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DROUGHT ADAPTATION IN COMMON BEAN (Phaseolus vulgaris L.)

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DROUGHT ADAPTATION IN COMMON BEAN (*Phaseolus vulgaris* L.)

By

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ABSTRACT

Drought Adaptation in Common Bean (*Phaseolus vulgaris* L.)

BY

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Increasing common bean (*Phaseolus vulgaris* L.) yields in drought prone environments requires a screening tool to identify resistant genotypes other than yield differences at harvest time. Both carbon isotope discrimination (CID) and osmotic adjustment (OA) have been suggested as screening criteria. However, the relationships of CID and OA to yield and its determinants (water use efficiency (WUE), harvest index (HI), and water use) under stress have not been elucidated. Two resistant and one susceptible genotypes were subjected to a vegetative water stress from 5-leaf stage (V5) to first flower (R1) and to reproductive water stress from R1 to harvest from 1991 to 1993. Seed yield varied from 28 to 352 g m^{-2}. Vegetative stress reduced yield by 15 to 30% while reproductive stress reduced yield by 35 to 50 % with one of the resistant genotypes producing a significantly greater yield than the susceptible genotype under reproductive stress. Reproductive stress significantly reduced CID by 9%. The drought susceptible genotype had 8 to 9% higher CID than the resistant genotypes under all water regimes. Similarly, the drought susceptible genotype had a higher level of osmotic adjustment under the reproductive stress. Relative reductions in biomass and water use efficiency strongly and positively correlated with relative CID reductions in both stress treatments. CID strongly and positively correlated with pod m^{-2} (r^2 =0.81 $p<0.01$ n=9). In addition, osmotic adjustment (r^2 =0.91 $p<0.01$ n=6) strongly and negatively correlated with yield as well as with yield determinants under the reproductive stress. It is concluded that both carbon isotope

discrimination and osmotic adjustment could be useful screening criteria for selections in drought resistance studies but need further verification with more diverse genotypes.

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important protein source worldwide and its consumption in the United States has been increasing recently (Holden and Haytowitz, 1998). Drought is the major constraint to bean production after diseases (White et al., 1990; Laing et al., 1984; Acosta-Gallegos et al.,1998).

The development of drought tolerant genotypes has been hampered by the lack of screening techniques that easily distinguish between tolerant and susceptible genotypes. Breeding for drought resistance in drought prone environments requires the existence of heritable genotypic variation as well as a screening trait that is visible to the breeder (Clarke and Townley-Smith, 1984). Obviously, such a trait must be directly linked to yield or to one of the yield determining factors. Yield determining factors such as water use or water use efficiency were reported (Doorenbos and Kassam, 1979; Stanhill, 1986; Menéndez and Hall, 1996; Barros and Hanks, 1993) and a multiplicative identity was proposed (Passioura, 1994) for this relationship as follows:

Yield=water transpired x water-use efficiency x harvest index

Field measurements of these factors usually take a whole season are not finalized before harvest. On the other hand, physiological or morphological traits can be observed early in the season. Besides, any trait that could be directly linked to any of the yield determinants could also be a useful screening tool for yield under drought. Such a relationship has, indeed, been suggested for traits like carbon isotope discrimination (Δ) and osmotic adjustment among others (Ludlow and Muchow, 1990; Ehleringer et al., 1991).

Seemann and Critchley (1985) reported that common beans do follow the

theoretically expected relationship between Δ and intercellular carbon dioxide concentration as proposed by Farquhar et al. (1982). In addition, genotypic differences among common bean cultivars for Δ have been reported (White et al., 1990; Ehleringer et al., 1991). However, results linking Δ to yield or to yield determinants under drought in common bean were not conclusive (White et al., 1990).

Similarly, there is no consensus on the relationship of osmotic adjustment to yield under drought (Morgan et al., 1991; Cortes and Sinclair, 1986; Li et al., 1993; Guei and Wassom, 1993; Kumar and Singh, 1998; Blum, 1989; Rodriguez et al., 1992) with some studies suggesting that osmotic adjustment may have a physiological cost to the plants and may result in yield reduction (Munns, 1988; Li et al., 1993). Compared to other crops, few studies reported osmotic adjustment for common bean (Parsons and Howe, 1984; Wullschleger and Oosterhuis, 1991; Raggi, 1994), and all three studies were conducted in a controlled environment. Osmotic adjustment of field grown common bean is not known.

This study investigated the effect of an intermittent (vegetative) and a terminal(reproductive) water stress on a drought susceptible (Seafarer) and two drought resistant bean genotypes (BAT 477 and LEF2RB).

The objectives of the study were to examine if relationships of water use efficiency, water use, and harvest index to yield in beans could be explained by the multiplicative yield model. Other main objectives included examining if beans osmotically adjust when field grown and the elucidation of the relationships of osmotic adjustment and carbon isotope discrimination to yield, root depth and to yield determinants such as water use efficiency and harvest index.

REFERENCES

Acosta-Gallegos, J.A., E. Acosta-Diaz, S. Padilla-Ramirez, E. Lopez-Salinas, R.A. Salinas-Perez, N. Mayek-Perez, and J.D. Kelly. 1998. Seed yield of dry bean cultivars under drought stress. Bean Improvement Cooperative 41:151-152.

Barros, L. C. G., and R. J. Hanks. 1993. Evapotranspiration and yield of beans as affected by mulch and irrigation. Agron. J. 85:692-697

Blum, A. 1989. Osmotic adjustment and growth of genotypes under drought. Crop Science 29:230-233.

Clarke, J. M., and T. F. Townley-Smith. 1984. Screening and selection techniques for improving drought resistance. In: P. B. Vose and S. G. Blixt. Crop breeding: a contemporary basis. Pergamon Press, New York.

Cortes, P.M. and T.R. Sinclair. 1986. Water relations of field -grown soybean under drought. Crop Science 26:993-998.

Doorenbos, J.and A. H. Kassam. 1979. Yield response to water. FAO Irrigation and Drainage Paper no. 33, Rome.

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

Farquhar, G. D., M. H. O'Leary, and J. A. Berry. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Aust. J. Plant Physiol 9:121-137.

Guei, R.G and C.E. Wassom. 1993. Genetics of osmotic adjustment in breeding maize for drought tolerance. Heredity 71(4):436-441.

Holden, J. and D. Haytowitz. 1998. The nutritional value of beans. Bean Improvement Cooperative 41:41-44.

Kumar, A and D.P. Singh. 1998. Use of physiological indices as a screening technique for drought tolerance in oil seed brassica species. Ann. Bot. 81(3):413-420.

Laing, D. R., P. G. Jones, and J. H. C. Davis. 1984. Common Bean (Phaseolus vulgaris L.). In: P. R. Goldsworthy and N. M. Fisher. The physiology of tropical field crops. John Wiley & Sons, New York.

Li, X., Y. Feng and L. Boersma. 1993. Comparison of osmotic adjustment responses to water and temperature stresses in spring wheat and sudangrass. Ann. Bot.

71(4):303-310.

Ludlow, M. M., and R. C. Muchow. 1990. A critical evaluation of traits for improving crop yields in water-limited environments. Adv. Agron. 43:107-153.

Menéndez, C., and A. E. Hall. 1996. Heritability of carbon-isotope discrimination and correllations with harvest index in cowpea. Crop Sci. 36:233-238.

Morgan, J.M., B. Rodriguez-Maribona, and E.J. Knights. 1991. Adaptation to water-deficit in chickpea breeding lines by osmoregulation:relationship to grain-yields in the field. Field Crops Research 27:61-70.

Munns, R. 1988. Why measure osmotic adjustment?. Australian Journal Plant Physiology 15:717-726.

Parsons, L. R and T. K. Howe. 1984. Effects of water stress on the water relations of *Phaseolus vulgaris* and the drought resistant *Phaseolus acutifolius*. Physiologia Plantarum 60:197-202.

