

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 ($\Delta^{13}\text{C}$) (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 \times 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 ($\Delta^{13}\text{C}$), 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 ($\Delta^{13}\text{C}$) technique (Ehleringer et al., 1990). Also, positive associations between $\Delta^{13}\text{C}$ 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 CO₂ 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