig. 23. The effect of drought stress imposed at early pod-filling stage on the ratio of leaf sucrose to starch concentrations of two common bean genotypes. The unit used to calculate the ratio was g (100 g DW)$^{-1}$. Vertical bars show ±S.E. of four replications.

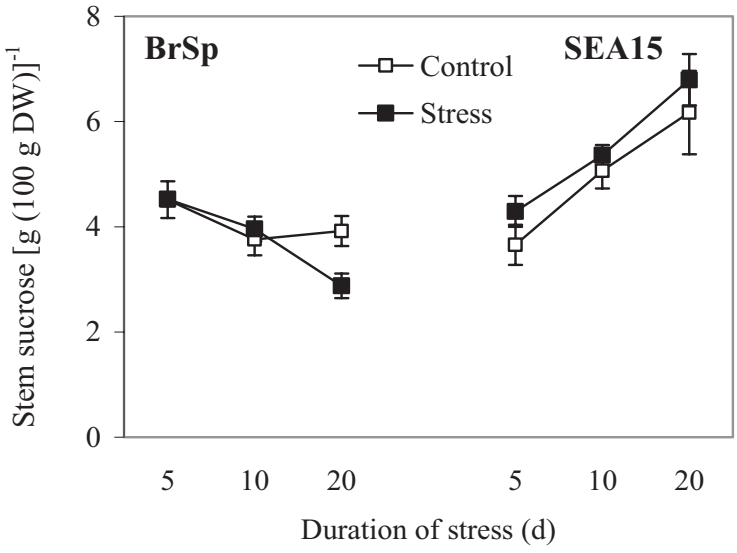

Fig. 24. The effect of drought stress imposed at early pod-filling stage on stem sucrose concentrations of two common bean genotypes. Vertical bars show ±S.E. of four replications.

Brown Speckled. As the duration of stress extended from 5 to 20 d, sucrose concentration in the stems of Brown Speckled tended to decline as opposed to a rise in concentration found for SEA 15 (Fig. 24). Stem hexose and total sugar concentrations of the bean genotypes followed a similar trend observed for stem sucrose concentration. The concentration levels of both parameters were comparable between drought-stressed and non-stressed plants of both genotypes (data not shown).

Drought stress did not alter the sucrose concentration in the productive pods of the two bean genotypes (Fig. 25A). On the other hand, productive pod hexose sugar concentration was negatively affected due to drought for Brown Speckled but not for SEA 15 (Fig. 25B). Relative to the corresponding non-stressed plants, drought stress kept for 5 and 10 d caused 28 and 30% reductions, respectively, in productive-pod hexose sugars concentrations of the drought-susceptible genotype, Brown Speckled (Fig. 25B). Productive pod total sugar (sucrose + hexose sugars) concentrations for Brown Speckled decreased in response to drought, whilst the concentration remained unaffected for the drought-resistant genotype, SEA 15 (Fig. 25C).

Drought stress increased the ratio of pod sucrose to hexose sugars (sucrolytic index) of both genotypes particularly during the first two harvests (data not presented). When compared with the productive pods, sucrose concentrations in the aborted pods of Brown Speckled were 16 - 36% and 23 - 62% found for the control and drought-stressed plants of the genotype, respectively (Fig. 26). Likewise, aborted pod sucrose concentrations for SEA 15 were only 10 - 23% and 14 - 33% of the concentrations found in the productive pods of the non-stressed and drought-stressed plants of the genotype, respectively.

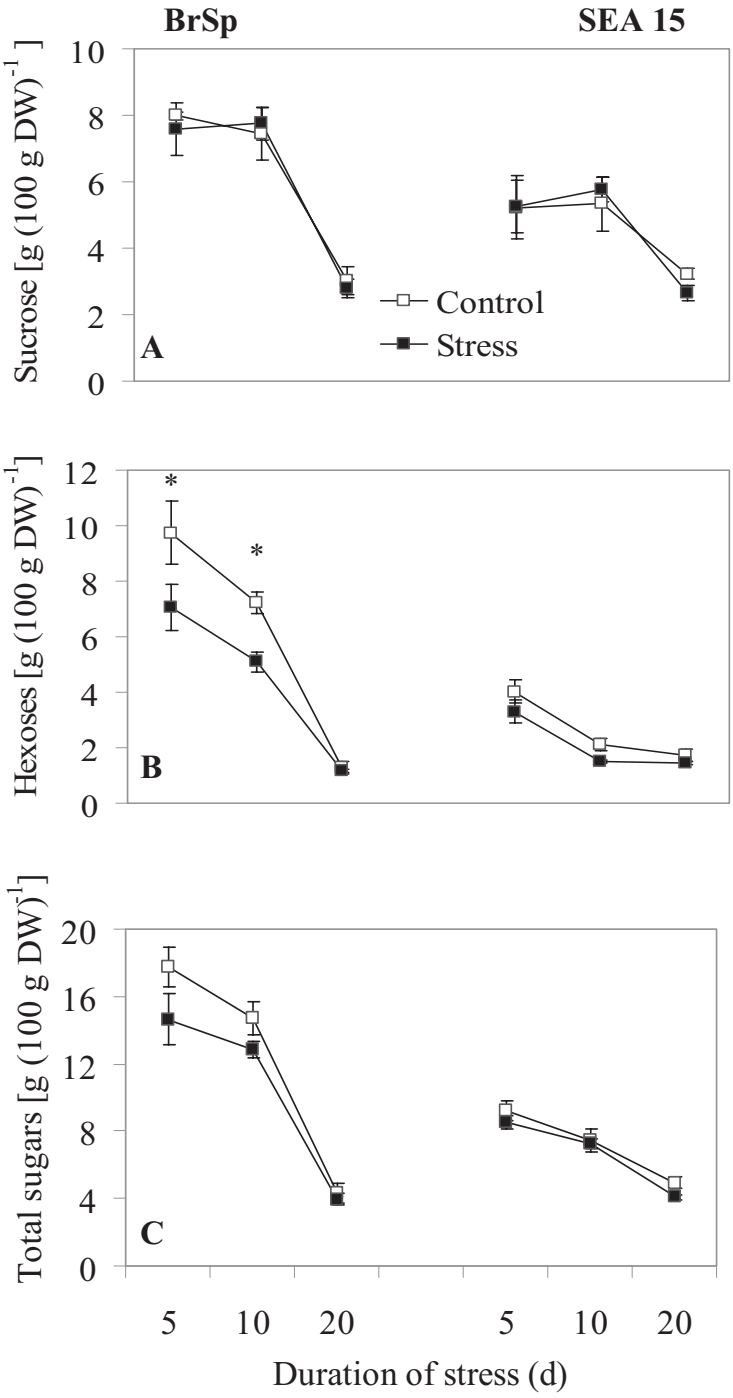

Fig. 25. The effect of drought stress imposed at early pod-filling stage on productive pod sucrose (A), hexose sugars (B) and total sugars (C) concentration of two common bean genotypes. Vertical bars show ±S.E. of four replications. * The difference between drought-stressed and non-stressed treatments is significant at 5% level of probability.

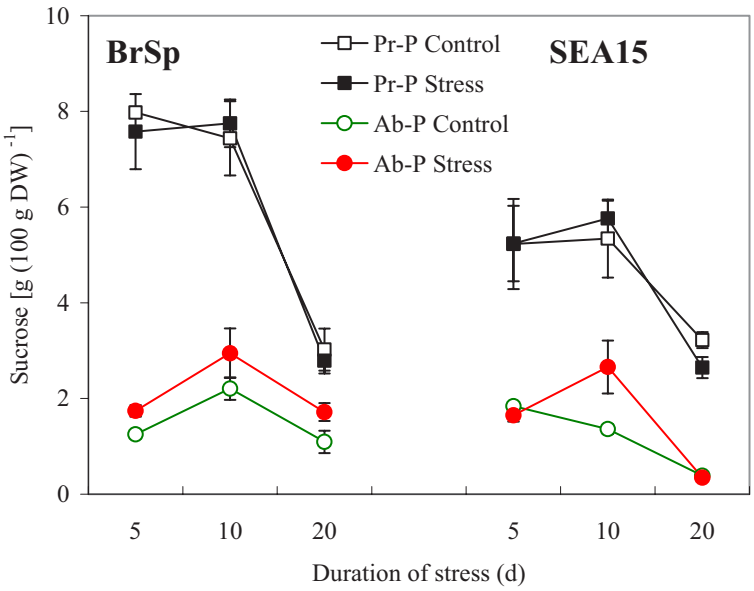

Fig. 26. Productive pods (Pr-P) and aborted pods (Ab-P) sucrose concentrations of two common bean genotypes grown under drought stress initiated at early pod-filling stage and non-stress growth conditions. Vertical bars show ±S.E. of four replications.

In addition to carbon compounds, drought stress imposed at early pod-filling stage of the crop had differential effects on the concentration of nitrogenous compounds in the pods of the two genotypes. Relative to non-stressed plants, pod free amino acid (α-amino N) concentrations for drought-stressed Brown Speckled were significantly higher during the last two harvesting times (Fig. 27). The concentration of α-amino-N in the pods of SEA 15 was comparable for the two contrasting soil moisture regimes at all harvesting times.

Profound genotypic differences were found in terms of the level of sucrose available for metabolism in the seeds under drought stress conditions. In Brown Speckled, drought initiated at early pod-filling stage caused ca. 29% (5 d stress) to 47% (10 d stress) reduction in seed sucrose concentration relative to the non-stressed plants (Fig. 28). On the contrary, seed sucrose concentrations for SEA 15 increased significantly by about 43

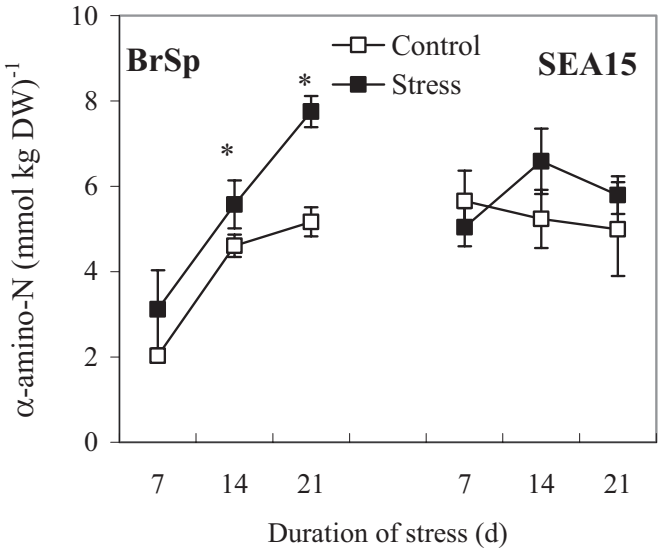

Fig. 27. The effect of drought stress imposed at pod-filling stage on pod α-amino-N concentrations of two common bean genotypes. Vertical bars show ±S.E. of three replications. * The difference between drought-stressed and non-stressed treatments are significant at 5% level of probability according to t-test.

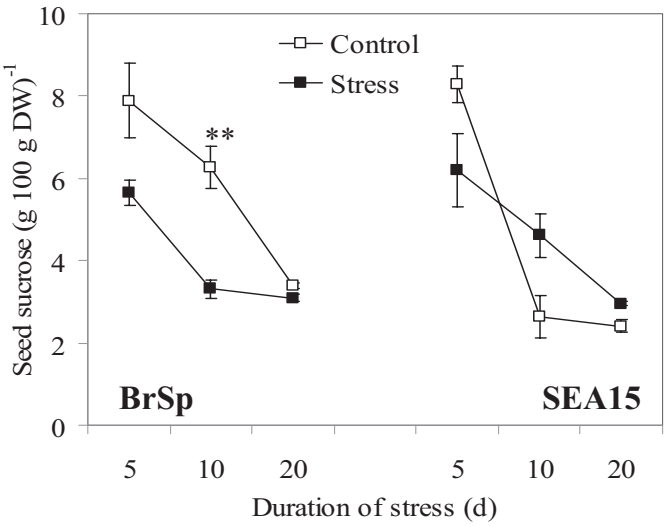

Fig. 28. The effect of drought stress imposed at early pod-filling stage on seed sucrose concentrations of two common bean genotypes. Vertical bars show ±S.E. of four replications. ** The difference between drought-stressed and non-stressed treatments is significant at 1% level of probability according to t-test.

(at 10 d stress) and 19% (20 d stress) as a consequence of the drought stress imposed during similar period. Seed hexose sugars concentrations of the genotypes were not only meager in quantity but also showed fluctuation during the course of the stress (data not presented). Whereas SEA 15 maintained comparable seed sucrose to hexose ratio between the two growth conditions, the ratio decreased markedly under drought stress (particularly at 10 d stress) for Brown Speckled (data not presented). Seed α-amino N concentrations of the genotypes under the contrasting growth conditions were parallel with concentrations found in the leaves and pods (Fig. 29). Relative to the control treatments, drought stress significantly increased seed α-amino N concentration by about 12 and 14% for Brown Speckled and SEA 15, respectively.

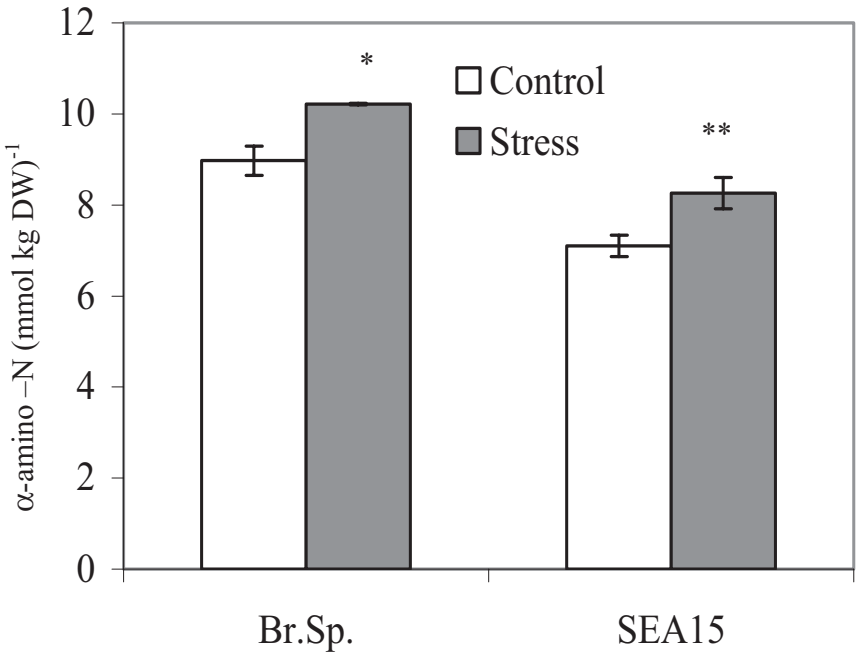

Fig. 29. The effect of drought stress imposed at pod-filling stage on seed α-amino-N concentrations of two common bean genotypes. Vertical bars show ±S.E. of four replications. *, ** The differences between drought-stressed and non-stressed treatments are significant at 5 and 1% levels of probability, respectively.

3.8.3. Assimilation of storage products in seeds

Although a genotypic difference was evident with regard to the length of the stress period at which the effects began to be manifested, seed starch concentrations of both bean genotypes were decreased under drought stress (Fig. 30). Drought-induced decrease in seed starch accumulation was more consistent across the stress period considered for Brown Speckled than for SEA 15. Plants of Brown Speckled subjected to drought stress commenced at pod-filling stage for the periods of 5 and 20 d had ca. 16 and 18% less seed starch concentrations than the corresponding non-stressed plants (Fig. 30). On the other hand, drought stress that lasted up to 10 d did not affect seed starch accumulation of the drought-resistant genotype, SEA 15. When the stress period was further prolonged (20 d stress), it decreased seed starch concentration of the genotype by ca. 20% relative to non-stressed plants (Fig. 30).