Passioura, J.B. 1994. The yield of crops in relation to drought. In: K. J. Boote (ed.). Physiology and determination of crop yield. ASA-CSSA-SSSA, Madison, Wisconsin.

Raggi, V. 1994. Changes in free amino acids and osmotic adjustment in leaves of water-stressed bean. Physiologia Plantarum 91:427-434.

Rodriguez-Maribona, B., J.L. Tenorio, J.R. Conde and L. Ayerbe. 1992. Correlations between yield and osmotic adjustment of peas (Pisum sativum L.) Under drought stress. Field Crop Research 29:15-22.

Seemann, J. R., and C. Critchley. 1985. Effects of salt stress on the growth, ion content, stomatal behavior and photosynthetic capacity of a salt-sensitive species, Phaseolus vulgaris L. Planta 164:151-62.

Stanhill, G. 1986. Water use efficiency. Adv. Agron. 39:53-83.

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

Wullschleger, S. D. and D. M. Oosterhuis. 1991. Osmotic adjustment and growth response of seven vegetable crops following water-deficit stress. HortScience 26(90:1210-1212.

CHAPTER 1

LITERATURE REVIEW

Bean Production and Its Importance

The highest population growth rates are found in Sub-Saharan Africa and Latin America (World Bank, 1992), two major bean (*Phaseolus vulgaris* L.) growing areas. The bean crop is important not only as a source of carbohydrate to this fast growing population but may be the only protein supplier in the diet of subsistence farmers. In addition, bean consumption in the developed countries is increasing (Holden and Haytowwitz, 1998) as people switch to vegetable protein for health reasons.

Bean yields have been disappointingly low, averaging less than 1 ton ha^{-1} in developing countries to 2 tons ha^{-1} in developed countries (Laing et al., 1984; Adams, 1996). Yet yields of 2.19 to 4.12 t ha^{-1} are reported from experiment stations indicating the enormous gap between the potential and actual production for this crop. The major constraint to bean production after diseases and other pests is drought. This occurs because almost all bean production is on dryland farming systems with frequent drought stress affecting more than 60 percent of the bean producing areas world-wide (White et al., 1990; Laing et al., 1984). The detrimental effect of drought stress on beans growing in semi-arid agroecological zones is known but bean yields are also reduced by intermittent drought stress in humid zones (Acosta-Gallegos et al., 1998).

The Challenge of Drought

This important problem of drought stress for food crops in general and for beans in particular has been examined at different levels of organization from cellular to whole plant. Agronomists usually try to change the environment while plant breeders focus on

adapting the plant to the particular (in this case water-limited) environment. Crop physiologists, on the other hand, try to elucidate the mechanisms of interaction between the plant and its environment, thus working with both.

The effect of drought stress on plant growth and survival has been measured and documented since the beginning of this century (Briggs and Shantz, 1914). More important for yield improvement, however, is the existence of an intraspecific variation in water use efficiency (Standhill, 1986).

Drought and drought resistance

Quantifying the notion of stress and drought resistance in crop plants has not been easy and is best considered as a relative rating of response compared to an optimally potential response under the same soil, weather and management conditions (Rana, 1986). Thus, drought resistance is defined as the ability of a genotype to reduce loss of yield resulting from a quantified level of moisture deficit relative to the maximum yield when there is no water deficit (Sinha, 1986; Doorenbos and Kassam, 1979). Based on this definition drought can then be quantified as relative reduction in evapotranspiration while resistance to drought resistance can be estimated as the relative yield reduction by the drought. However, since genotypes do not respond equally to the same quantity of drought researchers classify genotypes as follows (Fernandez, 1993):
High yielding resistant, high yielding susceptible, low yielding resistant, and low yielding susceptible.

Bean sensitivity to drought

There seems to be a consensus in the literature on the sensitivity of beans to water deficit (Laing et al., 1984; Halterlein, 1983; Ehleringer et al., 1991; Nielsen and Nelson,

1998). The available soil water content should not drop below 50 percent for optimum growth of beans (Vittum, and Gibbs, 1961; Maurer et al., 1969; Stegman and Olsen, 197; Miller and Burke, 1983). However, all growth stages are not equally affected by moisture deficit.

There are conflicting reports about the effect of water deficit during the vegetative period (Robins and Domingo, 1956; MacKay and Eaves, 1962; Dubetz and Mahalle, 1969; Walker and Hatfield, 1979; Acosta-Gallegos and Shibata, 1989; Castellanos et al., 1996). Moisture deficits at this growth stage seem to have only a small effect on yield (Robins and Domingo, 1956; Dubetz and Mahalle, 1969; Walker and Hatfield, 1979). In contrast, moisture deficits are always detrimental when they occur during the reproductive growth stages (Stoker, 1974; Walton et al., 1977; Acosta-Gallegos and Shibata, 1989; Castellanos et al.,1996; Nielsen and Nelson, 1998).

Breeding beans for drought resistance

It has not been easy to breed drought resistant genotypes for semi-arid environments based solely on grain yields. Plant traits that confer drought resistance are expressed from year to year because of the extreme variability in the rainfall pattern of these environments. Moreover, the genotypic variance of grain yield is low in such environments (Ludlow and Muchow, 1990). Breeders need both the existence of heritable genotypic variation and a screening tool to select for the desirable traits (Clarke and Townley-Smith, 1984). Considerable genotypic variation in drought resistance among common bean is does exist (CIAT, 1979; Singh, 1995). Most resistance is available in the small seeded black, cream, or coffee colored types. The lack of a suitable screening criterion seems to be the missing part of the puzzle.

Physiological and Morphological Traits

Selections are based on theoretical concepts, or correlations that may not be necessarily indicative of causal relationships (Ludlow and Muchow, 1990). In addition, selections rely on experiments conducted in controlled environments rather than field conditions. These authors suggested that it is critical to know if and how a particular trait contributes to yield.

Some of the traits proposed for crop improvement in drought prone environments were reviewed in detail (Clarke and Townley-Smith, 1984; Turner, 1986; Schulze, 1986; Ludlow and Muchow, 1990). Ludlow and Muchow (1990) in particular listed about seventeen traits that were carefully selected from prior research and based on empirical data. Each of these traits was then subjected to a set of criteria that included whether the trait has any demonstrated contribution to yield.

These reviews indicated a number of gaps in our knowledge about drought stress resistance in crop plants as well as opportunities for future progress based on existing conceptual models. The role of thermodynamically based concepts such as plant water potential to assess drought stress has been questioned (Sinclair and Ludlow, 1985; Passioura, 1988; Kramer, 1988).

CONCEPTUAL MODELS

The Yield Determinants Model

The first model for the yield component analysis was proposed by Passioura (1977) and considers grain yield in drought prone areas to be the product of three factors: the total water transpired (WU=total water use - evaporation), the Water Use Efficiency (WUE)=grain yield /water transpired), and the Harvest Index (HI=grain yield/total above

ground biomass). A trait that increases any one of these factors will contribute to yield as demanded by this multiplicative model. This model is applicable to determinate species but its usefulness to indeterminate or semi-determinate crops like beans is not clear.

Mechanisms of drought Resistance

The second conceptual model used by Ludlow and Muchow (1990) is based on Levitt (1980) and classifies the response of plants to drought stress. There are many such classifications that do not always agree (May and Milthorpe, 1962; Levitt, 1980; Turner, 1986). Generally, two plant responses are recognized.

Drought escape occurs when plants evade the drought period by having a short life span that ends before a terminal drought that comes at the end of the season or having developmental plasticity to hasten life cycle in the event of sensing a mid season drought.

Drought resistance occurs when the plants actually experience the drought but respond to it such that it does not adversely affect them. In this model (Levitt, 1980) drought resistance can be divided into drought avoidance and drought tolerance.

Drought Avoiders

Drought avoidance occurs when plants maintain high tissue water status by either saving water or by taking up more water to meet the evaporative demand. Water saving avoiders may close their stomates or activate leaf rolling, movement, or leaf senescence. Water spending avoiders, on the other hand, may grow more roots to absorb water from deeper profiles in the soil.

Drought avoiders also change tissue characteristics to maintain turgor. This happens through osmotic adjustment or by increasing tissue elasticity or by increasing the bound water fraction (Radin, 1983).

Drought Tolerators

Drought tolerance exists when the plant can cope with drought without maintaining high tissue water status. This can occur in such cases as plants having protoplasmic tolerance.

Screening for drought resistance in beans

The research reported in the following sections is based on the above working models. An attempt was made to study the most promising physiological and morphological traits together in one experiment.

REFERENCES

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

Acosta-Gallegos, J.A., E. Acosta-Diaz, S. Padilla-Ramirez, E. Lopez-Salinas, R.A. Salinas-Perez, N. Mayek-Perez, and J.D. Kelly. 1998. Seed yield of dry bean cultivars under drought stress. Bean Improvement Cooperative 41:151-152.

Adams, M. W. 1996. An historical perspective on significant accomplishments in dry bean research- the first 100 years. Bean Improvement Cooperative 39:32-45.

Briggs, L. J., and H. L. Shantz. 1914. Relative water requirement of plants. J. Agric. Research 3:1-63.

Castellanos, J. Z., J. J. Peña-Cabriales, and J. A. Acosta-gallegos. 1996. ^{15}N-determined dinitrogen fixation capacity of common bean (*Phaseolus vulgaris*) cultivars under water stress. J. Agric. Sci. 126:327-333.

Clarke, J. M., and T. F. Townley-Smith. 1984. Screening and selection techniques for improving drought resistance. In: P. B. Vose and S. G. Blixt. Crop breeding: a contemporary basis. Pergamon Press, New York.

CIAT. 1979. Annual report. Bean program. pp. C13 - C16, CIAT, Cali, Colombia.

Doorenbos, J.and A. H. Kassam. 1979. Yield response to water. FAO Irrigation and Drainage Paper no. 33, Rome.

Dubetz, S., and P. S. Mahalle. 1969. Effect of soil water stress on bush beans Phaseolus vulgaris L. at three stages of growth. J. Am. Soc. Hort. Sci. 94:479-481.

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

Fernandez, G.C.J. 1993. Effective selection criteria for assessing plant stress tolerance. In: C. G. Kuo (ed.) Adaptation of food crops to temperature and water stress. Proc. International Symposium, Taiwan 13-18 August 1992, AVRDC.

Halterlein, A. J. 1983. Bean. In: I. D. Teare and M. M. Peet. Crop-water relations. John Wiley & sons, New York.

Holden, J. and D. Haytowitz. 1998. The nutritional value of beans. Bean Improvement Cooperative 41:41-44.

Kramer, P. J. 1988. Changing concepts regarding plant water relations. Plant, cell, Environ. 11:565-568.

Laing, D. R., P. G. Jones, and J. H. C. Davis. 1984. Common Bean (Phaseolus vulgaris L.). In: P. R. Goldsworthy and N. M. Fisher. The physiology of tropical field crops. John Wiley & Sons, New York.

Levitt, J. 1980. Responses of plants to environmental stresses. Vol. II: water , radiation, salt, and other stresses. Academic press, New York.

Ludlow, M. M., and R. C. Muchow. 1990. A critical evaluation of traits for improving crop yields in water-limited environments. Adv. Agron. 43:107-153.

Mackay, D. C., and C. A. Eaves. 1962. The Influence of irrigation treatments on yields and on fertilizer utilization by sweet corn and snap beans. Can. J. Plant Sci. 42:219-228.

Maurer, A. R. , D. P. Ormrod, and N. J. Scott. 1969. Effect of five soil water regimes on growth and composition of snap beans. Can. J. Plant Sci. 49:271-278.

May, L. H. and F. L. Milthorpe. 1962. Drought resistance of crop plants. Field Crop Abstr. 15:171-179.

Miller, D. E., and D. W. Burke. 1983. Response of dry beans to daily deficit sprinkler irrigation. Agron. J. 75:775-778.

Passioura, J. B. 1977. Grain yield, harvest index, and water use of wheat. Aust. Inst. Agric. Sci. 43:107-153.

Passioura, J. B. 1988. Response to Dr. P. J. Kramer's article, 'Changing concepts regarding plant water relations', volume 11, number 7, pp.565-568. Plant, Cell, and Environ. 11:569-571.

Passioura, J.B. 1994. The yield of crops in relation to drought. In: K. J. Boote (ed.). Physiology and determination of crop yield. ASA-CSSA-SSSA, Madison, Wisconsin.

Radin, J. W. 1983. Physiological consequences of cellular water deficits: osmotic Adjustment. In: H.M. Taylor, W.R. Jordan, and T.R. Sinclair (eds.) Limitations to efficient water use in crop production. ASA, CSSA, SSSA, Madison, WI.

Rana, R. S. 1986. Breeding Crop varieties for salt-affected soils. In: V. L. Chopra and R. S. Paroda. Approaches for incorporating drought and salinity resistance in crop plants. Oxford &IBH Pub. Co. New Delhi.

Robins, J. S., and C. E. Domingo. 1956. Moisture deficits in relation to the growth and development of dry beans. Agron. J. 48:67-70.

Schulze, E. D. 1986. Whole-plant responses to drought. Aust. J. Plant Physiol. 13:127.

Sinclair, T. R., and M. M. Ludlow. 1985. Who taught plants thermodynamics? the unfulfilled potential of plant water potential. Aust. J. plant Physiol. 12:213-217.

Sinha, S. K. 1986. Drought resistance in crop plants: a physiological and biochemical analysis. In: V. L. Chopra and R.S. Paroda (Eds.) Approaches for incorporating drought and salinity resistance in crop plants. Oxford & IBH Publishing Co., New Delhi.

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

Stanhill, G. 1986. Water use efficiency. Adv. Agron. 39:53-83.

Stegman, E. C., and H. M. Olson .1976. Water management relationships for irrigated pinto beans. N. D. Res. Rep. No. 61.

Stoker, R. 1974. Effect on dwarf beans of water stress at different phases of growth. N. Z. J. Expl. Agric., 2:13-15.

Turner, N. C. 1986. Adaptation to water deficits: a changing perspective. Aust. J. Plant Physiol. 13:175-190.

Vittum, M. T., and G. H. Gibbs. 1961. Don't wait too long to irrigate snap beans. Farm Res. 27:15.

Walker, G. K., and J. L. Hatfield. 1979. Test stress-degree-day concept using multiple planting dates of red kidney beans. Agron. J. 71:967-971.

Walton, D. C., E. Galson, and M. A. Harris. 1977. The relationship between stomatal resistance and abscisic acid levels in leaves of water-stressed bean plants. Planta 133:145-148.

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

World Bank. 1992. Development and the environment. World Development Report 1992. Oxford University Press.

CHAPTER 2

BEAN ADAPTATION TO DROUGHT: YIELD COMPONENTS AND YIELD DETERMINANTS

ABSTRACT

Increasing bean (Phaseolus vulgaris L.) yields in drought prone environments requires the identification of drought resistant genotypes. The objective of this study was to evaluate the relationship of water use efficiency, harvest index and water use for beans growing under moisture deficit by comparing genotypes of known drought resistance characteristics. Two resistant genotypes BAT 477 and LEF2RB and a susceptible Seafarer were grown in a rain shelter in Michigan from 1991 to 1993 under a well watered conditions (control), a vegetative intermittent stress, and a reproductive terminal stress. Seed yield varied from 28 to 352 g m^{-2}. Vegetative stress reduced yield by 15 to 30% while reproductive stress reduced yield by 35 to 50%. Genotypic differences in yield were observed only under the reproductive stress. Water use and water use efficiency correlated positively with yield and biomass specially when examined under specific water treatment or by genotype but harvest index did not correlate with yield or biomass. It is concluded that beans follow a simpler model relating water use and water use efficiency to yield with harvest index being generally higher and more stable than those of other crops.