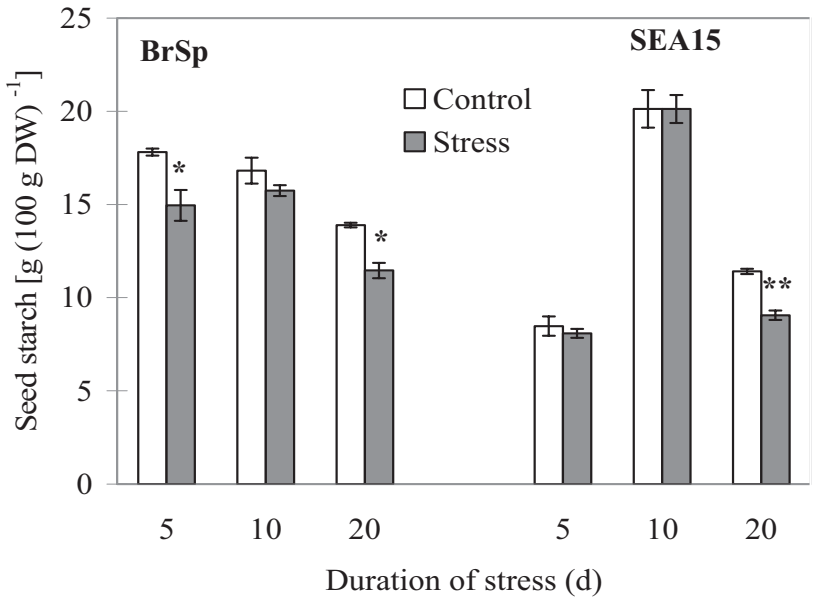

Fig. 30. The effect of drought stress imposed at early pod-filling stage on seed starch concentrations of two common bean genotypes. Vertical bars show ±S.E. of four replications. *, ** The differences between drought-stressed and non-stressed treatments are significant at 5 and 1% levels of probability, respectively.

3.8.4. Effect of drought stress on seed sink capacity

Apart from assimilate availability, we also investigated whether drought induces alterations in seed sink capacity of the genotypes with subsequent effect on the accumulation of storage products (i.e. starch in the seeds). Drought stress affected neither the number nor the volume of cotyledonary cells per seed of the two common bean genotypes (Table 9). On the other hand, drought stress decreased the number of starch granules (amyloplasts) per seed, although the reductions were not significant for both genotypes (Table 9). The total estimated area of the starch granules decreased significantly due to drought stress (ca. 42% for Brown Speckled vs. 33% for SEA 15) as compared with non-stresses plants of the genotypes.

The two genotypes exhibited significant differences for seed protein concentration. Nevertheless, the concentration of the storage product in the seeds of neither of the genotypes was affected due to drought stress imposed at early pod-filling stage relative to the corresponding non-stressed treatments (Fig. 31).

Table 9. The effect of drought stress imposed at early pod-filling stage on the numbers and volumes of cotyledonary cells and amyloplasts of two common bean genotypes. Data are means±S.E. of four replications.

Treatment		No. cotyledonary cell seed^{-1} (x 10^6)	Volume cotyledonary cell (nl) (x 10^{-3})	No. amyloplasts seed^{-1} (x 10^6)	Area of amylopast seed^{-1} (μm) x 10^7
Br.Sp.	Control	0.99±0.08 ^{b}	21.1±1.78 ^{a}	11.3±0.77 ^{b}	545.1±21.6^{b}
	Stress	0.99±0.09 ^{b}	20.4±1.94^{ab}	8.1±0.99 ^{b}	316.2±47.5^{c}
SEA15	Control	1.59±0.11 ^{a}	14.2±1.18 ^{b}	16.6±1.41 ^{a}	745.9±89.7^{a}
	Stress	1.33±0.16^{ab}	20.0±2.92^{ab}	13.3±1.40^{ab}	498.8±40.8^{b}

Means having similar letter within the same column are not significantly different according to LSD test at 5% level of probability.

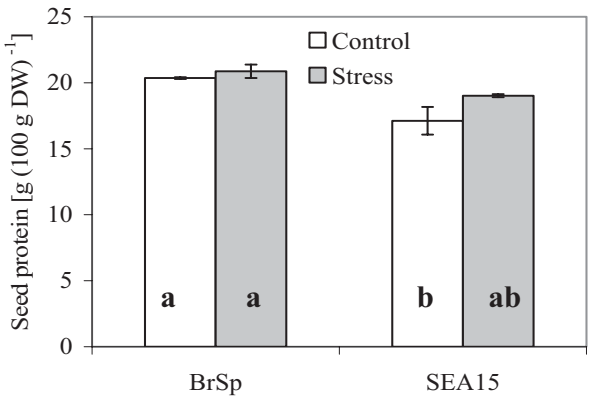

Fig. 31. The effect of drought stress imposed at pod-filling stage on seed protein concentrations of two common bean genotypes. Means followed by same letter are not significantly different according to LSD test at 5% level of probability. Vertical bars show ±S.E. of four replications.

3.9. Leaf protein changes under drought stress

A total of 550 different leaf proteins were detected by two-dimensional gel electrophoresis. Out of the total proteins detected, 230 of them were differentially expressed due to a 10-day drought stress initiated during the vegetative phase (Table 10). The number of down-regulated proteins (23.5% of total proteins detected) exceeded that of up-regulated ones (15.1% of total proteins). Furthermore, the stress induced the appearance of 10 new proteins, whereas 8 proteins disappeared compared with the non-stressed plants. Fig. 32 shows the positions of these proteins from the leaves of drought- stressed Brown Speckled.

Table 10. The effect of a 10 d drought stress initiated during the vegetative phase on quantitative and qualitative changes in leaf proteins of common bean (cv. Brown Speckled)

Regulation	Number	% of total
Total proteins detected	550	100.0
Differentially expressed (total)	230	41.8
Newly appeared	10	1.8
Up-regulated	83	15.1
Down-regulated	129	23.5
Disappeared	8	1.5

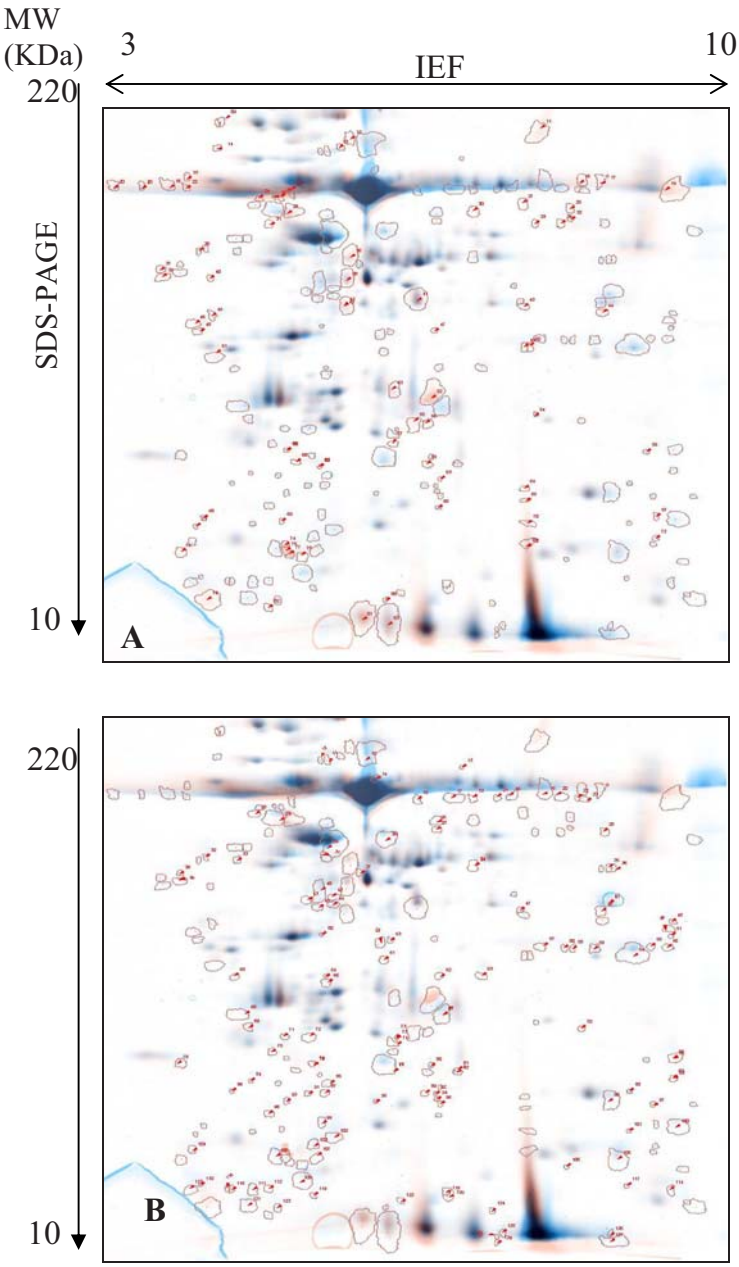

Fig. 32. Coomassie-stained 2D gel of total proteins extracted from mature leaves of drought-stressed cv. Brown Speckled. The proteins were separated by two-dimensional isoelectric focusing (IEF)/ SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Locations of the drought stress-responsive proteins (spots) are marked by circular boundaries. The arrows indicate 83 up-regulated proteins (A) and 129 down-regulated proteins (B) in drought-stressed plants in relation to non-stressed plants.

4. DISCUSSION

4.1. Effect of drought stress on seed yield

SEA 15 had the highest relative yield compared to other genotypes subjected to similar level of drought stress (Table 2). This genotype, therefore, could be regarded as the most drought-resistant of all tested genotypes in accordance with the suggestions of Hall (1993). Conversely, Brown Speckled produced the lowest relative yield (i.e. it was the most drought-susceptible genotype). Higher yields of the two inbred lines (SEA 15 and SEA 23) under both growth conditions were in accordance with their superior performance found under field conditions in selected drought-prone areas of south and central America (CIAT, 2002). Cultivars from the races Durango and Mesoamerica, represented by SEA 15 and SEA 23 in the present study, respectively, have previously been reported to possess significant levels of drought tolerance owing to their evolutionary origin in semi-arid and semi-humid regions of the Mexican highlands (Terán and Singh, 2002). A high level of consistency was retained for seed yield by the genotypes as demonstrated by the significant and positive correlation found between drought-stressed and non-stressed growth conditions asserting previous reports of Mohamed et al. (2002) and Frahm et al. (2004). Other studies have similarly shown that heritability estimates for yield of common bean grown under drought and non-stress were generally similar (Schneider et al., 1997; Singh, 1995) suggesting that selection should be equally effective under different levels of stress. Thus, if optimum performance and adaptation to drought stress are to be achieved, it is suggested that specific adaptation traits should be used in tandem with potential yield (yield under non-stress growth conditions) as selection criteria in breeding common bean.

The reduction in seed yield per plant due to drought stress imposed at reproductive stage of the tested genotypes was due to the adverse effect of the stress on individual yield components. Consistent with reports on other legumes including common bean (Leport et

al., 2006; Nunez Barrios et al., 2005; Boutraa and Sanders, 2001), the numbers of pods per plant followed by seeds per pod were the most affected yield components under drought stress (Table 6). Drought-induced abortion of pods for Brown Speckled and SEA 15 were approximately two-third and one-half of the initial pod set (total number of pods at 5 d stress), respectively (Fig. 1). In line with the suggestions of Daie (1996), the higher rate of pod abortion found for Brown Speckled (relative to SEA 15) may be due to limited assimilate supply (Fig. 20) as well as the marked decrease in pod water concentration (Fig. 9) under drought conditions. In soybean, decreased pod water potential caused by low moisture stress inhibited pod expansion and metabolism which eventually led to reduced pod set (Liu et al., 2003). Similarly, failure to set kernels at low water potential was correlated with loss of metabolic activity in the ovary of maize plant (Zinselmeier, 1991). Most aborted pods of the bean genotypes were late-initiated ones found on the upper parts of the main stem and branches. In fact, this is a common observation in cultivars of other legume species with indeterminate growth habit (Leport et al., 2006). The relationships between the rate of pod abortion and availability of assimilate at source and sink levels are discussed in more details in section 4.5.

Drought-induced increase in ABA concentration of immature (sink) leaves of the common bean genotypes (Fig. 13) was in accordance with previous reports on the accumulation of the plant hormone in young expanding leaves of other water-stressed plants (Alves and Setter, 2000; Hartung and Davies, 1994). Despite comparable ABA accumulation found in drought-stressed plants of the two genotypes, the increase was markedly higher for Brown Speckled (drought-susceptible) compared with SEA 15 (drought-resistant). In soybean subjected to drought stress, flower and pod ABA concentration linearly correlated with xylem and leaf ABA concentrations indicating that root-originated ABA and/or leaf ABA were the likely sources of ABA accumulated in the pods (Liu et al., 2003). Similarly, Nayyar et al. (2005) found that drought-susceptible chick pea cultivars that had higher rates of flower and pod abortion accumulated higher ABA than drought-tolerant ones when subjected to drought stress. Higher ABA levels in

stressed plants may restrict sucrose translocation to the developing sinks to induce their abortion (Liu et al. 2003, Nayyar et al. 2004, Nayyar and Walia 2004). Drought-induced accumulation of ABA inhibited maize endosperm cell division and kernel growth that led to kernel abortion and failure of seeds to form (Mambelli and Setter, 1998; Ober and Setter, 1992). Drought stress primarily affects developmentally younger reproductive structures in apical positions (where ABA accumulates in higher quantity) than those found in basal and middle positions (Wang et al., 2002; Setter et al., 2001). In line with this, higher abortion rates of pods found on the upper parts of the bean plants were probably enhanced due to the likely accumulation of ABA in those parts of the plant. We suppose that sink leaf ABA correlates with pod ABA concentration. The differences in ABA accumulation due to drought relative to non-stress conditions (higher for Brown Speckled compared with SEA 15) may partly explain the differences found in pod abortion (Fig. 1) between the two bean genotypes.

4.2. Source and sink limitations and their relationships to yield under drought stress

Limitations to crop yields are often sought in either source or sink restrictions. The source activity, which determines the availability of assimilates and the sink strength, which determines the ability of sink organs to import and utilize assimilate are the two processes involved in determining the yield of a crop (Egli and Bruening; 2001, Ho, 1988). As discussed in the preceding sections, drought stress initiated at different growth stages of the crop had differential effects on biomass production and reproductive sink establishment of the bean genotypes characterized by varying degrees of drought resistance. Whether these variations are related to differences in source and/or sink strength of the genotypes will be discussed in the following sections.

4.2.1. Assimilate synthesis, availability and supply – source strength

4.2.1.1. Photosynthesis

The strong correlation detected between A and g_s (Fig. 8) under the conditions of drought stress imposed at both growth phases of the crop suggests that drought-induced decline in

A was largely a consequence of stomatal limitation. Such robust association between the two variables, *A* and g_s, is commonly reported implying that the decrease in g_s is the dominant factor responsible for the decline in *A* until drought conditions become very severe (Monneveux et al., 2006; Zanella et al., 2004; Lawlor and Cornic, 2002). However, such a close correspondence can also be the consequence of the co-regulation of both parameters in response to drought (Osório et al., 2006; Escalona et al., 1999). Drought-sensitivity of gas-exchange was comparable between the two genotypes at both growth stages of the crop (see Fig. 14 and Fig. 15). This is in contrast to previous observations, where different rates of *A* and g_s were reported among drought-resistant and susceptible bean genotypes (Wentworth et al., 2006; Mencuccini and Comstock, 2000). According to these researchers, the differential response in gas-exchange of the studied genotypes was related to drought-mediated differences in leaf structure, stomatal density, hydraulic conductance and changes in leaf size. Drought-induced reduction in *A* corresponded with the decrease in leaf sugar concentrations of both genotypes during the vegetative phase (Fig. 19). Nevertheless, such relationship between *A* and leaf sugar levels was found only for Brown Speckled when the stress was initiated at pod-filling stage (Fig. 20). In line with direct associations often reported between photosynthetic rate and leaf carbohydrate levels (Amede and Schubert, 2003b; Scartazza et al., 2001), it is suggested that shortage of assimilates resulting from reduced carbon assimilation could be responsible for the observed reduction in growth and yield under the situations pointed out above.