INTRODUCTION

Drought is the major constraint to bean production after diseases. Almost all bean production is on dryland farming systems with frequent drought stress affecting more than

60 percent of the bean producing areas world-wide (White et al., 1990; Laing et al., 1984; Acosta-Gallegos et al.,1998).

It has also been proposed that yield under stressful environments is best characterized by a multiplicative model (Passioura, 1994) as follows:

Yield=water transpired x water-use efficiency x harvest index

Field measurement of the water transpired can be approximated by the evapotranspiration if similar evaporation is assumed for plots at the same site (Doorenbos and Kassam, 1979; Barros and Hanks, 1993). This model was used in cereal crops (Passioura, 1977) but its applicability to determinate genotypes of common bean has not been reported. It is critical to evaluate such a model for beans because research on yield determinants such as water use efficiency and harvest index would contribute to yield directly. Similarly, any trait that can be directly linked to any of the yield determinants can indirectly be a useful screening tool for yield under drought. Such a relationship has indeed been suggested for traits like carbon isotope discrimination and osmotic adjustment among others (Ludlow and Muchow, 1990; Ehleringer et al., 1991).

The objective of this study was to examine the relationships of water use, water use efficiency, and harvest index with yield and biomass in common bean genotypes that have contrasting drought resistance characteristics.

MATERIALS AND METHODS

Beans were planted on a Kalamazoo soil (Fine-loamy, mixed, mesic Typic Hapludalfs) under a rain shelter (Martin et al.,1988)at the Michigan State University's Kellog Biological Station at Hickory Corners, Michigan on 18 June 1991, 14 June 1992, and 4 June 1993. Based on soil tests about 98 kg ha^{-1} of urea 46% N was applied as a starter

fertilizer each year together with soil rhizobium to avoid any existing non-uniformity of rhizobium in the soil. The automatic rain shelter is set to close as soon as it starts raining. Maximum and minimum temperatures for the three seasons are shown in Figure 1.

The experimental design was a split plot with water as the main plot, genotypes as the subplot, and three (1991) or four (1992 and 1993) replications. Main plot was 6 m by 3.5 m which was sub divided into 3 sub-plots each 2 m long. The three genotypes in this experiment were two resistant checks: BAT 477 (Sponchiado et al., 1989) and LEF2RB (Schneider et al., 1997) and a susceptible control: Seafarer (Adams, 1996). The three water treatments were a well-watered control, no moisture from V5 to R1 (vegetative intermittent stress), and no moisture from R1 to harvest (reproductive terminal stress). The irrigation amounts and times are presented in Table 1 and in Figure 2.

Water stress treatments were matched with phenological growth stages to elucidate the differential effect of moisture deficit on physiological and morphological traits. The phenological stages were measured by counting leaf number during vegetative growth. The first flower was considered as R1 and the details of other growth stages were based on those proposed by Nuland and schwartz (1989).

Water use was calculated from neutron probe readings before and after the water treatment. The access tube for each plot was placed on one of the two center rows of each plot. The count ratios of the neutron probe were calibrated with gravimetrically measured soil moisture for each depth.

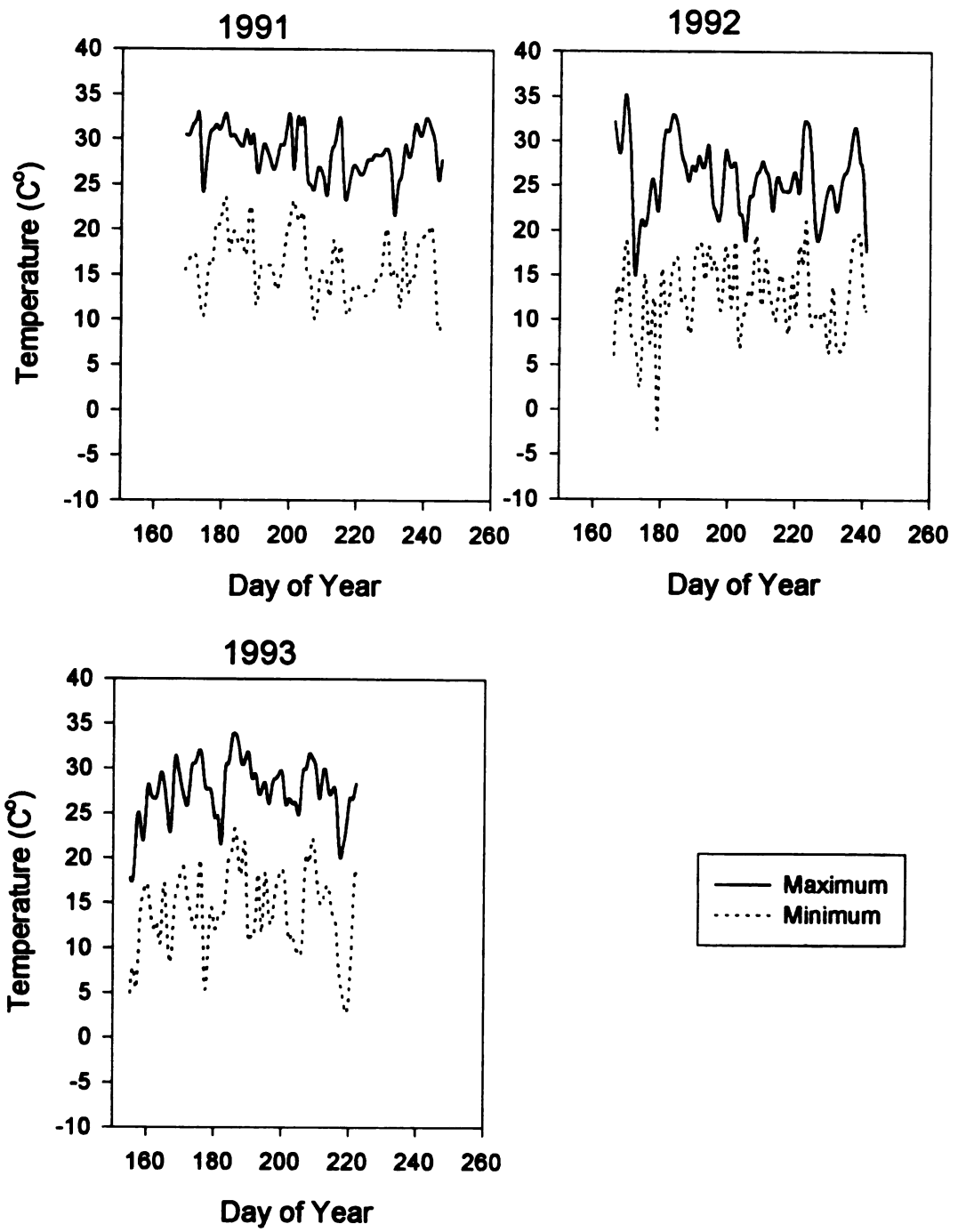

Figure 1. Maximum and minimum air temperatures for the growing season at experimental site 1991-1993.

Table 1. Irrigation treatment and application for three common bean genotypes grown in a rain shelter at Hickory Corners, Michigan 1991-1993.

Year	Irrigation Treatment	Growth Stages: Planting to 5-leaf	5-leaf to R1†	R1 to Harvest	Sum	% of Control
		mm				
1991	Control (W1)	114	102	89	305	100
	Stress (W2)	114	0	89	203	67
	Stress (W3)	114	102	0	216	71
1992	Control (W1)	152	51	90	294	100
	Stress (W2)	152	0	90	242	83
	Stress (W3)	152	51	0	203	69
1993	Control (W1)	114	51	85	250	100
	Stress (W2)	114	0	85	199	80
	Stress (W3)	114	51	0	165	66

†R1=First open flower in any node.