Although stomatal closure appears to be the predominant factor limiting *A* as observed in other C_3 plants subjected to comparable intensity of drought stress we used (Flexas et al. 2004, Lawlor and Cornic, 2002), non-stomatal inhibition of *A* was also evident from the ratio C_i/C_a (Fig. 16) and A/C_i (Fig. 17). A lower ratio of C_i/C_a under drought stress relative to control treatment for SEA 15 suggests that the decline in *A* was due to limited availability of CO_2 caused by stomatal closure. Contrary to this, the decrease in g_s due to drought was not accompanied by reduced C_i available for photosynthesis (i.e. the ratio C_i/C_a was unaffected) for Brown Speckled. According to Lawlor and Cornic (2002), C_i

levels similar or higher in values under drought stress relative to control treatments imply metabolic limitations to A caused by non-stomatal effects under drought conditions. However, there are questions about whether assessments of metabolic limitations based on C_i analysis are reliable under drought. Two main problems have been described related to C_i calculations in stressed leaves: patchy stomatal closure (Laisk, 1983; Buckley et al., 1997) and the increase of the relative importance of cuticular transpiration when stomata are closing in drying leaves (Boyer et al., 1997). Other researchers, on the other hand, stressed that even if patchiness occurs, it probably is much less important than once thought (Lawlor and Cornic, 2002). Assuming that uniform stomatal closure did occur in response to drought in the bean plants due to slow imposition of the stress as has been observed by Gimenez et al. (1992) and Gunasekera and Berkowitz (1992), the difference in the ratio C_i/C_a found between the bean genotypes implies that the stress thresholds at which stomatal and metabolic limitations to A occur varied between the two common bean genotypes.

The stronger decrease in carboxylation efficiency (A/C_i) due to drought stress for Brown Speckled compared with SEA 15 (Fig. 17) demonstrated that metabolic limitations to A were relatively more important for the drought-susceptible genotype. According to Osório et al. (2006), higher C_i values found for Brown Speckled implied that an inhibition of carboxylation may limit CO_2 assimilation in drought-stressed plants. However, it is worth mentioning that the ratio A/C_i under drought stress may also be underestimated whenever C_i is proportionally overestimated (Zhang et al., 2005). Several reports have shown that differences in the estimates of carboxylation efficiency observed among genotypes subjected to drought stress could be related to the degrees of metabolic impairments (e.g. photophosphorylation, RuBP regeneration and Rubisco activity) and/or to differences in mesophyll conductance to CO_2 (Zhang et al., 2005; Flexas et al., 2004; Chaves et al., 2002, and the references therein).

The difference in photosynthetic change under drought stress observed between the bean genotypes, therefore, may be useful in identifying drought-resistant cultivars. While

photosynthetic inhibition due to stomatal closure is largely reversible upon re-watering, metabolic inhibition involves an impairment of biochemical processes, which may retard CO_2 fixation even after recovery (Loreto et al. 1995) or even cause irreversible effects leading to death of the leaf tissues (Lawlor and Cornic 2002). This could therefore be an important consideration in determining the ability of plants to withstand drought. Although genotypic differences were not significant for photosynthesis inhibition under similar levels of drought stress, the causes that have led to inhibition of photosynthesis may differ and may have significant consequences with respect to the productivity of the bean genotypes under water limiting conditions.

4.2.1.2. Assimilate availability and supply at source level

The reduction in leaf sugar concentrations of the two bean genotypes due to drought stress imposed at the vegetative growth stage (see Fig. 19) was parallel to the decreases in NAR and RGR (Table 3) determined during the same period. A study carried out to investigate the responses of several *Phaseolus* species exposed to salinity at early vegetative growth stage yielded similar results that the decreases in growth rate and net assimilation rate were attributed to the decline in photosynthesis (Bayuelo-Jiménez et al., 2003). In agreement with the reports of Amede and Schubert (2003b), drought-induced decrease in leaf sugar concentrations of both bean genotypes (Fig. 19) could be ascribed to the significant decrease in carbon assimilation rate (Fig. 14). These results suggest that shortage of assimilates (source limitation) is the prime factor responsible for the observed inhibition of growth and biomass accumulation in both bean genotypes subjected to drought stress during the vegetative phase of the crop.

Starch and sucrose are the principal end products of carbon assimilation in common beans (Sharkey et al., 1985). Starch is the major storage form of carbohydrate in mature leaves (Huber et al., 1984). In sucrose-transporting plants such as beans, its concentration in leaves represents the current availability of assimilates for reproductive development (Westgate and Thomson Grant, 1989). Subjecting the bean genotypes to drought stress

Table 11. Analysis of source-sink relationships under drought stress (imposed during the reproductive phase) relative to control conditions for the genotypes SEA 15 and Brown Speckled (percent reductions of various parameters). The analysis was based on Ho (1988).

Parameter	**BrSp**	**SEA 15**
Source strength		
Net photosynthetic rate (μmol $m^{-2}s^{-1}$)	62.0	50.0 ^{ns}
Leaf total nonstructural carbohydrates [g (100 g DW)$^{-1}$]	38.5	17.4 **
Leaf sucrose concentration [g (100 g DW)$^{-1}$]	24.7	- 5.3 *
Leaf starch concentration [g (100 g DW)$^{-1}$]	46.6	26.3 *
Pod sucrose concentration [g (100 g DW)$^{-1}$]	2.8	3.3 ^{ns}
Seed sucrose concentration [g (100 g DW)$^{-1}$]	28.1	- 24.1 **
Sink activity		
Seed starch concentration (20 d stress) [g (100 g DW)$^{-1}$]	17.5	20.6 ^{ns}
Seed sink strength (seed starch accumulation per plant)† (g g^{-1})	84.0	37.0 *
Sink capacity		
No. cotyledonary cells per seed (No. plant^{-1})	0.0	14.4 ^{ns}
Volume of cotyledonary cells per seed (nl)	3.3	- 40.1 *
No. amyloplasts per seed (No. seed^{-1})	28.3	18.1 ^{ns}
Volume (area) of amyloplasts per seed (μm)	42.0	33.1 ^{ns}

† Seed sink strength = seed sink activity × seed sink capacity (size)

*, ** Differences between the genotypes are significant at 5 and 1% levels of probability, respectively, according to t-test; ns not significant.

during the pod-filling stage did not consistently alter leaf sucrose concentration of SEA 15 (except at 10 d stress), whereas consistent reductions were found for Brown Speckled at all durations of stress monitored (Fig. 20A). Interestingly, the genotypic differences observed were in spite of comparable decline in carbon assimilation rate measured under drought conditions (Fig. 15A). Consistent with our findings for the drought-susceptible genotype, drought-induced decrease in leaf sucrose concentrations had previously been

observed in other legumes (Liu et al., 2004; Keller and Ludlow, 1993). According to these reports and others (Scartazza et al., 2001; Huber et al., 1989), reduced availability of sucrose has a direct and leading role in limiting the establishment of new sink organs under drought conditions. In the same line, we conclude that drought-induced decreases in photosynthetic rate and leaf sucrose concentration may have led to a reduced rate of sucrose export to the sink organs thereby inhibiting reproductive development of the drought-susceptible genotype (see Table 11).

Drought stress decreased the net photosynthetic rate of SEA 15 on the same scale it affected Brown Speckled (Fig. 15A). Consequently, comparable leaf sucrose levels found under the contrasting growth conditions for SEA 15 do not imply that the genotype maintained relatively higher carbon assimilation rate than Brown Speckled under drought conditions. According to da Silva and Arrabaça (2004), such leaf sucrose accumulation under drought stress could be due to the inhibition of growth and subsequent decreased rate of sucrose export to sink organs. At 10 d stress, the significant decrease in leaf sucrose concentration of drought-stressed SEA 15 corresponded with a concurrent increase in leaf hexose sugars concentration (Fig. 20B). Such a modification in leaf sugar composition (hydrolysis of sucrose to hexose) is often linked to drought-induced increase in the activities of sucrose hydrolyzing enzymes (acid invertase and sucrose synthase) (Liu et al., 2003; Kim et al., 2000; Keller and Ludlow, 1993). Accumulation of hexose in plant leaves under drought stress may contribute to turgor maintenance as part of plant strategies to adapt to drought stress (Turner, 1997).

Reduced leaf starch concentrations due to drought stress for both genotypes presented in Fig. 22A are consistent with the findings reported for other crops including common bean (Osório et al., 2006; Patakas and Noitsakis, 2001; Vassey and Sharkey, 1989). According to Paul and Foyer (2001), starch synthesis is promoted when sucrose synthesis is restricted and in many plant species leaf starch serves as a transient sink to accommodate excess photosynthate that cannot be converted to sucrose and exported. Changes in leaf

starch level may reflect changes in source-sink relationships and, hence could be used to evaluate potential sink limitations during seed filling (Liu et al., 2003; Egli, 1999). In common bean, Nakano et al. (2000) reported that reducing sink demand by depodding increased leaf starch concentrations, whereas decreasing source-sink ratio by shading decreased leaf starch concentrations. According to Egli (1999), the consistent decrease in leaf starch concentration found for both bean genotypes (Fig. 22A) implies that drought-induced sink limitation was not substantial.

A linear relationship of assimilation rate with both starch and sucrose synthesis has been found in common bean leaves, although sucrose is the more preferred product than starch at very low assimilation rates (Sharkey et al., 1985). Drought-induced increase in leaf sucrose to starch ratio found for Brown Speckled (Fig. 23) is consistent with the above report. The modification in carbon partitioning between the two carbohydrates in favor of sucrose could be due to the fact that sucrose is the exclusive form of carbohydrate required for export to the various sink organs for metabolism and storage. Similar findings of increased ratio of leaf sucrose to starch as adaptive feature to different types of stresses including drought (e.g. da Silva and Arrabaça, 2004), cold (e.g. Savitch et al., 2000) and salinity (e.g. Rathert, 1984) have been reported. Modification in carbon partitioning under drought stress (favoring sucrose rather than starch synthesis) during photosynthesis is primarily due to the up-regulation of the enzyme of sucrose synthesis, sucrose phosphate synthase (SPS) (Baxter et al., 2001; Geigenberger et al., 1999).

Unlike carbon compounds, drought stress imposed during the reproductive phase increased amino acid concentrations in various plant parts (Fig. 21, Fig. 27, Fig. 29) demonstrating that the stress did not limit the availability and supply of nitrogenous compounds. According to Schubert et al. (1995), such an accumulation of nitrogenous compounds under drought stress could be due to reduced growth, which decreases the demand for the assimilates.

4.2.2. Carbohydrate import and utilization - sink strength

As pointed out earlier, drought stress initiated at pod-filling stage had a differential effect on the availability of sucrose in the leaves of the bean genotypes (see Fig. 20). Nevertheless, the concentration of the assimilate in the pods of both bean genotypes was comparable between drought-stressed and non-stressed plants (Fig. 25A). Previous studies have demonstrated a linear relationship between sucrose availability in the source and rate of export to sink organs (Komor, 2000; Huber et al., 1984). Corresponding to these reports, comparable pod sucrose concentrations found between drought-stressed and non-stressed SEA 15 plants reflected the availability of the assimilate at source level presented in Fig. 20. In Brown Speckled, similar level of sucrose found in the pods of stressed plants with those grown under non-stress conditions could be due to the inhibition of the hydrolysis of incoming sucrose, because hexose sugar concentration in the same reproductive organ was significantly lowered under drought stress (Fig. 25B). In fact, a drought-induced increase in pod sucrose to hexose sugars ratio was found for both genotypes, although it was higher for Brown Speckled compared with SEA 15 (data not presented). Sucrose accumulation with a concomitant increase in sucrose to hexose sugars ratio (sucrolytic index) has been observed previously in soybean pods (Liu et al., 2004), tomato fruit (Balibrea et al., 2000) and maize ovaries (Setter et al., 2001; Zinselmeier et al., 1995) of drought-stressed plants. The failure to set pods/kernels/fruits by these crops under drought stress was associated with higher sucrose to hexose ratio caused by decreased invertase activities in the reproductive structures.

In the above context, we suppose that in addition to sucrose availability, the capacity for utilizing the assimilate may have been differentially affected in the two bean genotypes under drought stress. The variation in sink strength (ability to metabolize imported sucrose by the pods) may, therefore, partly explain the observed genotypic difference in the establishment and growth of reproductive structures under drought conditions. According to Liu et al. (2004), decreased sucrose utilization in the pods of drought-

stressed plants inhibits cell division in young ovules and pod walls leading to higher suppression of growth and eventual abortion of pods.

As depicted in Fig. 26, sucrose concentrations in aborted pods of the genotypes were 2-3 times lower than the concentrations in the corresponding productive pods under both soil moisture regimes. Therefore, it is possible that the underlying mechanisms controlling pod abortion in non-stressed bean plants are simply enhanced under drought stress. Tanaka and Fujita (1979) previously reported that bean pods developed at the top of main stem and branches nearby smaller leaves tended to abscise as assimilates produced by these leaves hardly meet higher demands for expansion growth of the young pods. Likewise, Mauk and Breen (1986) found for snap beans that 34% of ^{14}C assimilates translocated from a labeled mature leaf was recuperated in the nearby inflorescence as compared to 2% recuperated on a distant raceme node. i.e. most of the photosyntates of a given leaf ended up in the flowers, pods and axis of the same leaf. In maize subjected to low moisture stress at post-pollination stage, the carbohydrate levels in apical ear zone was much lower than the basal zone, resulting in reduced kernel number in the former region (Setter et al., 2001). Ho (1988) and Alkio et al. (2002), on their part mentioned that in addition to the availability of assimilates and the intrinsic ability to attract the assimilates, proximity of the sink to the source (i.e. transport conductivity) would affect the actual sink strength. Because early formed pods and seeds in the same basal region of the plant become priority sink for assimilate delivery, sufficient assimilates may not reach the late-initiated pods from mature bean leaves during the critical abortion sensitive growth stage of pods.

Drought stress imposed at pod-filling stage highly reduced pod water concentration of Brown Speckled more than it did in SEA 15 (Fig. 9). The decreases in pod water concentration of the drought-susceptible genotype was markedly higher at 20 d stress, when seed dry matter accumulation was perhaps at its maximum. According to Daie (1996) such a decrease in tissue water status of the sink organs may reduce sink strength

through arresting expansion growth or assimilate metabolism and utilization, both of which may enhance reproductive sink abortion. Studies on soybean (Liu et al. 2003; Westgate and Peterson 1993) and maize (Zinselmeier 1991) also showed that the failure/decrease in pod/kernel set under drought stress were related to low water potentials of the reproductive structures, which directly inhibited expansion growth and metabolic activity of the organs.

4.2.3. Storage carbohydrates

Sucrose metabolism is pivotal in seed development and is particularly susceptible to drought stress (Pinheiro et al., 2005). The decrease in seed sucrose concentration due to drought at all durations of stress considered for Brown Speckled (Fig. 28) reflected the lower availability of the assimilate at source level (Fig. 20). We were not able to show a direct relationship between reproductive sink establishment and photosynthate flux from leaves to pods or seeds. Direct relationship between sucrose availability and export rate at source level and the establishment of new sink organs has been shown for other crops (Liu et al., 2004; Setter et al., 2001). In line with these reports, we suppose that the higher decrease in sink size (number of pods and seeds) of the drought-susceptible genotype due to drought stress is partly attributed to reduced availability of the assimilate at source level (Minchin et al., 1993; Ho, 1988). For the drought-resistant genotype, SEA 15, seed sucrose concentration was unaffected due to drought, reflecting its unaltered availability in the source leaves (Fig. 20), stems (Fig. 24) and pods (Fig. 25A).