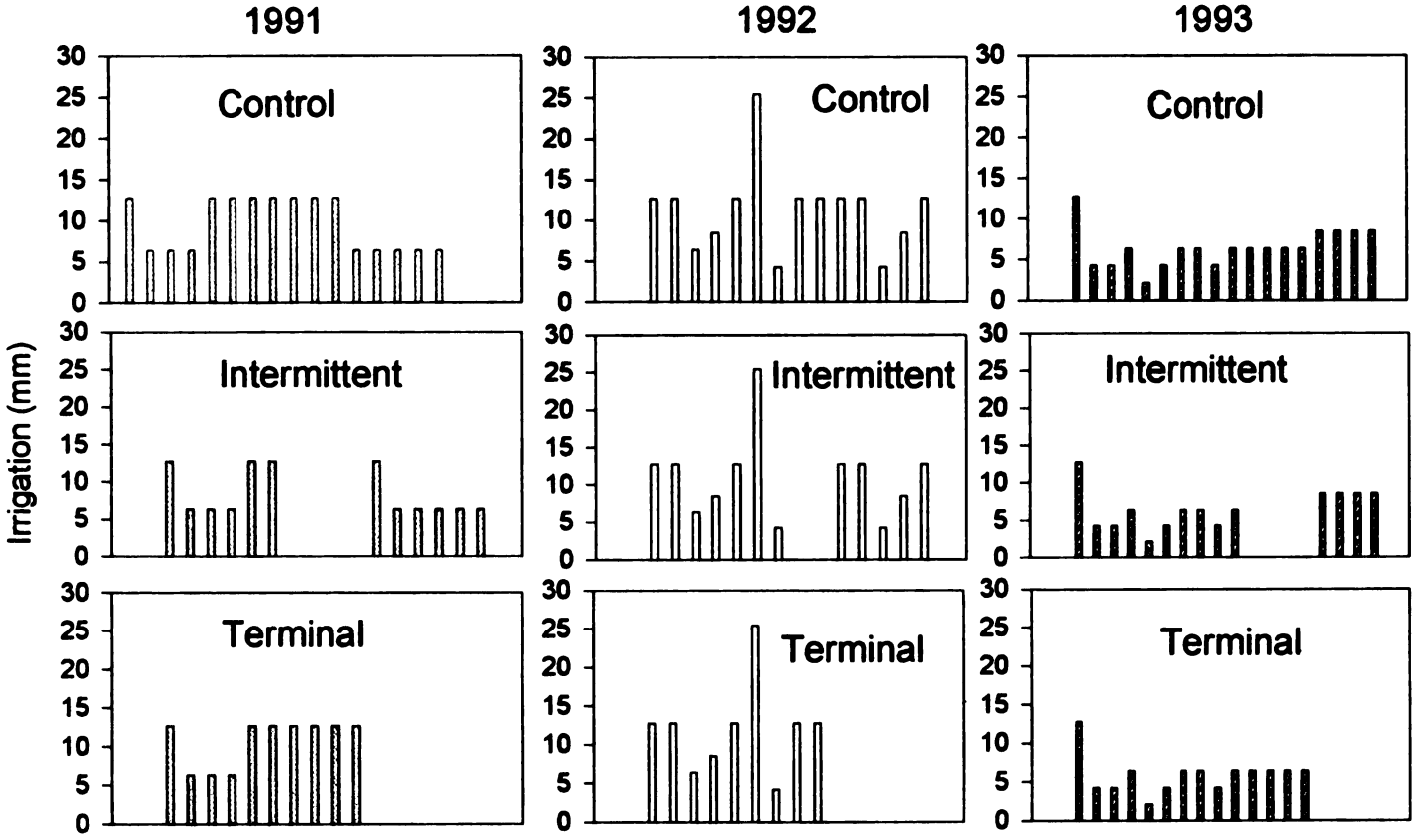

Figure 2. Different irrigation treatments for three common bean genotypes grown in a rain shelter at Hickory Corners, Michigan 1991-1993.

Grain yield, yield components and the above ground dry weight (Biomass) data were recorded from the harvested samples of 1.5 m of the two center rows. Growth stage biomass yield was also measured from 5 randomly selected plants per plot through out the growing season. At any sampling date, the number of pods and seeds per plot were obtained by counting. Water use efficiency (WUE) was measured from the ratio of biomass yield and Seasonal cumulative water use.

The data were analyzed by the SAS General Linear Model (GLM) (SAS Institute, 1993). Correlation analysis was also done to examine any associations among parameters. A combined analysis over all the years was done whenever there were no interactions with years in addition to individual yearly analysis to show the general trend. Years are considered random variables and represent environments as the plots rotated each year around the shelter and a new randomization was done for each site. Thus, years were confounded with sites. Means from different treatments were compared by using Fisher's ANOVA protected Least Significant Difference (LSD).

RESULTS AND DISCUSSION

Yield determinants

Both vegetative and reproductive stress reduced bean yields. The highest yield of 352.16 g m^{-2} was attained in 1991 under well watered conditions by LEF2RB. The lowest yield of 28.35 gm^{-2} was also attained by the same genotype under the reproductive terminal stress in 1993. Intermittent vegetative stress reduced yield by 15% in 1991 and by 30% in 1992 (Table 2). Similar reductions for vegetative stress have been reported (Acosta-Gallegos and Shibata, 1989). There are conflicting reports about the effect of water deficit during the vegetative period for beans (Robins and Domingo, 1956; MacKay

Table 2. Yield , water use efficiency (WUE), harvest index (HI), and seasonal water Use of three common bean genotypes grown under well water conditions (W1), intermittent vegetative water stress (W2), and terminal reproductive stress (W3) at Hickory Corners, Michigan 1991-1993.

Year	Treatment	Yield ($g\ m^{-2}$)	WUE ($g\ Kg^{-1}$)	HI	Water Use (mm)
1991	Control-W1	270.46	0.74	0.57	638.60
	Stress-W2	190.63	0.87	0.56	406.06
	Stress-W3	160.03	0.63	0.52	483.92
	LSD(0.05)	55.82	NS+	NS	58.89
1992	Control-W1	254.99	0.88	0.50	583.74
	Stress-W2	217.48	0.89	0.51	485.11
	Stress-W3	166.85	0.79	0.47	444.01
	LSD(0.05)	31.32	NS	NS	19.65
1993	Control-W1	181.27	0.62	0.58	498.21
	Stress-W2	119.32	0.57	0.54	387.44
	Stress-W3	90.36	0.46	0.52	368.03
	LSD(0.05)	64.72	NS	0.04	24.37

+significant at the $p<0.10$ level.

and Eaves, 1962). Moisture deficits at this growth stage seem to have only a small effect on yield (Robins and Domingo, 1956; Dubetz and Mahalle, 1969; Walker and Hatfield, 1979; Castellanos et al., 1996).

Reproductive terminal stress reduced yields greatly every year with reductions ranging from 35% in 1992 to 50% in 1993. These results are comparable to those of Acosta-Gallegos and shibata (1989). Generally, moisture deficits are always detrimental when they occur during the reproductive growth stages (Stoker, 1974; Walton et al., 1977;Acosta-Gallegos and Shibata, 1989; Castellanos et al.,1996). The two stress treatments did not significantly differ from each other except in 1992 (Table 2). Moderately stressed beans grown under plastic covers yielded similarly as those under well watered control (Raeini-Sarjaz and Barthakur, 1997).

Across water treatment, there was no significant genotypic yield differences at any year but Seafarer ranked lowest every year (Table 3). It was under the reproductive terminal stress that the resistant BAT 477 could be clearly distinguished from the susceptible Seafarer both in yield as well as in some of the yield determinants. Under this stress, Seafarer had 24% lower yield than BAT 477 in part due to more reductions in water use efficiency and water use (Table 4).

There was no significant differences between water treatments in water use efficiency (WUE) in any year at the $p<0.05$ level across genotypes. Seafarer had lower WUE than the other two genotypes in 1992. In mung beans, three irrigations increased water use efficiency over four irrigations but harvest index increased in the treatment with two irrigations only (Pannu et al.,1993).

Harvest index (HI) was significantly reduced by both stress treatments only in

Table 3. Yield , water use efficiency (WUE), harvest index (HI), and seasonal water Use of three common bean genotypes grown under different water stress treatments at Hickory Corners, Michigan 1991-1993.

Year	Genotypes	Yield ($g\ m^{-2}$)	WUE ($g\ Kg^{-1}$)	HI	Water Use (mm)
1991	Seafarer	190.66	0.68	0.56	497.00
	LEF2RB	198.94	0.69	0.55	522.31
	BAT 477	231.51	0.87	0.55	509.26
	LSD(0.05)	NS	NS	NS	NS
1992	Seafarer	200.76	0.76	0.52	500.69
	LEF2RB	226.94	0.91	0.50	506.29
	BAT 477	211.62	0.90	0.47	505.88
	LSD(0.05)	NS	0.10	0.03	NS
1993	Seafarer	120.44	0.52	0.55	410.12
	LEF2RB	136.73	0.57	0.55	417.30
	BAT 477	133.79	0.55	0.55	426.26
	LSD(0.05)	NS	NS	NS	11.30

Table 4. Yield , water use efficiency (WUE), harvest index (HI), and seasonal water Use of three common bean genotypes grown under different water stress treatments at Hickory Corners, Michigan 1991-1993.

Treatment	Genotypes	Yield ($g\ m^{-2}$)	WUE ($g\ Kg^{-1}$)	HI	Water Use (mm)
Control-W1	Seafarer	221.32	0.70	0.56	558.13
	LEF2RB	254.95	0.82	0.55	571.79
	BAT 477	220.93	0.72	0.54	572.87
	LSD(0.05)	NS	NS	NS	NS+
Stress-W2	Seafarer	165.14	0.67	0.58	423.67
	LEF2RB	169.91	0.76	0.52	430.33
	BAT 477	188.34	0.88	0.51	430.09
	LSD(0.05)	NS	0.16	0.04	NS
Stress-W3	Seafarer	119.93	0.57	0.48	418.45
	LEF2RB	134.63	0.60	0.52	432.76
	BAT 477	156.96	0.71	0.51	430.58
	LSD(0.05)	27.29	0.12	NS	10.41

+Significant at the $p< 0.10$ level only.

1993 (Table 2). Harvest index ranged from 0.34 to 0.61 across the years and that range agrees with prior research (Scully and wallace, 1990; Pilbeam, 1996). BAT 477 scored lowest in HI in 1992 than both of the genotypes but had higher water use than Seafarer (table 3). Seafarer had higher harvest index under the vegetative stress treatment (Table 4). Bush forms like Seafarer produce less due to shorter remobilization period (Fernandez, 1981). De costa et al. (1997) reported the post flowering stress increased harvest index in faba beans.

Seasonal water use was reduced by the vegetative by 17% in 1992 to 36% in 1991. Reproductive stress reduced seasonal water use by an average of 25% every year (Table 2).

Genotype by treatment interactions were found for yield and yield determinants except water use when the data was pooled over the years. Similar interactions were reported in beans (Samper, 1984) indicating the existing potential in selecting for drought resistant genotypes. In this study, there was no genotypic differences under well watered conditions indicating a measure of similarity in yield potential (Table 4).

Yield Components

Vegetative stress reduced pod m^{-2} by 16% in 1991 and by 22% in 1993. Similarly, pods $plant^{-1}$ were reduced by 15 to 28% for the same years, respectively. This stress did not affect seed weight or seeds per pod (Table 5). Reproductive terminal stress reduced pods m^{-2} in all three years. In addition, this stress was detrimental to seed weight relative to the control (Table 5). Seeds pod^{-1} were not reduced by any stress at any year contrary to the findings of Nielsen and Nelson (1998). These results agree with those of Samper (1984) for both seed weight and seed number.

Table 5. Yield components of three common bean genotypes grown under well water conditions (W1), intermittent vegetative water stress (W2), and terminal reproductive stress (W3) at Hickory Corners, Michigan 1991-1993.

Year	Treatment	Pods m^{-2}	Pods $Plant^{-1}$	Seeds pod^{-1}	Seed wt (mg $Seed^{-1}$)
1991	Control-W1	344.74	13.75	4.50	187.45
	Stress-W2	288.89	11.70	3.59	188.12
	Stress-W3	251.26	10.37	6.62	169.79
	LSD(0.05)	47.43	1.78	NS	NS
1992	Control-W1	343.78	12.53	5.05	153.77
	Stress-W2	329.39	11.76	4.55	152.79
	Stress-W3	269.39	10.55	4.51	141.56
	LSD(0.05)	57.77	NS	NS	6.69
1993	Control-W1	237.94	10.28	3.88	201.65
	Stress-W2	184.50	7.37	3.38	185.80
	Stress-W3	166.00	7.31	3.24	166.48
	LSD(0.05)	51.10	2.13	NS	22.82

Table 6. Yield components of three common bean genotypes grown under different moisture stress treatments at Hickory Corners, Michigan 1991-1993.

Year	Genotypes	Pods m^{-2}	Pods $Plant^{-1}$	Seeds pod^{-1}	Seed wt (mg $Seed^{-1}$)
1991	Seafarer	347.93	14.98	3.39	155.74
	LEF2RB	271.11	10.73	3.31	214.46
	BAT 477	265.85	10.10	5.02	175.16
	LSD (0.05)	48.26	1.95	0.72	14.46
1992	Seafarer	374.11	13.78	4.54	117.47
	LEF2RB	299.28	11.03	3.95	190.81
	BAT 477	269.17	10.04	5.62	139.84
	LSD (0.05)	39.19	1.39	0.47	9.30
1993	Seafarer	244.39	12.14	2.91	162.59
	LEF2RB	184.89	7.46	3.18	212.10
	BAT 477	159.17	5.35	4.41	179.24
	LSD (0.05)	25.06	0.84	0.36	13.24

Table 7. Yield components of three common bean genotypes grown under different moisture stress treatments at Hickory Corners, Michigan 1991-1993.

Treatment	Genotypes	Pods m^{-2}	Pods $Plant^{-1}$	Seeds pod^{-1}	Seed wt (mg $Seed^{-1}$)
Control-W1	Seafarer	378.12	16.32	4.05	151.26
	LEF2RB	297.82	11.12	4.00	217.29
	BAT 477	240.73	8.69	5.38	172.54
	LSD (0.05)	49.07	1.79	0.60	9.83
Stress-W2	Seafarer	315.88	12.47	3.74	142.26
	LEF2RB	243.52	9.54	3.16	211.02
	BAT 477	237.58	8.43	4.69	170.01
	LSD (0.05)	29.25	1.16	0.49	11.78
Stress-W3	Seafarer	265.39	11.7	3.12	139.42
	LEF2RB	208.67	8.28	3.32	186.69
	BAT 477	206.49	7.94	4.97	148.85
	LSD (0.05)	28.18	0.96	0.37	14.60

Table 8. Biomass and its components of three common bean genotypes grown under well water conditions (W1), intermittent vegetative water stress (W2), and terminal reproductive stress (W3) at Hickory Corners, Michigan 1991-1993.