Irrespective of genotypic differences found for seed sucrose concentrations owing to drought, seed starch accumulation of the genotypes decreased diffrently under the stress conditions. The drought-induced decreases in seed starch concentration of Brown Speckled were observed at all harvesting times (5 to 20 d stress) (Fig. 30) corresponding with seed sucrose levels measured during similar periods. In wheat endosperm, Jenner et al. (1991) found a similar relationship between the two seed carbohydrates that the rate of storage starch accumulation was a function of the concentration of sucrose. *In vitro*

cotyledon dry weight accumulation in *Vicia faba* (Barratt and Pullen, 1984) and pea (Wang and Hedley, 1993) were also dependent on high sucrose levels. Based on these relationships, it is appears that shortage of assimilate (sucrose) could be one of the prime factors responsible for the reduced starch accumulation in the seeds of the drought-susceptible bean genotype. On the contrary, reduced seed starch concentration found for SEA 15 (only at 20 d stress) (Fig, 29) was not accompanied by a decrease in seed sucrose level concurring similar results reported for barley (Brooks et al., 1982) and maize (Andersen et al., 2002; Zinselmeier et al., 1999). These results imply that apart from assimilate availability *per se*, drought stress may induce other factors that contribute to decreased seed starch synthesis. Limitations of sink activities due to the inhibition of the activities of key enzymes of sucrose metabolism (invertases and sucrose synthase) (Weber et al., 2005; Heim et al., 1993) and starch synthesis (ADP-glucose pyrophosphorylase and starch synthase) (Zinselmeier et al., 1999; Sheoran and Saini, 1996; Ho, 1988) have been cited as principal factors responsible for reduced starch synthesis under drought situations. Reduced rate of starch synthesis due to drought stress may in turn lead to the accumulation of sucrose in the seed and slows the rate of export by the leaves (Ho, 1988). Pinheiro et al. (2005) and Zinselmeier et al. (1999) observed that young embryos abort when starch is depleted during early ovary development. Consistent with this, the decrease in seed starch concentration found for Brown Speckled from the first harvesting time (5 d stress) onwards probably may explain the higher rate of seed abortion observed for this genotype compared with SEA 15.

By measuring the number of storage cells and the storage organelles within the sink, the physical constraint upon a sink organ's assimilate import and metabolism (sink size) can be determined (Ho, 1988). Under both soil moisture regimes, SEA 15 had larger numbers of cotyledonary cells and amyloplasts per seed than Brown Speckled (Table 9). In accordance with the proposal of Gleadow et al. (1982), both features may have accounted for the higher grain weight and seed starch content (data not presented) of SEA 15 compared with Brown Speckled. The numbers and volume of cotyledonary cells were

unaffected by drought stress for both genotypes (see Table 9). Our findings are in agreement with the reports of Brooks et al. (1982) but deviate from that reported by Nicolas et al. (1985) for wheat plants subjected to low moisture stress during the reproductive growth stage. Drought stress caused a slight reduction in the number of amyloplasts per seed but substantially decreased the area of the starch granules per seed for both genotypes (Table 9). Restriction of starch granule size expansion, therefore, appears to be the major limiting factor of seed starch accumulation under drought stress, attesting similar results reported for cassava (Santisopasri et al., 2001). All in all, our study demonstrated that the difference in yield of the two bean genotypes under drought conditions are primarily determined by source strength (availability of assimilates in the leaves) rather than sink attributes.

4.3. Growth, biomass accumulation and partitioning

Biomass reduction due to drought stress imposed during the vegetative phase (Table 4) was proportional to the drought-induced impairment of leaf area measured on individual leaves of the genotypes (Fig. 4A). The higher rate of decrease in leaf area for SEA 15 caused higher degrees of reduction in total leaf area and leaf biomass weight per plant of the genotype compared with Brown Speckled. In spite of the strong decrease in above-ground biomass, drought-stressed SEA 15 maintained competitive advantage over Brown Speckled by higher biomass accumulation during the same period (Table 4). Our results concur recent reports on *Salix* species in which higher biomass reduction was found for fast growing species than for slow growing counterparts when subjected to drought stress at early growth stage (Turtola et al., 2006). Diversion of biomass to plant parts other than leaves is considered an adaptational response to drought stress of resistant genotypes during early growth stage of plants (Fernández et al., 2002; Charzoulakis et al., 1993). In addition to leaf area reduction, several other mechanisms operating simultaneously or on different time scale (e.g. decline in photosynthetic rate, reduced growth of stems and branches, decreased rate of new leaf production and reduced relative growth rate)

contributed to reduced growth and biomass accumulation of the common bean genotypes under drought stress.

In contrast to the vegetative phase, the decrease in above-ground biomass due to drought initiated at pod-filling stage was comparable between the genotypes except at 20 d stress (Table 4). The genotypic difference observed at 20 d stress was related to the variation found in partitioning of biomass stored in vegetative structures to reproductive parts (Fig. 3). Following the approach used by Xue et al. (2006), the rate of assimilate remobilization by the genotypes was estimated from dry weight losses of stem and leaf biomass between the first (5 d stress) and last (20 d stress) harvest. Leaf dry weight loss was ca. 40% for Brown Speckled compared with more than 90% for SEA 15 under both soil moisture supply regimes. During the same period the stem dry weight loss of Brown Speckled (13% under control vs. 20% under stress) was smaller than SEA 15 (46% under control vs. 38% under stress). The higher decrease in total above-ground biomass yield of SEA 15 was accompanied by a remarkable increase in reproductive to vegetative biomass ratio (Fig. 3). On the other hand, continued vegetative growth after the start of the reproductive phase resulted in relatively lower reproductive to vegetative mass ratio for Brown Speckled. These results demonstrate that the drought-susceptible genotype (Brown Speckled) has an inherently lower sink strength than SEA 15. Losses in stem and leaf dry weights owing to drought stress occurring during grain filling coupled with important gains in harvest index have been reported for drought-resistant genotypes of several crops including some legumes (Chaves et al., 2002; Rodrigues et al., 1995).

According to Zhang et al. (2005), mobilization of reserves is dependent on sink strength, which varies with the genotype and is affected by the environment (e.g. water availability). In line with this and other available reports (e.g. Monneveux et al., 2005), it is suggested that the mechanisms underlying differences in drought resistance (yielding ability under drought stress) of the bean genotypes are primarily related to the selections made for efficient biomass partitioning to reproductive structures rather than biomass

accumulation ability *per se.* Our findings, therefore, do not support the common generalization that a strong correlation exists between plant biomass and seed yield in common beans when grown across a wide range of environments (Shenkut and Brick, 2003). In major bean-growing regions of Central America and the highlands of Mexico, field studies have also shown that the only traits that have proven to be valuable for both terminal and intermittent drought situations are earliness and partitioning toward reproductive structure (greater harvest index) (Foster et al., 1995; Acosta-Gallegos and Adams, 1991).

As presented in Fig. 4A, the drought-induced reduction in leaf area was much higher for the drought-resistant genotype compared with the susceptible one. The restriction in leaf area expansion is one of the earliest morphological reactions of plants to drought, which is employed as an avoidance mechanism to limit further water losses when the stomata are closed (Maroco et al., 2000; Ruiz-Sanchez et al., 2000). The decrease in leaf area caused by osmotic stress (due to either salinity or drought) has often been associated with a decrease of leaf turgor, changes in cell wall properties, ABA accumulation and decreased photosynthetic rate (Bacon, 1999; Franco et al., 1997). Under our conditions, it could be due to the accumulation of ABA (Fig. 13).

Specific leaf weight (SLW, dry weight per unit area of leaf) as surrogate tool for selecting cultivars with higher biomass production and water-use efficiency (WUE) under drought stress has been suggested for a range of crops (Upadhyaya, 2005; Subrahmanyam, 2002; Thumma et al. 2001). Consistent with these reports, drought-induced increase in SLW (Fig. 4B) corresponded with higher WUE of the drought-resistant genotype (SEA 15) during the vegetative phase. Studies have shown that the increases in SLW of plants under drought conditions are related to the increment in leaf thickness and specific leaf nitrogen content (Nageswara Rao and Wright, 1994), increased accumulation of nonstructural carbohydrates (Brown and Byrd, 1997), larger numbers of mesophyll cells (Nelson, 1988), increased mesophyll conductance to CO_2 (Peña-Rojas et al., 2005) and

higher amount of Rubisco (Nageswara Rao et al., 1995). Because these traits contribute positively to an increased carbon assimilation rate, their expression would enhance WUE under drought situations. In the present study, drought stress imposed during the vegetative phase caused a comparable reduction in leaf gas-exchange between the bean genotypes. Thus, the genotypic difference observed for SLW and WUE under our conditions appears to be independent of photosynthetic rate.

The drought-induced decrease in vegetative relative growth rates (RGR) of the genotypes was accompanied by a reduction in net assimilation rate (NAR) (Table 5). This is consistent with several reports that drought and salinity-induced decrease in plant growth rates are mainly related to NAR rather than to LAR (Bayuelo-Jiménez et al. 2003; Ryser and Wahl, 2001). The reduction in NAR under osmotic stress is usually caused by (i) reduced rate of photosynthesis; (ii) reduced ability to utilize photosynthates for growth; (iii) increased utilization of photosynthates in respiration (Bayuelo-Jiménez et al. 2003, Cramer et al., 1990; Poorter, 1989). The significant correlation of NAR with single-leaf carbon exchange rate found in the present experiment implied that factors inhibiting photosynthesis under drought stress may be the primary factors explaining the inhibition of growth in both genotypes. In contrast to comparable reduction of growth rate found for the two bean genotypes under our conditions, Lizana et al. (2006) and Costa Franća et al. (2000) reported significant differences in growth rates among common bean genotypes varying in drought resistance. Genotypic differences observed in those studies were explained in terms of variation in net assimilation rate under drought, which was apparently not the case in our study. The remarkably similar qualitative and quantitative effect of drought stress on vegetative growth rates of the common bean genotypes support previous suggestions of Fernández and Reynolds (2000) that growth potential and drought resistance may not always exhibit a direct association.

Although LAR did not affect growth rate as pointed out earlier, apparent differences were observed between the two genotypes in partitioning dry matter to different plant parts in

response to drought. In SEA 15, drought stress resulted in an increase of stem dry weight but reduced leaf weight ratio (LWR) as compared with Brown Speckled. Such a shift in allocation pattern of the drought-resistant genotype by accumulating proportionally more biomass in stems and perhaps in roots than in the leaves is considered as an adaptive mechanism of plants to water-limited drought situations. Slower growth under stress allows plants to divert assimilates and energy, otherwise used for shoot growth, into protective molecules to fight stress (Zhu, 2002) and/or to maintain root growth, improving water acquisition (Chaves et al., 2003).

As opposed to the vegetative growth rate, the analyses of growth rate of reproductive structures (Fig. 6) better explained the actual genotypic difference found in drought resistance degrees (determined based on relative seed yield production under drought stress). Our results further underscored that for crops such as common bean that are principally grown for seed yield, selection for drought resistance should capitalize on reproductive growth potential (capacity to develop new reproductive sink) rather than mere drought survival during early growth phase. In accordance with Balibrea et al. (2003), higher dry matter accumulation (equivalent to AGR) and RGR of reproductive structures found for SEA 15 compared with Brown Speckled suggested that the resistant genotype had higher sink strength than the susceptible one under both growth conditions. The superiority of SEA 15 under the contrasting soil moisture supply regimes was on the account of rapid early growth coupled with higher water consumption during the vegetative phase and higher efficiency of biomass remobilization to the reproductive sinks during the reproductive phase.

4.4. Leaf-water relations and water-use efficiency

When plants of both genotypes were subjected to drought stress, the leaf dehydration (expressed by leaf relative water content, RWC) was smaller for Brown Speckled than for SEA 15 (Fig. 7A, B). This is in contrast to the findings of Costa Franća et al. (2000) which disclosed higher leaf tissue water retention capacity by a drought-resistant cultivar

compared with a susceptible common bean genotype. The bean genotypes exhibited marked differences in terms of the response of stomatal conductance (g_s) and photosynthetic rate (A) to leaf RWC. i.e. the role of leaf water status in driving stomatal closure under drought stress appeared to differ between the two bean genotypes. The decrease in leaf RWC of the drought-resistant genotype, SEA 15, was accompanied by a smaller decrease relative to Brown Speckled in g_s and A (Fig. 15A, B) implying that leaf water status had a leading (feedback control) role over stomatal closure. Leaf gas-exchange parameters (g_s and A) of Brown Speckled decreased earlier (at 2 d stress) than the decrease in RWC, which was detected only 6 d after drought stress was initiated (Fig. 7B). According to Schulze (1986), closure of stomata under dehydrating conditions could result either from a feedback response to the generation of water deficits in the leaf itself that is transmitted to the guard cells, or from a feed-forward control before any alteration in leaf tissue water status takes place (perhaps the case with Brown Speckled here). Our finding supports the proposal of Flexas et al. (2004) that g_s rather than RWC is a more reliable indicator of the level of stress in plant leaves and hence, g_s determined the rate of photosynthetic rate under drought stress more than RWC did.

The decreases in ψ and ψ_s values (Table 6) observed for the tested bean genotypes under drought stress were within the range of that reported for common bean (Costa Franca et al., 2000; Scotti Campos, 1999; Ismail and Davis, 1997). Drought-induced decreases in the osmotic potential (ψ_s) found for most of the genotypes were related to higher solute accumulation. However, it is unclear if the decrease of ψ_s is true osmotic adjustment or if it results from a concentration of the cell sap due to tissue dehydration, as previously reported for common bean (Amede and Schubert, 2003a; Markhart, 1985). In spite of significant genotypic differences found for ψ_s, drought stress did not affect the turgor pressure (ψ_p) of most of the genotypes studied. Such lack of the ability to completely express leaf turgor by the genotypes was presumably due to the comparable water potential (ψ) maintained under the contrasting soil moisture supply regimes. Consistent

with our finding, comparable cellular turgor between drought-stressed and non-stressed common bean plants had previously been reported (Brerstic et al., 1994). No correlation was detected between water potential and yield-related parameters implying that the leaf water-relation parameters have limited significance as selection criteria for drought resistance.

The results of this study showed that substantial improvement of WUE_{SY} occurred in inbred lines (represented by SEA 15 and SEA 23) selected for specific adaptation to drought stress compared with the old adapted cultivars (represented by Mex. 142, Roba 1 and Brown Speckled) (Table 8). Higher WUE_{SY} under both soil moisture supply regimes for the inbred lines was associated with higher harvest index and relatively smaller quantity of water consumed during the entire growth period (Table 7). Consistent with higher WUE_{SY} found for the inbred lines over the adapted bean cultivars in the present study, Siddique et al. (1990) reported similar differences between modern and old wheat cultivars. In both improved (modern) bean and wheat genotypes, higher WUE_{SY} was augmented by two key features: early flowering and the subsequent use of a larger proportion of available water for the maintenance of reproductive growth (see Table 7).

Relative to non-stressed treatments, water consumption per unit leaf area for drought-stressed (imposed at vegetative stage) Brown Speckled and SEA 15 was ca. 58 and 44%, respectively. This implies that SEA 15 has much greater transpirational water control than Brown Speckled under drought situations. Faster and higher vegetative biomass accumulation of SEA 15 (Table 4) was achieved through luxurious consumption of water when grown under non-limiting soil moisture supply regime (Fig. 10). When the genotype was subjected to drought during the same growth phase, biomass yield (weight) and water consumption were much more depressed than in Brown Speckled. This demonstrates that the drought-resistant genotype was an ‘opportunist’ in relation to available water, having higher rates of transpiration and growth when soil moisture was adequate but having marked reductions of both water loss and growth when soil moisture was limiting.

Higher correlation of IWUE with g_s than with A (Fig. 14) implied that drought-induced increase in water-use efficiency of the bean genotypes was attained mainly due to efficient stomatal closure as a water conservation strategy. The predominance of stomatal control of IWUE over that by carbon assimilation capacity has been reported for several crops (Impa et al., 2005; Anyia and Herzog, 2004; van den Boogaard et al., 1997). In line with the reports of Zhang et al. (2005) and Heschel et al. (2002), higher WUE of the inbred lines (represented by SEA 15) compared with old adapted cultivars (represented by Brown Speckled) when grown under drought stress could be ascribed to their derivation from parents, which were evolved in dry environments of central and south American highlands. Systematic screenings of several hundred germplasm accessions, breeding lines, and cultivars of common bean of diverse origins have shown that cultivars obtained from these regions generally have the highest level of drought resistance and WUE (Terán and Singh, 2002; Acosta-Gallegos and Adams, 1991; Acosta-Gallegos and Kohashi-Shibata, 1989).