Year	Treatment	Biomass	Pods†	Straw	Stover
		($g\ m^{-2}$)	($g\ m^{-2}$)	($g\ m^{-2}$)	($g\ m^{-2}$)
1991	Control-W1	473.38	81.89	121.03	202.92
	Stress-W2	352.37	63.76	97.98	161.74
	Stress-W3	300.35	56.95	83.36	140.31
	LSD(0.05)	56.14	10.82	23.08	20.35
1992	Control-W1	514.80	98.91	160.90	259.82
	Stress-W2	430.42	84.81	128.13	212.94
	Stress-W3	350.06	64.56	118.65	183.21
	LSD(0.05)	67.14	13.99	NS	54.82
1993	Control-W1	309.22	63.86	50.59	114.45
	Stress-W2	216.89	43.88	43.86	87.74
	Stress-W3	169.52	35.90	35.72	71.62
	LSD(0.05)	108.15	19.23	NS	NS+

†Empty pods + Significant at the $p<0.10$ level only.

The three genotypes in this study had each one yield component over which it excelled over the two genotypes no matter what water stress treatment they were in. Seafarer excelled in pod numbers, BAT 477 in seeds pod^{-1}, and LEF2RB in seed weight. This was consistent in all the years (Table 6). Moreover, BAT 477 consistently scored over Seafarer in seed weight while LEF2RB had higher pod numbers than BAT 477 in 1993 only.

Yield component adjustment per genotype did not seem to have occurred based on the above but genotype by water interactions did take place. For example, BAT 477 had lower pod numbers than both Seafarer and LEF2RB under the well watered conditions but was similar to LEF2RB under stress (Table 7). In addition, LEF2RB was more sensitive to vegetative stress regarding seeds pod^{-1} than Seafarer. Correlation analysis did not indicate any negative cross correlations for seed yield components when the treatment means were used for any year (data not presented). Seed weight negatively but weakly correlated with seeds per pod ($r=-0.62$ $p<0.10$ $n=9$) and with pods m^{-2} ($r=-0.62$ $p<0.10$ $n=9$) under the vegetative stress if the year by genotype means were used. Seafarer was the only genotype that indicated any negative correlations between seeds pod^{-1} and seed weight ($r=-0.69$ $p<0.05$ $n=9$)and only when the year by water means were used in the analysis. Similar results were found in faba beans (De costa et al., 1997).

Biomass Components

Biomass was reduced by the intermittent vegetative stress in two of the three years (Table 8). These reductions varied from 16% (1992) to 26% (1991). Further more, empty pod weights were severely lowered by this stress treatment in all the years as well

Table 9. The biomass components of three common bean genotypes grown under different moisture stress treatments at Hickory Corners, Michigan 1991-1993.

Year	Genotypes	Biomass ($g\ m^{-2}$)	Pods† ($g\ m^{-2}$)	Straw ($g\ m^{-2}$)	Stover ($g\ m^{-2}$)
1991	Seafarer	334.53	62.84	81.03	143.87
	LEF2RB	365.34	59.31	107.10	166.40
	BAT 477	426.22	80.45	114.26	194.71
	LSD(0.05)	NS	16.20	NS	NS
1992	Seafarer	379.56	82.28	96.52	178.80
	LEF2RB	461.43	76.46	158.02	234.49
	BAT 477	454.29	89.54	153.14	242.68
	LSD(0.05)	53.03	NS+	21.33	26.53
1993	Seafarer	216.92	47.08	39.48	86.56
	LEF2RB	240.79	47.18	45.96	93.14
	BAT 477	237.92	49.38	44.74	94.12
	LSD(0.05)	NS	NS	NS	NS

†Empty pods. + Significant at the $p<0.10$ level.

but straw weights were not (table 8). In contrast, reproductive stress reduced biomass and all of its components except straw weights in all the years. Biomass reductions varied from 32% in 1992 to 45% in 1993. In other studies biomass reductions reached up to 50% for some genotypes (Samper, 1984). Straw weights were adversely affected by the terminal reproductive stress only in 1991 (Table 8).

Seafarer had significantly lower biomass, straw weight and stover weight than both BAT 477 and LEF2RB in 1992. On the other hand, BAT 477 had significantly higher empty pod weight than the other two genotypes in 1991. The differences in empty pod weight between Seafarer and BAT 477 in 1992 were significant at the $p<0.10$ level only (Table 9). The Samper (1984) study examined twenty two genotypes and described LEF2RB as on of two genotypes that increased their biomass under stress which is one of the reasons why LEF2RB was thought of as a drought resistant genotype.

The susceptible Seafarer had lower straw and stover weights than LEF2RB even under well watered conditions (Table 10). In addition, BAT 477 was not different than Seafarer and was in fact lower in straw weight than LEF2RB under the control. In contrast, BAT 477 was similar to LEF2RB but significantly higher yielding in biomass and straw weight than Seafarer under the intermittent vegetative stress (Table 10). Moreover, BAT 477 had higher pod and stover weights than both Seafarer and LEF2RB under the vegetative stress. Similarly, BAT 477 had significantly higher biomass yield than Seafarer under the terminal reproductive stress. Under this stress, LEF2RB produced lower stover and pod weights than BAT 477 (Table 10).

Table 10. The biomass components of three common bean genotypes grown under different moisture stress treatments at Hickory Corners, Michigan 1991-1993.

Treatment	Genotypes	Biomass ($g\ m^{-2}$)	Pods† ($g\ m^{-2}$)	Straw ($g\ m^{-2}$)	Stover ($g\ m^{-2}$)
Control-W1	Seafarer	396.82	81.50	89.00	170.50
	LEF2RB	474.80	79.14	135.56	214.70
	BAT 477	414.62	83.93	105.19	189.12
	LSD(0.05)	NS+	NS	17.97	28.40
Stress-W2	Seafarer	285.01	59.94	56.74	116.67
	LEF2RB	329.49	59.62	95.89	155.51
	BAT 477	379.95	73.00	115.16	188.16
	LSD(0.05)	63.67	10.22	29.30	37.26
Stress-W3	Seafarer	242.59	51.10	68.93	120.02
	LEF2RB	260.67	44.65	78.69	123.34
	BAT 477	309.29	60.45	89.00	149.44
	LSD(0.05)	49.07	8.14	NS	23.99

†Empty pods + Significant at the $p<0.10$ level.

Yield determinants relationships with yield

Across treatments and genotypes, water use efficiency modestly and positively correlated with yield (Figure 3-a,d). The relationship between yield and WUE was stronger when the data was organized under water treatment or genotypes.

In contrast, harvest index did not correlate with yield or biomass (Figure 3-b, e). In other studies, harvest index correlated weekly (Fernandez, 1981) or strongly (Acosta-Gallegos and Adams, 1991)with yield .

Seasonal water use, on the contrary, strongly correlated with yield and biomass (Figure 3-c, f). Because the data for water use were from plots that were either well watered or stressed there are two clusters of data with no points in between them (Figure 3-c,f). Generally, assuming a linear relationship in such types of data may seem questionable but in the case of well watered versus stressed plots this spread of the data is common (Mahalakshmi et al., 1990)and the assumption of a linear relationship is justified based on results from other methods (Barros and Hanks, 1993; Miller and Burke, 1983).

The relationship between yield and WUE was stronger when the data was organized under water treatments and specially so under the stress treatments (Figure 4-a). Again, harvest index did not correlate with yield under any water treatment (Figure 4-b). In contrast to WUE, seasonal water use correlated more strongly with yield under the control treatment (Figure 4-c). These results indicate that of the three yield determinants only water use efficiency may predict yield under stress. This is in line with the concept that WUE acts like the link between drought response and drought stress (Stanhill, 1986).