4.5. Proteome changes in bean leaves

The changes in leaf proteins of Brown Speckled detected using 2D gel electrophoresis (see Table 10 and Fig. 32) corroborate previous reports on the responses of plants to drought stress (Salekdeh et al., 2002; Costa et al., 1998; Riccardi et al., 1998). Furthermore, the wide variation in sizes of the drought-responsive proteins detected was within the range reported for the dehydrin family of proteins (9 to 200 kDa) that are differentially expressed under dehydration stress (Close, 1996). Drought regulation of dehydrin gene expression was observed in both drought-tolerant and drought-susceptible cultivars (Cellier et al., 1998; Wood and Goldsbrough, 1997). In the present study, the drought stress-responsive proteins were neither identified nor compared with stress-regulated proteins isolated from other plant species. However, recent identification and characterization studies have demonstrated that most of the drought-responsive proteins are related to metabolism, energy, protein biosynthesis, cell defense, signal transduction, transport, and lignification (Rodríguez et al., 2006; Jiang and Huang, 2002; Salekdeh et

al., 2002). Also, evidences are unfolding in favor of a relationship between the accumulation of drought-induced proteins and physiological adaptation traits to water limitation (Bray, 1993; Han and Kermode, 1996; Riccardi et al., 1998).

5. CONCLUSIONS

Drought stress imposed during different growth phases of the crop reduced the above-ground biomass yields of the inbred lines as well as the old adapted cultivars at more or less comparable rates. Nevertheless, the inbred lines developed for specific adaptation to drought conditions maintained higher rate of biomass partitioning to reproductive organs than the adapted cultivars developed for wider agro-ecological adaptations. Compared with the drought-susceptible adapted cultivars (represented by Brown Speckled), the drought-resistant inbred lines maintained higher sink strength (larger number of pods and seeds per plant), which was largely responsible for their higher relative seed yield under drought stress. Drought-resistant inbred lines also exhibited higher water-use efficiency (WUE) than the drought-susceptible old adapted cultivar under drought stress commenced at different growth stages of the crop. Relatively smaller transpiration rate (less water used) per unit leaf area, efficient stomatal regulation, higher rates of leaf area and leaf biomass weight reduction (reduced LWR) and higher absolute biomass and seed yield attained accounted for the observed higher WUE of the drought-resistant genotype. These features may confer a fitness advantage for the drought-resistant inbred lines over the drought-susceptible cultivars under drought conditions.

Drought stress decreased net photosynthetic rate (*A*) of the bean genotypes differing in drought resistance at both growth phases of the crop. Drought-induced stomatal closure (limited availability of CO_2) was the main factor responsible for the reduced carbon assimilation rate of both genotypes, although there was evidence of non-stomatal inhibition (metabolic impairments) of photosynthesis for the drought-susceptible genotype. The decrease in *A* due to drought stress initiated during the vegetative phase

was parallel to reduced leaf carbohydrate concentrations of the bean genotypes determined under similar growth conditions. Thus, shortage of assimilate as a consequence of reduced carbon assimilation rate contributed to the reduction in growth and biomass accumulation of the bean genotypes subjected to drought stress during early vegetative growth.

Carbohydrate concentration in the source (leaf) and sink (seed) organs of the drought-susceptible genotype was lower under drought stress (imposed at pod-filling stage) compared with non-stress growth conditions. Thus, sink strength (the capacity to establish new sink) and yield of the genotype under drought stress was primarily source-limited. Moreover, higher pod sucrose to hexose sugars ratio found for Brown Speckled demonstrates that the capacity to utilize the imported sucrose was inhibited due to drought. For the drought-resistant genotype the stress did not affect the concentration of assimilates in both source and sink organs despite the decrease in carbon assimilation rate. The results suggest that at whole plant level of SEA 15, total *A* is less affected than total carbohydrate demand because the genotype was able to adjust sink demand with source supply under drought conditions.

In addition to assimilate synthesis, availability and metabolism, the differences found in drought-induced ABA accumulation and the decrease in pod water concentration could also be responsible for the differential drought-sensitivity of the bean genotypes. Drought stress also negatively affected seed starch accumulation of the bean genotypes mainly through arresting the expansion growth of starch granules (amyloplasts). In summary, although apparent genotypic differences were observed for sink strength under drought stress, the underlying variation in sink establishment and ultimate yield of the bean genotypes reside in the capacity to supply assimilates by the source (source-strength).

6. SUMMARY

Drought stress is a major constraint to common bean (*Phaseolus vulgaris* L.) production worldwide. Understanding the physiological basis of drought resistance may help to target the key traits that limit yield of the crop under drought situations. The objective of this study was to test the hypotheses that I) differences exist in biomass accumulation, yield and water-use efficiency among common bean cultivars developed for wider agro-ecological adaptation and inbred lines selected for specific adaptation to drought situations when subjected to drought stress; II) a drought-resistant genotype has a higher sink strength than a susceptible genotype and the difference between the genotypes is related to the ability to maintain assimilate synthesis and availability of assimilates for metabolism in the reproductive sink organs under drought stress; III) drought stress induces higher accumulation of ABA in sink leaves of a drought-susceptible genotype than in the leaves of a resistant genotype; and IV) relative to non-stressed plants, drought stress alters the protein pattern in a mature bean leaf.

Three adapted cultivars and three inbred lines of common bean were initially screened to assess seed yield-based drought resistance and water-use efficiency of the genotypes. A drought-resistant (SEA 15) and a susceptible genotype (Brown Speckled) were selected and used for subsequent experiments carried out thereafter. Drought stress was initiated at different growth stages (vegetative or reproductive phases) by withholding the amount of water applied in order to keep the moisture level at about 30% of the maximum water-holding capacity (WHC) of the soil. For control treatments, the soil moisture was maintained at 70% of the maximum WHC. Parameters related to growth, yield, water-use efficiency, leaf-water relations, gas-exchange, seed sink size, and the concentration of assimilates in various source and sink organs were determined. Changes in mature leaf proteins due to drought stress imposed during the vegetative phase were detected using 2D gel electophoresis.

Drought stress initiated during the reproductive phase significantly reduced the seed yields of the tested genotypes. However, seed yield reductions owing to the stress imposed were considerably higher for the old adapted cultivars than for the inbred lines selected for specific adaptation to drought conditions. With ca. 30 and 53% decrease in seed yield, SEA 15 and Brown Speckled were the most drought-resistant and susceptible genotypes, respectively. Drought stress initiated during different growth phases of the crop adversely affected biomass accumulation, although apparent genotypic differences were lacking. However, the harvest index of the drought-susceptible genotype (Brown Speckled) was reduced by about 29% due to drought, whereas that of SEA 15 remained unaffected. The maintenance of higher sink strength (larger numbers of pods and seeds per plant) under drought stress of SEA 15 was partly contributed by the higher efficiency of the genotype to remobilize biomass from vegetative parts to reproductive organs. SEA 15 maintained higher water-use efficiency (WUE) than Brown Speckled when subjected to drought stress. Relatively smaller transpiration rate (less water consumption), efficient stomatal regulation, faster vegetative biomass accumulation and higher seed yield production accounted for the observed higher WUE of the drought-resistant genotype. These features may confer a fitness advantage for drought-resistant inbred lines over drought-susceptible cultivars under drought conditions.

Drought stress commenced at the vegetative as well as reproductive growth phases decreased net photosynthetic rate of the bean genotypes differing in drought resistance. Drought-induced stomatal closure (limited availability of CO_2) was the main factor responsible for the reduced carbon assimilation rate of both genotypes, although there was evidence of non-stomatal inhibition of photosynthesis for the drought-susceptible genotype. During the vegetative phase, drought-induced reductions in *A* corresponded with the decreases in leaf sugar concentrations of the bean genotypes. Thus, shortage of assimilate as a consequence of reduced carbon assimilation could be the growth-limiting factor during the vegetative phase. Similarly, drought initiated during the pod-filling stage reduced not only *A* but also the concentrations of sucrose in the leaves and seeds of

Brown Speckled demonstrating that reproductive sink establishment and yield of the susceptible genotype was primarily source-limited. Moreover, higher pod sucrose to hexose sugars ratio found for Brown Speckled demonstrates that the capability to utilize the imported sucrose was inhibited due to drought. Drought stress initiated during the same period did not alter the availability of assimilates for the drought-resistant genotype (SEA 15) both at source and sink levels. The results suggest that at whole plant level of SEA 15, total *A* is less affected than total carbohydrate demand because the genotype was able to adjust sink demand with source supply under drought conditions.

In addition to assimilate synthesis, availability and metabolism, the differences found in drought-induced ABA accumulation and the decrease in pod water concentration could also be responsible for the differential drought-sensitivity of the bean genotypes. Drought stress also negatively affected seed starch accumulation of the bean genotypes mainly through arresting the expansion growth of starch granules (amyloplasts). In summary, although apparent genotypic differences were observed for sink strength under drought stress, the underlying variation in sink establishment and ultimate yield of the bean genotypes reside in the capacity to supply assimilates by the source (source-strength).

Drought stress imposed during the vegetative phase of Brown Speckled resulted in the differential expression of ca. 42% of the total leaf proteins detected. Out of these proteins, 1.5% disappeared, 1.8% were newly produced, 23.5% were down-regulated and 15.1% were up-regulated in response to drought. The sizes of the drought-responsive proteins were widely variable.

7. ZUSAMENFASSUNG

Weltweit ist Dürrestress der stärkste ertragslimitierende Produktionfaktor für die Bohne (*Phaseolus vulgaris* L.). Das Verständis der physiologischen Grundlagen der Dürreresistenz trägt dazu bei Ertragsminderungen bei Trockenheit zu reduzieren. Ziel der vorliegenden Arbeit war es, die nachstehenden Hypothesen zu prüfen: I) Bestehen genotypische Unterschiede in der Biomasseproduktion von Bohnensorten und Bohnenlinien, die aufgrund ihrer Biomasseakkumulation und Wassernutzungseffizienz unter Dürre selektiert wurden? II) Hat ein trockenresistenter Genotyp eine größere Sinkkapazität als ein trockenempfindlicher Genotyp, und steht dieser Unterschied zwischen den Genotypen in Zusammenhang zur Assimilatsynthese und zum Vermögen bei Dürrestress die Assimilate in den reproduktiven Sinkorganen zu metabolisieren? III) Verursacht Dürrestress eine höhere Akkumulation von ABA in den Sinkblättern eines dürreempfindlichen Genotyps als in den Sinkblättern eines dürreresistenten Genotyps? IV) Verändert ein Dürrestress das Proteinmuster in einem vollentwickelten Bohnenblatt?

Drei angepasste Sorten und drei Linien von Bohnen wurden zuerst selektiert, um eine auf den Samenertrag begründete Dürreresistenz und Wassernutzungseffizienz der Genotypen festzustellen. Für die folgenden Versuche wurde ein dürreresistenter (SEA 15) und ein dürreempfindlicher Genotyp (Brown Speckled) ausgewählt. Dürrestress wurde in verschiedenen Wachstumsphasen (vegetative oder reproduktive Phase) durch Wasserentzug appliziert, so dass die Bodenfeuchte bei ca. 30% von der maximalen Wasserhaltekapazität (WHK) des Bodens lag. In der Kontrolevariante wurde die Bodenfeuchte auf 70% der maximalen WHK eingestellt. Bezogen auf das Wachstum wurden die Parameter Ertrag, Wassernutzungseffizienz, Wassergehalt des Blattes, Gasaustausch, Sink-Größe der Samen und die Assimilat-Konzentration in verschiedenen Organen von Source und Sink bestimmt. Eine mögliche Änderung des Proteinmusters in den vollentwickelten Blättern infolge von Dürrestress wurde mit der 2D-Gelelektrophorese analysiert.

Ein während der reproduktiven Phase induzierter Dürrestress führte zur Verminderung der Samenerträge bei den getesteten Genotypen. Die aufgrund von Dürrenstress beobachtete Reduktion im Samenertrag war bei der alten angepassten Sorte stärker ausgeprägt als bei den Inzuchtlinien, die auf Dürreresistenz selektiert worden waren. Mit ca. 30 und 53% Abnahme im Samenertrag war SEA 15 bzw. Brown Speckled der jeweilig dürreresistenteste und dürreempfindlichste Genotyp. Dürrestress, der während der unterschiedlichen Wachstumphasen induziert wurde, beeinflusste die Biomasseakkumulation ungünstig, obwohl deutliche genotypische Unterschiede verlagen. Jedoch wurde der Ernteindex des dürreempfindlichen Genotyps (Brown Speckled) um ungefähr 29% aufgrund von Dürre verringert, während der von SEA 15 unbeeinflusst blieb. Bei Dürrestress trug die Sinkgröße (größere Anzahl von Hülsen und Samen pro Pflanze) von SEA 15 dazu bei, dass Assimilate effizienter aus der vegetativen Biomasse in die reproduktiven Organen verlagert wurden. Bei Dürrestress zeigte SEA 15 eine höhere Wassernutzungseffizienz (WUE) als Brown Speckled. Die höhere WUE von SEA 15 beruhte auf einer kleineren Transpirationsrate (geringer Wasserverbrauch), effizienten Stomataregelung, einem raschen vegetativen Wachstum und einem hohen Samenertrag. Diese Eigenschaften könnten die Dürreresistanz der dürreresistenten Genotypen erklären.

Dürrestress, der während der vegetativen sowie reproduktiven Wachstumsphasen induziert wurde, reduzierte die Nettophotosyntheserate (*A*) der Bohnengenotypen, die sich in der Dürrenresistenz unterschieden. Dürreverursachtes Stomataschließen (limitierende Verfügbarkeit von CO_2) war der Hauptfaktor, der für die reduzierte Assimilationsrate beider Genotypen verantwortlich war. Dennoch gab es Hinweise auf eine nicht-stomatäre Hemmung der Photosynthese beim dürreempfindlichen Genotyp. Während der vegetativen Phase stand die durch Dürre bedingte Abnahme von *A* mit der Reduktion der Blattzuckerkonzentrationen der Bohnengenotypen in Beziehung. Folglich könnte ein Assimilatmangel, der durch eine verminderte CO_2-Assimilation bedingt war, ein Grund für die Wachstumshemmung in der vegetativen Phase sein. Dürrestress, der während der Hülsenfüllungsphase induziert wurde, reduziert nicht nur Nettophotosyntheserate,

sondern auch die Saccharosekonzentrationen in den Blättern und in den Samen von Brown Speckled. Diese Ergebnisse zeigen, dass die Anlage von reproduktiven Sinkorganen und der Ertrag des empfindlichen Genotyps hauptsächlich vom Source begrenzt waren. Ferner weist das höhere Verhältnis der Saccharosekonzentration zur Hexosekonzentration in den Hülsen von Brown Speckled daraufhin, dass die Kapazität zur Nutzung von importierter Saccharose durch Trockenheit gehemmt wurde. Die Ergebnisse deuten darauf hin, dass bei SEA 15, bezogen auf die Gesamtpflanze, Nettophotosyntheserate weniger beeinflusst wurde als der Gesamt-Kolenhydratbedarf. Dieser Genotyp ist in der Lage, die Sinknachfrage durch eine Sourceversorgung auszugleichen.

Neben Synthese, Verfügbarkeit und Stoffwechsel von Assimilaten, könnte die Dürreempfindlichkeit der Bohnengenotypen mit einer dürreinduzierten ABA-Akkumulation und einer Abnahme der Hülsenwasserkonzentration erklärt werden. Dürrestress reduzierte die Stärkeakkumulation in den Samen von beiden Bohnengenotypen infolge einer Wachstumshemmung der Stärkekörnchen (Amyloplasten). Zusammenfassend ist festzustellen, dass, obwohl deutliche genotypische Unterschiede in der Sinkgröße unter Dürrestress beobachtet wurden, die Variation in der Sinkanlage und der Ertrag der Bohnengenotypen besonders auf dem Vermögen des Sources beruht Assimilate bereitzustellen (Sourcegröße).

Der während der vegetativen Phase von Brown Speckled induzierte Dürrestress führte zu einer differentiellen Expression von ca 42% der bestimmten Blattproteine. Von diesen Proteinen waren 1,5% verschwunden, 1,8% neu synthetisiert, 23,5% herunterreguliert und 15,1% hochreguliert. Die Größe der Proteine, die infolge von Dürre auftraten, war sehr variabel.

8. REFERENCES

Abebe, A., Brick, M.A., Kirkby, R.A. (1998): Comparison of selection indices to identify productive dry bean lines under diverse environmental conditions. Field Crops Res. **58**, 15-23

Acosta-Gallegos, J. A. (1988): Selection of common bean (*Phaseolus vulgaris* L.) genotypes with enhanced drought tolerance and biological nitrogen fixation. PhD Diss., Michigan State University, East Lansing

Acosta-Gallegos, J.A., White, J.W. (1995): Phenological plasticity as an adaptation by common bean to rainfed environments. Crop Sci. **35**, 199-204

Acosta-Gallegos, J.A., Adams, M.W. (1991): Plant traits and yield stability of dry bean (*Phaseolus vulgaris* L.) cultivars under drought stress. J Agric Sci. **117**, 213–219

Acosta-Gallegos, J.A., Kohashi-Shibata, J. (1989): Effect of water stress on growth and yield of indeterminate dry-bean (*Phaseolus vulgaris* L.) cultivars. Field Crops Res. **20**, 81–93

Adams, M.W., Coyne, D. P., Davis, J. H. C., Graham, P. H., Francis, C.A. (1985): Common bean (*Phaseolus vulgaris* L.). *In*: Summerfield, R. J., Roberts, E. H. (Eds.). Grain Legume Crops, Collins, London. pp. 433-476

Alkio, M., Diepenbrock, W., Grimm, E. (2002): Evidence for photoassimilate supply in the capitulum of sunflower (*Helianthus annuus* L.). New Phytol. **156**, 445-456

Alves, A.A.C., Setter, T.L. (2000): Response of cassava to water deficit: Leaf area growth and abscisic acid. Crop Sci. **40**, 131-137

Amede, T., Schubert, S. (2003): Mechanisms of drought resistance in grain legumes I: Osmotic adjustment. SINET: Ethiop J Sci. **26(1)**, 37-46

Amede, T., Schubert, S. (2003): Mechanisms of drought resistance in grain legumes II: Stomatal regulation and root growth. SINET: Ethiop J Sci. **26(2)**, 137-144

Andersen,M.N., Asch, F., Wu, Y., Jensen, C.R., Næsted, H., Mogensen, V.O., Koch, K.

E. (2002): Soluble invertase expression is an early target of drought stress during the critical, abortion-sensitive phase of young ovary development in maize. Plant Physiol. **130**, 591-604

Anyia, A. O., Herzog, H. (2004): Water-use efficiency, leaf area and leaf gas exchange of cowpeas under mid-season drought. Eur J Agron. **20(4)**, 327-339

Araus, J. L., Slafer, G. A., Reynolds, M. P., Royo, C. (2002): Plant breeding and drought in C_3 cereals: what should we breed for?. Ann Bot. **89**, 925-940

Babu, R.C., Nguyen, B.D., Chamarerk, V., Shanmugasundaram, P., Chezhian, P., Jeyaprakash, P., Ganesh, S.K., Palchamy, A., Sadasivam, S., Sarkarung, S., Wade, L.J., Nguyen, H.T. (2003): Genetic analysis of drought resistance in rice by molecular markers: association between secondary traits and field performance. Crop Sci. **43**, 1457-1469

Bacon, M.A. (1999): The biochemical control of leaf expansion during drought. Plant Growth Regul. **29,** 101-112

Balibrea, M.E., Cuartero, J., Bolarín, M.C., Pérez-Alfocea, F. (2003): Sucrolytic activities during fruit development of *Lycopersicon* genotypes differing in tolerance to salinity. Physiol Plant. **118** (1), 38-46

Balibrea, M.E., Dell'Amico, J., Bolarín, M.C., Pérez-Alfocea, F. (2000): Carbon partitioning and sucrose metabolism in tomato plants growing under salinity. Physiol Plant. **110**, 503-511

Barratt, D.H.P., Pullen, A. (1984): Control of seed protein accumulation in field bean. Ann Bot. **54**, 31-38

Baxter, C.J., Foyer, C.H., Rolfe, S.A., Quick, W.P. (2001): A comparison of the carbohydrate composition and kinetic properties of sucrose-phosphate synthase (SPS) in transgenic tobacco (*Nicotiana tabacum*) leaves expressing maize SPS protein with untransformed controls. Ann Appl Biol. **138,** 47-55

Bayuelo-Jiménez, J. S., Debouck, D.G., Lynch, J.P. (2003): Growth, gas exchange, water relations, and ion composition of *Phaseolus* species grown under saline conditions. Field Crops Res. **80 (3)**, 207-222

Beadle, C.L. (1993): Growth analysis. *In*: Hall, D.O., Scurlock, J.M.O., Bolhar-Nordentrampf, H.R., Leegod, R.C., Long, S.P. (Eds.), Photosynthesis and Production in a Changing Environment. Chapman and Hall, London, pp. 36-45

Bidinger, F.R., Witcombe, J.R. (1989): Evaluation of specific drought avoidance traits as selection criteria for improvement of drought resistance. *In*: Baker, F.W.G. (ed). Drought resistance in cereals. Wallingford, UK:CABI. pp. 151-164

Blum, A. (1988): Plant Breeding for Stress Environments, CRC Press, London.

Bohnert, H. J., Jensen, R. G. (1996): Strategies for engineering water stress tolerance in plants. Trends Biotechnol. **14**, 89-97

Bohnert, H.J., Nelson, D.E., Jensen, R.G. (1995): Adaptation to environmental stresses. Plant Cell. **7**, 1099-1111

Boutraa, T., Sanders, F.E. (2001): Influence of water stress on grain yield and vegetative growth of two cultivars of bean (*Phaseolus vulgaris*, L.). J Agron Crop Sci. **187**, 251-257

Boyer, J.S., Chin-Wong, S., Farquhar, G.D. (1997): CO_2 and water vapour exchange across leaf cuticle (epidermis) at various water potentials. Plant Physiol. **114**, 185-191

Bray, E. (1997): Plant responses to water deficit. Trends Plant Sci. **2**, 48-54

Bray, E.A. (1993): Molecular responses to water deficit. Plant Physiol. **103**, 1035-1040

Brestic, M., Cornic, G., Freyer, M.J., Baker, N.R. (1994): Does photorespiration protect the photosynthetic apparatus in french bean leaves from photoinhibition during drought stress? Planta. **196(3),** 450 - 457

Brooks, A., Jenner, C. F., Aspinall, D. (1982): Effects of water deficit on endosperm starch granules and on grain physiology of wheat and barley. Aust J Plant Physiol. **9**, 423-436

Brown, R.H., Byrd, G.T. (1997): Relationships between specific leaf weight and mineral concentration among peanut (*Arachis hypogaea* L.)genotypes. Field Crops Res. **54(1)**, 19-28

Buckley, T.N., Farquhar, G.D., Mott, K.A. (1997): Qualitative effects of patchy stomatal

conductance distribution features on gas-exchange calculations. Plant Cell Environ. **20**, 867-880

Cellier, F., Conejero, G., Breitler, J.C., Casse, F. (1998): Molecular and physiological responses to water defi cit in drought-tolerant and drought-sensitive lines of sunflower. Plant Physiol. **116**, 319-328

Chartzoulakis, K., Noitsakis, B., Therios, I. (1993): Photosynthesis, plant growth and dry matter distribution in kiwifruit as influenced by water deficits. Irrigation Sci. **14 (1)**, 1-5

Chaves, M.M., Maroco, J.P., Pereira, J.S. (2003): Understanding plant response to drought: from genes to the whole plant. Funct Plant Biol. **30**, 239-264

Chaves, M.M., Pereira, J.S., Maroco, J.P., Rodrigues, M.L., Ricardo, C.P.P., Osório, M.L., Carvalho, I., Faria, T., Pinheiro, C. (2002): How plants cope with water stress in the field: photosynthesis and growth. Ann Bot. **89**, 907-916

Chimenti C.A., Pearson, J., Hall, A.J. (2002): Osmotic adjustment and yield maintenance under drought in sunflower. Field Crops Res. **75**, 235-246

CIAT (Centro International Agriculture de Tropical). (2002): Bean Annual Report. Cali, Columbia. p.96

Close, T.J. (1996): Dehydrins: emergence of a biochemical role of a family of plant dehydration proteins. Physiol Plant. **100**, 291-296

Colmenero-Flores, J.M., Campos, F., Garciarrubio, A., Covarrubias, A.A. (1997): Characterization of *Phaseolus vulgaris* L. cDNA clones responsive to water deficit: identification of a novel late embryogenesis abundant-like protein. Plant Mol Biol. **35**, 393-405

Costa, P., Bahrman, N., Frigerio, J., Kremer, A., Plomion , C. (1998): Water-deficit-responsive proteins in maritime pine. Plant Mol Biol. **38**, 587-596

Costa França, M.G., Pham-Thi, A.T., Pimentel, C., Pereyra Rossiello, R.O., Zuily-Fodil, Y., Laffray, D. (2000): Differences in growth and water relations among *Phaseolus vulgaris* cultivars in response to induced drought stress. Environ Exp Bot. **43**, 227-237

Cramer, G.R., Epstein, E., Läuchli, A. (1990): Effects of sodium, potassium and calcium on salt-stressed barley. 1. Growth analysis. Physiol Plant. **80**, 83-88

Da Silva, J. M., Arrabaça, M. C. (2004): Contributions of soluble carbohydrates to the osmotic adjustment in the C_4 grass *Setaria sphacelata*: A comparison between rapidly and slowly imposed water stress. J Plant Physiol. **161**, 551-555

Daie, J. (1996): Metabolic adjustments, assimilate partitioning, and alterations in source-sink relations in drought-stressed plants. *In*: Zamski, E., Schaffer, A. (Eds.). Photoassimilate Distribution in Plants and Crops. Marcel Dekker, Inc. New York. pp. 407-420

Damerval, C., de Vienne, D., Zivy, M., Thiellement, H. (1986): Technical improvements in two-dimensional electrophoresis increase the level of genetic variation detected in wheat-seedling proteins. Electrophoresis.**7**, 52–54

Egli, D.B. (1999): Variation in leaf starch and sink limitations during seed filling in soybean. Crop Sci. **39**, 1361-1368

Egli, D.B., Bruening, W. P. (2001): Source-sink relationships, seed sucrose levels and seed growth rates in soybean. Ann Bot. **88**, 235-242

Egli, D.B., Leggett, J.E., Cheniae, A. (1980): Carbohydrate levels in soybean leaves during reproductive growth. Crop Sci. **20**, 468-472

Ehleringer, J.R., Hall, A.E., Farquhar, G.D. (1993): Stable isotopes and plant carbon-water relations. Academic Press, San Diego, CA.

Ehleringer, J.R., White, J.W., Johnson, D.A., Brick, M. (1990): Carbon isotope discrimination, photosynthetic gas exchange, and transpiration efficiency in beans and range grasses. Acta Oncol. **11**, 611-625.

Ehleringer, J.R., Klassen, S., Clayton, C., Cherrill, D., Fuller-Holbrook, M., Fu, Q., Cooper, T. A. (1991): Carbon isotope discrimination and transpiration efficiency in common bean. Crop Sci. **31**, 1611-1615

Escalona, J.M., Flexas, J., Medrano, H. (1999): Stomatal and non-stomatal limitations of photosynthesis under water stress in field-grown grapevines. Aust J Plant Physiol. **26**, 421-433

Farquhar, G.D., Richards, R.A. (1984): Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Aust J Plant Physiol. **11**, 539-552

Fernández, R.J., Reynolds, J.F. (2000): Potential growth and drought tolerance of eight desert grasses: lack of a trade-off? Oecologia, **123 (1)**, 90-98

Fernández, R.J., Wang, M., Reynolds, J.F. (2002): Do morphological changes mediate plant responses to water stress? A steady-state experiment with two C_4 grasses. New Phytol. **155 (1)**, 79-88

Flexas, J., Bota, J., Cifre, J., Escalona, J. M., Galmés, J., Gulías, J., Lefi, E., Martínez-Cañellas, S. F., Moreno, M.T., Ribas-Carbó, M., Riera, D., Sampol, B., Medrano, H. (2004): Understanding down-regulation of photosynthesis under water stress: future prospects and searching for physiological tools for irrigation management. Ann Appl Biol. **144**, 273-283

Foster, E. F., Pajarito, A. J. A., Acosta, G. (1995): Moisture stress impact on N partitioning, N remobilization and N-use efficiency in beans (*Phaseolus vulgaris* L.). J Agric Sci. **124**, 27-37

Frahm, M.A., Rosas, J.C., Mayek-Pérez, N., López-Salinas, E., Acosta-Gallegos, J.A., Kelly, J.D. (2004): Breeding beans for resistance to terminal drought in the Lowland tropics. Euphytica. **136(2),** 223-232

Franco, J.A., Fernández, J.A., Bañón, S., González, A. (1997): Relationship between the effects of salinity on seedling leaf area and fruit yield of six muskmelons cultivars. J Hortic Sci. **32**, 642-647

Geigenberger, P., Reimholz, R., Deiting, U., Sonnewald, U., Stitt, M. (1999): Decreased expression of sucrose phosphate synthase strongly inhibits the water stress-induced synthesis of sucrose in growing potato tubers. Plant J. **19,** 119-129

Geiger, D.R., Fondy, B.R. (1991): Regulation of carbon allocation and partitioning: status and research agenda. *In*: Bonnemain, J.I., Delrot, S., Dainty, J., Lucas, W. J. (eds.). Recent Advances in Phloem Transport and Assimilate Compartimentation.

Nantes, Questedns, pp. 1- 9

Gimenez, C., Mitchell, V.J., Lawlor, D.W. (1992): Regulation of photosynthetic rate of two sunflower hybrids under water stress. Plant Physiol. **98**, 516-524

Gleadow, R.M., Dalling, M.J., Halloran, G.M. (1982): Variation in endosperm characteristics and nitrogen content in six wheat lines. Aust J Plant Physiol. **9**, 539-551

Graham, P.H., Ranalli, P. (1997): Common Bean (*Phaseolus vulgaris* L.). Field Crop Res. **53**, 131-146

Gunasekera, D., Berkowitz, G.A. (1992): Heterogenous stomatal closure in response to leaf water deficits is not a universal phenomenon. Plant Physiol. **98**, 660-665

Gutschick, V.P., Currier, C.G. (1992): Increased water-use efficiency in alfalfa by selection for two key, heritable physiological traits. New Mexico Water Resources Research Institute Report No. 263, New Mexico State University, Las Cruces, NM.

Hall, A.E. (1993): Is dehydration tolerance relevant to genotypic differences in leaf senescence and crop adaptation to dry environments? *In*: Close, T.J., Bray, E.A. (Eds.). Plant Responses to Cellular Dehydration During Environmental Stress. The American Soc. Plant Pathologists, Rockville, Maryland. pp. 1-10

Han, B., Kermode, A.R. (1996): Dehydrin-like proteins in castor bean seeds and seedlings are differentially produced in responses to ABA and water-deficit-related stresses. J Exp Bot. **47**, 933-939

Hartung, W., Zhang, J.H., Davies, W.J. (1994): Does abscisic acid play a stress physiological-role in maize plants growing in heavily compacted soil." J Exp Bot. **45**, 221-226

Haterlein, A.J. (1983): Bean. *In*: Tearce, T.P., Peet, M..M.Jr. (Eds.). Crop Water Relations. Willey, New York. pp. 157-185

Hattendorf, M.J., Evans, D.W., Peaden, R.N. (1990): Canopy temperature and stomatal conductance of water-stressed dormant and nondormant alfalfa types. Agron J. **82**, 873-877

Heim, U., Weber, H., Bäumlein, H., Wobus, U. (1993): A sucrose-synthase gene of *Vicia*

faba: Expression pattern in developing seeds in relation to starch synthesis and metabolic regulation. Planta. **191**, 394-401

Heschel, M.S., Donohue, K., Hausmann, N., Schmitt, J., 2002. Population differentiation and natural selection for WUE in *Impatiens capensis* (Balsaminaceae). Int J Plant Sci. **163(6)**, 907-912

Ho, L.C. (1988): Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. Ann Rev Plant Physiol Mol Biol. **39**, 355-378

Hsiao, T. C. (1973): Plant responses to water stress. Ann Rev Plant Physiol. **24**, 519-570

Hsiao, T. C., Acevedo, E., Fereres, E., Henderson, D.W. (1976): Water stress, growth and osmotic adjustment. Philosophical Transactions Royal Society of London, Series B 273-479-500

Huber, J.L.A., Huber, S.C., Nielsen, T.H. (1989): Protein phosphorylation as a mechanism for regulation of spinach leaf sucrose phosphate synthase. Arch Biochem Biophys. **270**, 681-690

Huber, S.C. (1989): Biochemical mechanism for regulation of sucrose accumulation in leaves during photosynthesis. Plant Physiol. **91**, 656-662

Huber, S.C., Rogers, H., Mowry, F.L. (1984): Effects of water stress photosynthesis and carbon partitioning in soybean plants grown in the field at different CO_2 levels. Plant Physiol. **76**, 244-249

Hunt, R. (1990): Basic growth analysis. Unwin Hyman Limited, London, UK.

Impa, S.M., Nadaradjan, S., Boominathan, P., Shashidhar, G., Bindumadhava, H., Sheshshayee, M. S. (2005): Carbon isotope discrimination accurately reflects variability in WUE measured at a whole plant level in rice. Crop Sci. **45**, 2517-2522

Ingram, J., Bartels, D. (1996): The molecular basis of dehydration tolerance in plants. Ann. Rev. Plant Physiol. Plant Mol Biol. **47**, 377-403

Ishitani, M., Nakamura, T., Han, S.Y., Takabe, T. (1995): Expression of the betaine

aldehyde dehydrogenase gene in barley in response to osmotic stress and abscisic acid. Plant Mol Biol. **27**, 307-315

Ismail, M.R., Davies, W.J. (1997): Water relations of *Capsicum* genotypes under water stress. Biol Plant. **39**, 293-297

Ismail, A.M., Hall, A.E. (1993): Correlation between water-use efficiency and carbon isotope discrimination in diverse cowpea genotypes and isogenic lines. Crop Sci. **32**, 7 - 12

Jat, K. R., Muralia, R.N., Kumar, A. (1991): Physiology of drought tolerance in wheat (*Triticum aestivum* L.). II Water potential and its components . J Agron Crop Sci. **167**, 73-80

Jenner, C.F., Uglade, T.D., Aspinall, D. (1991): The physiology of starch and protein deposition in the endosperm of wheat. Aust. J. Plant Physiol. **18**, 211-226

Jiang, Y., Huang, B. (2002): Protein alterations in tall fescue in response to drought stress and abscisic acid. Crop Sci. **42**, 202-207

Johnson, R.C., Tieszen, L.L. (1994): Variation for water-use efficiency in alfalfa germplasm. Crop Sci. **34**, 452-458

Jones, H.G., Corlett, J.E. (1992): Current topics in drought physiology. J Agric Sci. **119**, 291-296

Jones, M.M., Turner, N.C. (1980): Osmotic adjustment in expanding and fully expanded leaves of sunflower in response to water deficits. Aust J Plant Physiol. **7**, 181-192

Jongdee, B., Fukai, S., Cooper, M. (2002): Leaf water potential and osmotic adjustment as physiological traits to improve drought tolerance in rice. Field Crops Res. **26**, 153-163

Kaiser, W.M. (1987): Effects of water deficit on photosynthetic capacity. Physiol Plant. **71**, 142-249

Kawasaki, S., Miyake, C., Kohchi, T., Fujii, S., Uchida, M., Yokota, A. (2000): Responses of wild watermelon to drought stress: accumulation of an ArgE homologue and citrulline in leaves during water deficits. Plant Cell Physiol. **41(7)**, 864-873

Keller, F., Ludlow, M.M. (1993): Carbohydrate metabolism in drought-stressed leaves of pigeonpea (*Cajanus cajan*). J Exp Bot. **44**, 1351-1359

Kim, J.Y., Mahé, A., Brangeon, J., Prioul, J.L. (2000): A maize vacuolar invertase, *IVR2*, is induced by water stress. Organ/tissue specificity and diurnal modulation of expression. Plant Physiol. **124**, 71-84

Kiyosue, T., Yamaguchi-Shinozaki, K., Shinozaki, K. (1994): Cloning of cDNAs for genes that are early-responsive to dehydration stress (ERDs) in *Arabidopsis thaliana* L.: identification of three ERDs as HSP cognate genes. Plant Mol Biol. **25**, 791-798

Koç, M., Barutçular, C., Genç, I. (2003): Photosynthesis and productivity of old and modern durum wheats in a Mediterranean environment. Crop Sci. **43**, 2089-2098

Koizumi, M., Yamaguchi-Shinozaki, K., Tsuji, H., Shinozaki, K. (1993): Structure and expression of two genes that encode distinct drought-inducible cysteine proteinases in *Arabidopsis thaliana*. Gene. **129**, 175-182

Komor, E. (2000): Source physiology and assimilate transport: the interaction of sucrose metabolism, starch storage and phloem export in source leaves and the effects on sugar status in phloem. Aust J Plant Physiol. **27**, 497-505

Kramer, P.J., Boyer, J.S. (1995): Water relation of plants and soils. Academic Press, London

Kubiske, M.E., Abrams, M.D. (1993): Stomatal and non-stomatal limitations of photosynthesis in 19 temperate tree species on contrasting sites during wet and dry years. Plant Cell Environ. **16**, 1123-1129

Laffray, D., Louguet, P. (1990): Stomatal responses and drought resistance. Bull Soc Bot Fr. 137. Actual Bot. **1**, 47-60

Laisk, A. (1983): Calculation of leaf photosynthetic parameters considering the statistical distribution of stomatal apertures. J Exp Bot. **34**, 1627-1635

Lawlor, D.W. (1995): The effects of water deficit on photosynthesis. *In*: Smirnoff N. Environment and plant metabolism. Oxford: Bios Scientific Publishers, pp. 129-160.

Lawlor, D.W., Cornic, G. (2002): Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell Environ. **25**, 275-294

Leport, L., Turner, N.C., Davies, S.L., Siddique, K.H.M. (2006): Variation in pod production and abortion among chickpea cultivars under terminal drought. Eur J Agron. **24 (3)**, 236-246

Levitt, J. (1980): Responses of plants to environmental stresses. Vol. II. Academic Press, London

Lisse, T., Bartels, D., Kalbitzer, H.R., Jaenicke, R. (1996): The recombinant dehydrin-like desiccation stress protein from the resurrection plant *Craterostigma plantagineum* displays no defined three-dimensional structure in its native state. Biol Chem. **377**, 555 -561

Liu, F., Jensen, C.R., Andersen, M. N. (2004): Drought stress effect on carbohydrate concentration in soybean leaves and pods during early reproductive development: its implication in altering pod set. Field Crops Res. **86(1)**, 1-13

Liu, F., Andersen, M.N., Jensen, C.R. (2003): Loss of pod set caused by drought stress is associated with water status and ABA content of reproductive structures in soybean. Funct Plant Biol. **30**, 271-280

Lizana, C., Wentworth, M., Martinez, J.P., Villegas, D., Meneses, R., Murchie, E.H., Pastenes, C., Lercari, B., Vernieri, P., Horton, P., Pinto, M. (2006): Differential adaptation of two varieties of common bean to abiotic stress. I. Effects of drought on yield and photosynthesis. J Exp Bot. **57(3)**, 685-697

Loreto, F., Tricoli, D., Di Marco, G. (1995): On the relationship between electron transport rate and photosynthesis in leaves of the C_4 plant *Sorghum bicolor* exposed to water stress, temperature changes and carbon metabolism inhibition. Aust J Plant Physiol. **22**, 885-891

Mambelli, S., Setter, T.L. (1998): Inhibition of maize endosperm cell division and endoreduplication by exogenously applied abscisic acid. Physiol. Plant. **104**, 266-272

Markhart, A.H. (1985): Comparative water relations of *Phaseolus* v*ulgaris* L.and *Phaseolus acutifolius* Gray. Plant Physiol. **77**, 113-117

Maroco J.P., Pereira, J.S., Chaves, M.M. (2000): Growth, photosynthesis and water-use efficiency of two C_4Sahelian grasses subjected to water deficits. J Arid Environ. **45(2)**, 119-137

Martin, M.A., Brown, J.H., Ferguson, H. (1989): RWC and leaf water potential differentiated drought tolerant and drought susceptible genotypes of barley. Agron J. **81**, 100-105

Mauk, C.S., Breen, P.J. (1986): Partitioning of ^{14}C-photosyntate among competitive sinks during flowering and early fruiting in snap beans. J Am Soc Hort Sci. **111**, 416-421

McCree, K.J. (1986): Measuring the whole-plant daily carbon balance. Photosynthetica. **20**, 82-93

Medrano, H., Escalona, J.M., Bota, J., Gulías, J., Flexas, J. (2002): Regulation of photosynthesis of C_3 plants in response to progressive drought: the interest of stomatal conductance as a reference parameter. Ann Bot. **89**, 895-905

Mekbib, F. (2003): Yield stability in common bean (*Phaseolus vulgaris* L.) genotypes. Euphytica. **130,** 147-153

Mencuccini, M., Comstock, J. (2000): Variability in hydraulic architecture and gas exchange of common bean (*Phaseolus vulgaris*) cultivars under well-watered conditions: interactions with leaf size. Aust J Plant Physiol. **26(2),** 115-124

Menendez, C.M., Hall, A.E. (1995): Heritability of carbon isotope discrimination and correlations with earliness in cowpea. Crop Sci. **35**, 673–687

Mertens, R.J., Deus-Neumann, B., Weiler, E.W. (1985): Monoclonal antibodies for the detection and quantification of the endogenous plant growth regulator, abscisic acid. FEBS Letters. **160**, 269-272.

Minchin, P.E.H., Thorpe, M.R., Farrar, J.F. (1993): A simple mechanistic model of phloem transport which explains sink priority. J Exp Bot. **44**, 947-955

Miyashita, K., Tanakamaru, S., Maitani, T., Kimura, K. (2005): Recovery responses of

photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. Environ Exp Bot. **53(2)**, 205-214

Mohamed, M.F., Keutgen, N.A., Tawfika, A., Noga, G. (2002): Dehydration-avoidance responses of tepary bean lines differing in drought resistance. J Plant Physiol. **159(1)**, 31-38

Monneveux, P., Rekika, D., Acevedo, E., Merah, O. (2006): Effect of drought on leaf gas exchange, carbon isotope discrimination, transpiration efficiency and productivity in field grown durum wheat genotypes. Plant Sci. **170(4)**: 867-872

Monneveux, P., Sánchez, C., Beck, D., Edmeades, G.O. (2005): Drought tolerance improvement in tropical maize source populations: Evidence of progress. Crop Sci. **46**, 180-191

Morgan, J..M. (2000): Increases in grain yield of wheat by breeding for an osmoregulation gene: relationship to water supply and evaporative demand. Aust J Agric Res. **50**, 971-978

MSTAT-C. (1989): MSTATC-C, a microcomputer program for the design, management, and analysis of agronomic research experiments. Michigan State Univ., East Lansing, MI

Nageswara Rao, R.C., Wright, G.C. (1994): Stability of the relationship between specific leaf area and carbon isotope discrimination across environments in peanut. Crop Sci. **34**, 98-103

Nageswara Rao, R.C., Udaykumar, M., Farquhar, G.D., Talwar, H.S., Prasad, T.G. (1995): Variation in carbon isotope discrimination and its relationship to specific leaf area and ribulose-1, 5-bisphosphate carboxylase content in groundnut genotypes. Aust J Plant Physiol. **22**, 545-551

Nakano, H., Muramatsu, S., Makino, A., Mae, T. (2002): Relationship between the suppression of photosynthesis and starch accumulation in the pod-removed bean. Aust J Plant Physiol. **27(2)**, 167-173

Nayyar, H., Kaur, S., Smita, K., Singh, J., Dhir, K. K., Bains, T. (2005): Water stress-

induced injury to reproductive phase in chickpea: evaluation of stress sensitivity in wild and cultivated species in relation to abscisic acid and polyamines. J Agron Crop Sci. **191**, 450-457

Nayyar, H., Walia, D. P. (2004): Genotypic variation in wheat in response to water stress and abscisic acid induced accumulation of osmolytes in developing grains. J Agron Crop Sci. **190**, 39-45

Nayyar, H., Bains, T., Kumar, S. (2004): Low temperature induced floral abortion in chickpea: relationship to abscisic acid and cryoprotectants in reproductive organs. Environ Exp Bot. **53**, 39-47

Nelson, C.J. (1988): Genetic association between photosynthetic characteristics and yield: review of evidence. Plant Physiol Biochem. **26,** 543-554

Neslihan-Ozturk, Z. Talam´e1,V., Deyholos, M., Michalowski, C. B. Galbraith, D.W. Gozukirmizi, N., Tuberosa, R., Bohnert, H. J. (2002): Monitoring large-scale changes in transcript abundance in drought- and salt-stressed barley. Plant Mol Biol. **48**, 551-573

Nicolas, M.E., Gleadow, R.M., Dalling, M.J. (1985): Effect of post-anthesis drought on cell division and starch accumulation in developing wheat grains. Ann Bot. **55**, 433-444

Niu, X., Bressan, R.A., Hasegawa, P.M., Pardo, J.M. (1995): Ion homeostasis in NaCl stress environments. Plant Physiol. **109**, 735-42

Norman, M.J.T. Pearson, C.J., Searle, P.G.E. (1995): The ecology of tropical food crops. Cambridge University Press, Cambridge. pp. 208-224

Nunez Barrios, A., Hoogenboom, G., Nesmith, D.S. (2005): Drought stress and the distribution of vegetative and reproductive traits of a bean cultivar. Sci. agric. (Piracicaba, Braz.) **62 (1)**, 18-22

Ober, E. S., Bloa, M.L. Clark, C. J. A. , Royal, A., Jaggard, K.W., Pidgeon, J. D. (2005): Evaluation of physiological traits as indirect selection criteria for drought tolerance in sugar beet. Field Crops Res. **91(2)**, 231-249

Ober, E.S., Setter, T.L. (1992): Water deficit induces abscisic acid accumulation in

endosperm of maize viviparous mutants. Plant Physiol. **98**, 353-356

Osório, M.L., Breia, E., Rodrigues, A., Osório, J., Le Roux, X., Daudet, F.A., Ferreira, I., Chaves, M.M. (2006): Limitations to carbon assimilation by mild drought in nectarine trees growing under field conditions. Environ Exp Bot. **55(3)**, 235-247

Pantuwan, G., Fukai, S., Cooper, M., Rajatasereekul, S., O`Toole, J.C., (2002): Yield response of rice (*Oryza sativa*, L.) genotypes to drought under rainfed lowlands. 1. Grain yield and yield components. Field Crops Res. **73**, 153-168

Pantuwan, G., Fukai, S., Cooper, M., Rajatasereekul, S., O'Toole, J.C., Basnayake, J. (2004): Yield response of rice (*Oryza sativa* L.) genotypes to drought under rainfed lowlands 4. Vegetative stage screening in the dry season. Field Crops Res. **89**, 281-297

Passioura J.B. (2002): Environmental biology and crop improvement. Funct Plant Biol. **29**, 537-546

Pastenes, C., Pimentel, P., Lillo, J. (2005): Leaf movements and photoinhibition in relation to water stress in field-grown beans. J Exp Bot. **56(411)**, 425-433

Patakas, A., Noitsakis, B. (2001): Leaf age effects on solute accumulation in water-stressed grapevines. J Plant Physiol. **158(1),** 63-69

Paul, M.J., Foyer, C.H. (2001): Sink regulation of photosynthesis. J Exp Bot. **52**, 1383-1400

Pelleschi, P., Rocher, J.P., Prioul, J.L. (1997): Effect of water restriction on carbohydrate metabolism and photosynthesis in mature maize leaves. Plant Cell Environ. **20**, 493-503

Peña-Rojas, K., Aranda, X., Joffre, R., Fleck, I. (2005): Leaf morphology, photochemistry and water status changes in resprouting *Quercus ilex* during drought. Funct Plant Biol. **32(2),** 117-130

Pinheiro, C., Rodrigues, A.P., de Carvalho, I.S., Chaves, M.M., Ricardo, C.P. (2005): Sugar metabolism in developing lupin seeds is affected by a short-term water deficit. J Exp Bot. **56(420)**, 2705-2712

Poorter, H. (1989): Plant growth analysis: towards a synthesis of the classical and the

functional approach. Physiol Plant. **75**, 237-244

Ramirez-Vallejo, P., Kelly, J.M. (1998): Traits related to drought resistance in common bean. Euphytica. **99**, 127-136

Rathert, G. (1984): Sucrose and starch content of plant parts as a possible indicator for salt tolerance of crops. Aust J Plant Physiol. **11**, 491-495

Riccardi, F., Gazeau, P. de Vienne, D., Zivy, M. (1998): Protein changes in response to progressive water deficit in maize. Quantitative variation and polypeptide identification. Plant Physiol. **117**, 1253-1263

Rodrigues, M.L., Pacheco, C.M.A., Chaves, M.M. (1995): Soil–plant water relations, root distribution and biomass partitioning in *Lupinus albus* L. under drought conditions. J Exp Bot. **46**, 947-956

Rodríguez, M., Canales, E., Borroto, C.J., Carmona, E., López, J., Pujol, M., Borrás-Hidalgo, O. (2006): Identification of genes induced upon water-deficit stress in a drought-tolerant rice cultivar. J Plant Physiol. **163(5)**, 577-584

Rosales-Serna, R., Kohashi-Shibata, J., Acosta-Gallegos, J.A., Trejo-López, C., Ortiz-Cereceres, J., Kelly, J.D. (2002): Yield and phenological adjustment in four drought-stressed common bean cultivars. Ann Rep Bean Improv Coop. **45**, 198-199

Ruiz-Sanchez, M.C., Domingo, R., Torrecillas, A., Perez-Pastor, A. (2000): Water stress preconditioning to improve drought resistance in young apricot plants. Plant Sci. **156**, 245-251

Ryser, P., Wahl, S. (2001): Interspecific variation in RGR and the underlying traits among 24 grass species grown in full daylight. Plant Biol **3**, 426-436

Salekdeh, G.H., Siopongco, J., Wade, L.J., Ghareyazie, B., Bennett, J. (2002): Proteomic analysis of rice leaves during drought stress and recovery. Proteomics. **2(9)**, 1131-45

Santisopasri, V., Kurotjanawong, K., Chotineeranat, S., Piyachomkwan, K., Sriroth, K., Oates, C.G. (2001): Impact of water stress on yield and quality of cassava starch. Ind Crops Prod. **13,** 115-129

Saranga, Y., Flash, I., Yakir, D. (1998): Variation in water-use efficiency and its relation to carbon isotope ratio in cotton. Crop Sci. **38 (3)**, 782-787

Savitch, L.V., Harney, T., Huner, N.P. A. (2000): Sucrose metabolism in spring and winter wheat in response to high irradiance, cold stress and cold acclimation. Physiol Plant. **108** (3), 270-278

Saxena, C.M., Silim, S.N., Singh, B.K. (1990): Effect of supplementary irrigation during reproductive growth on winter and spring chickpea (*Cicer arietinum*) in a Mediterranean environment. J Agric Sci. **114**, 285-293

Scartazza, A., Proietti, S., Moscatello, S., Augusti, A., Monteverdi, M.C., Brugnoli, E., Battistelli, A. (2001): Effect of water shortage on photosynthesis, growth and storage carbohydrate accumulation in walnut *(Juglans regia* L.*)*. Acta Hort. (ISHS) **544**, 227-232

Schneider, K.A., Rosales-Serna, R., Ibarra-Perez, F.J., Cazares-Enriquez, B., Acosta Gallegos, J.A., Ramirez-Vallejo, P., Wassimi, N., Kelly, J.D. (1997): Improving common bean performance under drought stress. Crop Sci. **37**, 43-50

Scholander, P.F., Hammel, H.T., Bradstreet, E.D., Hemmingsen, E.A. (1965): Sap pressure in vascular plants. Science. **148**, 339-346

Schubert, S., Serraj, R., Plies-Balzer, E., Mengel, K. (1995): Effect of drought stress on growth, sugar concentrations and amino acid accumulation in N_2-fixing alfalfa (*Medicago sativa*). J Plant Physiol. **146,** 541-546

Schulze, E.D. (1986): Carbon dioxide and water vapour exchange in response to drought in the atmosphere and in the soil. Annu Rev Plant Physiol. **37**, 247-274

Schussler, J.R., Westgate, M.E. (1995): Assimilate flux determines kernel set at low water potential in maize. Crop Sci. **35**, 1196-1203

Schussler, J.R., Westgate, M.E. (1991): Maize kernel set at low water potential. II. Sensitivity to reduced assimilates at pollination. Crop Sci. **31**, 1196-1203

Scotti Campos, P., Ramalho, J.C., Lauriano, J.A., Silva, M.J., do Ceu Matos, M. (1999): Effects of drought on photosynthetic performance and water relations of four *Vigna* genotypes. Photosynthetica. **36(1-2),** 79-87

Setter, T.L., Flannigan, B.A. Melkonian, J. (2001): Loss of kernel set due to water deficit and shade in maize: carbohydrate supplies, abscisic acid, and cytokinins. Crop Sci. **41**, 1530-1540

Sharkey, T.D., Berry, J.A., Raschke, K. (1985): Starch and sucrose synthesis in *Phaseolus vulgaris* as affected by light, CO_2 and abscisic acid. Plant Physiol. **77**, 617-620

Shen, L., Courtois, B., McNally, K.L., Robin, S., Li, Z. (2001): Evaluation of near-isogenic lines of rice introgressed with QTLs for root depth through marker-aided selection. Theor Appl Genet. **103**, 75-83

Shenkut, A. A., Brick, M.A. (2003): Traits associated with dry edible bean (*Phaseolus vulgaris* L.) productivity under diverse soil moisture environments. Euphytica. **133 (3)**, 339-347

Sheoran, I.S., Saini, H.S. (1996): Drought-induced male sterility in rice: changes in carbohydrate levels and enzyme activities associated with the inhibition of starch accumulation in pollen. Sex Plant Reprod. **9**, 161-169

Sibounheuang, V. , Basnayake, J., Fukai, S. (2001): Genotypic consistency in the expression of leaf water potential in rice (*Oryza sativa* L.). Field Crops Res. **97(2-3),** 142-154

Siddique, K.H.M., Regan, L.K., Tennant, D., Thompson, B.D. (2001): Water use and water use efficiency of cool season grain legumes in low rainfall Mediterranean-type environments. Eur J Agron. **15**, 267-280

Siddique, K.H.M., Tennant, D. M., Perry, W., Belford, R.K. (1990): Water-use and water-use efficiency of old and modern wheat cultivars in a Mediterranean-type environment. Aust J Agric Res. **41**, 431-447

Siddique, M.R.B., Hamid, A., Islam, M.S. (2000): Drought stress effects on water relations of wheat. Bot Bull Acad Sin. **41**, 35-39

Sinclair, T.R. and M.M. Ludlow. 1985. Who taught plants thermodynamics? The unfulfilled potential of plant water potential. Aust J Plant Physiol. **33**, 213-217

Singh, S.P. (2001): Broadening the genetic base of common bean cultivars. Crop Sci. **41**,

1659-1675

Singh, S.P. (1995): Selection for water stress tolerance in interracial populations of common bean. Crop Sci. **35**, 118-124

Singh, S.P. (1982): A key for identification of different growth habits of *Phaseolus vulgaris* L. Annu Rep Bean Improv. Coop. **25**, 92-94

Slafer, G. A., Satorre, E. H., Andrade, F. H. (1994): Increases in grain yield in bread wheat from breeding and associated physiological changes. *In*: Slafer, G.A. (ed.). Genetic Improvement of Field Crops. Marcel Dekker Inc, New York, pp. 1-68

Smirnoff, N., Cumbes, Q.J. (1989): Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry. **28**, 1057-1060

Socías, F.X., Correia, M.J., Chaves, M.M., Medrano, H. (1997): The role of abscisic acid and water relations in drought responses of subterranean clover. J Exp Bot. **48**, 1281-1288

Subrahmanyam, D. (2002): Interrelationship between leaf gas-exchange characteristics, area leaf mass, and yield in soybean (*Glycine max* L. Merr) genotypes. Photosynthetica. **40(3)**, 445-447

Tanaka, A., Fujita, K. (1979): Photosynthesis and yield components in relation to grain yield of the field beans. Journal of the Faculty of Agriculture. Hokkaido University. **59**, 145-238

Terán, H., Singh, S. (2002): Comparison of sources and lines selected for drought resistance in common bean. Crop Sci. **42**, 64-70

Thumma, B.R., Naidu, B.P., Chandra, A., Cameron, D.F., Bahnisch, L.M., Liu, C. (2001): Identification of causal relationships among traits related to drought resistance in *Stylosanthes scabra* using QTL analysis. J Exp Bot. **52(355)**: 203-214

Thung, M., Rao, I.M. (1999): Integrated management of abiotic stresses. *In*: Singh, S.P. (ed). Common Bean Improvement in the Twenty-First Century. Dordrecht: Kluwer Academic Publishers. pp. 331-370

Turner, N.C. (1997): Further progress in crop water relations. Adv Agron. **58**, 293-338

Turner, N. C. (1986): Crop water deficits: a decade of progress. Adv Agron. **39**, 1-51

Turner, N. C. (1982): The role of shoot characteristics in drought resistance of crop plants. *In*: Drought Resistance in Crops with Emphasis on Rice, Los Banos, Leguna, Philippines: IRRI pp. 115-134

Turtola, S., Rousi, M., Pusenius, J., Yamaji, K., Heiska, S., Tirkkonen, V., Meier, B., Julkunen-Tiitto, R. (2006): Genotypic variation in drought response of willows grown under ambient and enhanced UV-B radiation. Environ Exp Bot. **56(1)**, 80-86

Udayakumar, M., Sheshshayee, M.S., Nataraj, K.N., Madhava, H. B., Devendra, R., Aftab Hussain, I.S., Prasad, T.G. (1998): Why has breeding for water-use efficiency not been successful? An analysis and alternate approach to exploit this trait for crop improvement. Current Sci. **74**, 994 -1000

Upadhyaya, H.D. (2005): Variability for drought resistance related traits in the mini core collection of peanut. Crop Sci. **45**, 1432-1440

Van den Boogaard, R., Alewijnse, D., Veneklaas, E. J., Lambers, H. (1997): Growth and water-use efficiency of 10 *Triticum aestivum* cultivars at different water availability in relation to allocation of biomass. Plant Cell Environ. **20**(2), 200-210

Vassey, T.L., Sharkey, T.D. (1989): Mild water stress of *Phaseolus vulgaris* plants leads to reduced starch synthesis and extractable sucrose phosphate synthase activity. Plant Physiol. **89,** 1066-1070

Wang, Z., Mambelli, S., Setter, T.L. (2002): Abscisic acid catabolism in maize kernels in response to water deficit at early endosperm development. Ann Bot. **90**, 623-630

Wang, T.L., Hedley, C.L. (1993): Genetic and developmental analysis of the seed. *In* Casey, R., Davies, D.R. (Eds.), Peas: Genetics, Molecular Biology and Biochemistry. Wallingford, UK. CAB International. pp. 83-120

Wardlaw, I.F. (2002): Interaction between drought and chronic high temperature during kernel filling in wheat in a controlled environment. Ann Bot. **90**, 469-476

Weber, H., Borisjuk, L., Wobus, U. (2005): Molecular physiology of legume seed development. Ann Rev Plant Biol. **56,** 253-279

Wentworth, M., Murchie, E.H., Gray, J.E., Villegas, D., Pastenes, C., Pinto, M., Horton,

P. (2006): Differential adaptation of two varieties of common bean to abiotic stress: II. Acclimation of photosynthesis. J Exp Bot. **57(3)**, 699-709

Wessel-Beaver, L., Beck, R. H., Lambert, R. J.(1984): Rapid method for measuring kernel density. Agron J. **76**, 307-309

Westermeier, R., Naven, T. (2002): Proteomics in Practice, A laboratory manual of proteome analysis, Amersham Pharmacia Biotech, Wiley-VHC, Little Chalfont, UK

Westgate, M.E., Peterson, C.M. (1993): Flower and pod development in water-deficient soybeans (*Glycine maxs* L. Merr). J Exp Bot. **44**, 109-117

Westgate, M.E., Thomson Grant, G.L. (1989): Water deficits and reproduction in maize. Responses of the reproductive tissue to water deficits at anthesis and mid-grain fill. Plant Physiol. **91**, 862-867

White, J.W., Singh, S.P. (1991): Breeding for adaptation to drought. *In*: van Schoonhoven, A., Voysest, O. (Eds.). Common Beans: Research for Crop Improvement. CAB Int CIAT, Colombia. pp. 501-560

White, J.W., Castillo, J.A. (1989): Relative effect of root and shoot genotypes on yield of common bean under drought stress. Crop Sci. **29**, 360-362

White, J.W., Castillo, J.A., Ehleringer , J. (1990): Associations between productivity, root growth and carbon isotope discrimination in *Phaseolus vulgaris* under water deficit. Aust J Plant Physiol. **17**, 189-198

Wood, A.J., and P.B. Goldsbrough. 1997. Characterization and expression of dehydrins in water-stressed *Sorghum bicolor*. Physiol Plant. **99**, 144-152

Xue, Q., Zhu, Z., Musick, J.T., Stewart, B.A., Dusek, D.A. (2006): Physiological mechanisms contributing to the increased water-use efficiency in winter wheat under deficit irrigation. J Plant Physiol. **163(2)**, 154-164

Zanella, F., Watanabe, T.M., Lima, A.L.S., Schiavinato, M.A. (2004): Photosynthetic performance in jack bean (*Canavalia ensiformis* L.) under drought and after rehydration. Braz J Plant Physiol. **16(3)**, 181-184

Zhang, X., Wu, N., Li, C. (2005): Physiological and growth responses of *Populus davidiana* ecotypes to different soil water contents. J Arid Environ. **60(4)**, 567-579

Zhu, J.K. (2002): Salt and drought stress signal transduction in plants. Annu Rev Plant Biol. **53**, 247-273

Zinselmeier, C. (1991): The role of assimilate supply, partitioning and metabolism in maize kernel development at low water potentials. Ph.D. Thesis, University of Minnesota, St. Paul.

Zinselmeier, C., Jeong, B.R., Boyer, J.S. (1999): Starch and the control of kernel number in maize at low water potentials. Plant Physiol. **121**, 25-35

Zinselmeier, C., Schussler, J.R., Westgate, M.E., Jones, R.J. (1995): Low water potential disrupts carbohydrate metabolism in maize ovaries. Plant Physiol. **107**, 385-391

Zörb, C., Schmitt, S., Neeb, A., Karl, S., Linder, M., Schubert, S. (2004): The biochemical reaction of maize (*Zea mays* L.) to salt stress is characterized by a mitigation of symptoms and not by a specific adaptation. Plant Sci. **167**, 91-100

9. APPENDIX

CURRICULUM VITAE

Personal data

Name	Setegn Gebeyehu Endire
Date of birth	09.01.1971
Place of birth	Guduru (Oromia), Ethiopia
Sex	Male
Marital status	Married, two children
E-mail:	setegng@yahoo.co.uk

Education

Since April 2003	Doctoral study at the Justus-Liebig University of Giessen, Germany
1995/96 - 1998	MSc in Agriculture (Agronomy) at Alemaya University, Ethiopia
1987/88 - 1991	BSc in Agriculture (Plant Sciences) at Alemaya University, Ethiopia
1983/84 - 1987	Secondary School at Ambo Comprehensive Senior Secondary School, Ambo, Ethiopia
1976/77- 1983	Gebete Wolkite Gudena Primary School, Guduru (Oromia), Ethiopia

Career after graduation

	Duration (Month and Year)		
Position	**From**	**To**	**Field of Work**
Junior Researcher	February 1992	March 1995	Crop Improvement (small cereals)
Assistant Researcher	March 1995	August 1998	Crop Improvement (lowland pulses)
Lecturer	September 1998	December 2000	Agronomy
Associate Researcher	December 2000	September 2002	Bean Breeding & Genetics

DECLARATION

I hereby declare that the Ph.D. thesis entitled “Physiological response to drought stress of common bean (*Phaseolus vulgaris* L.) genotypes differing in drought resistance” submitted to the University of Giessen, Germany, is an independent work carried out by me and the thesis has not formed previously the basis for the award of any degree.

Sincerely,

Setegn Gebeyehu Endire