All three genotypes had significant positive associations between water use

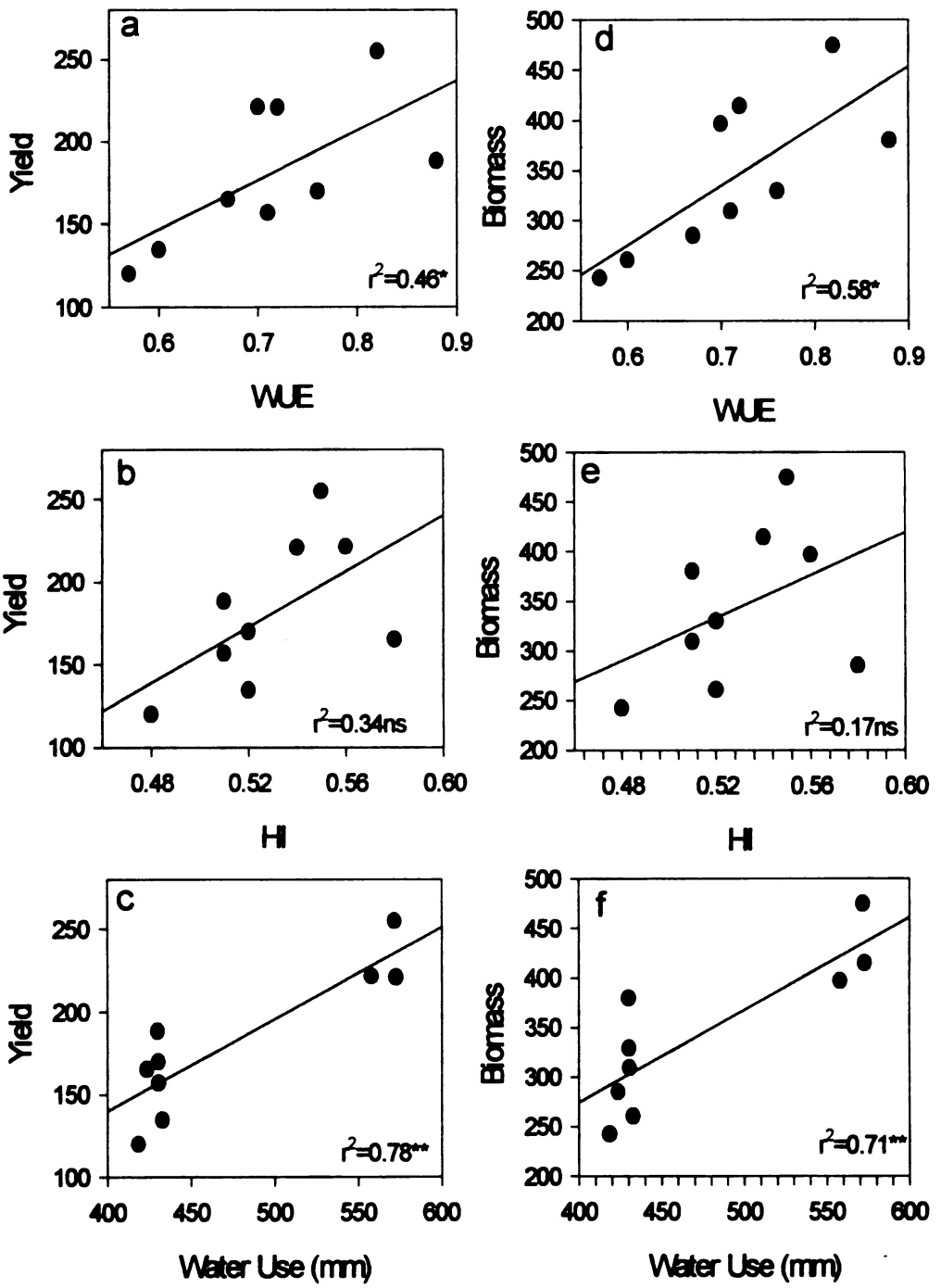

Figure 3. Water use efficiency (WUE), harvest index (HI), and seasonal water use and their relationship with yield and biomass of common bean genotypes grown at Hickory Corners, Michigan 1991-1993. Data are the pooled water by genotype means.

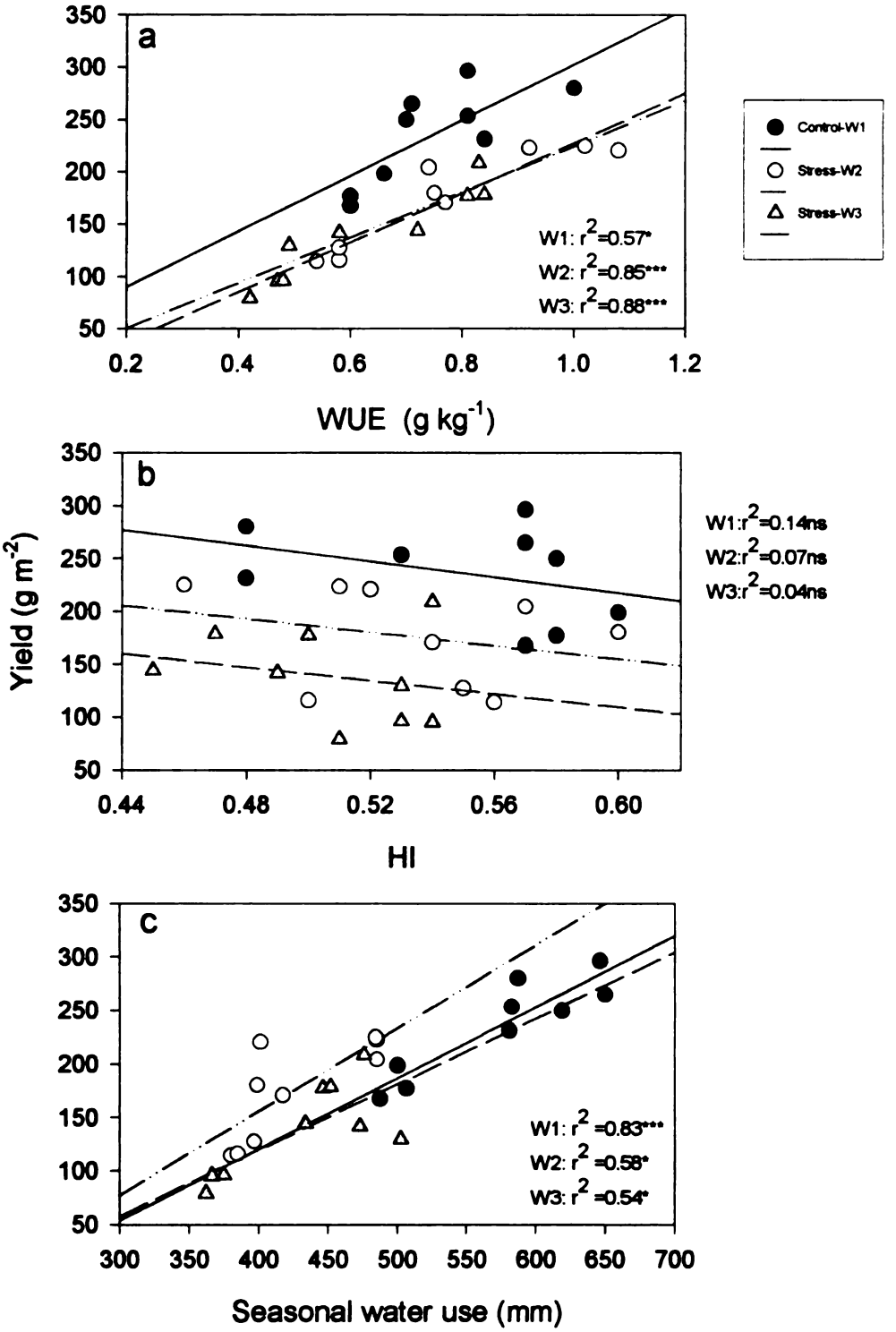

Figure 4. The relationship of water use efficiency (WUE), harvest index (HI), and water use to yield as affected by different moisture stress treatments of common bean genotypes grown at Hickory Corners, Michigan 1991-1993. Data are from the pooled year by genotype means.

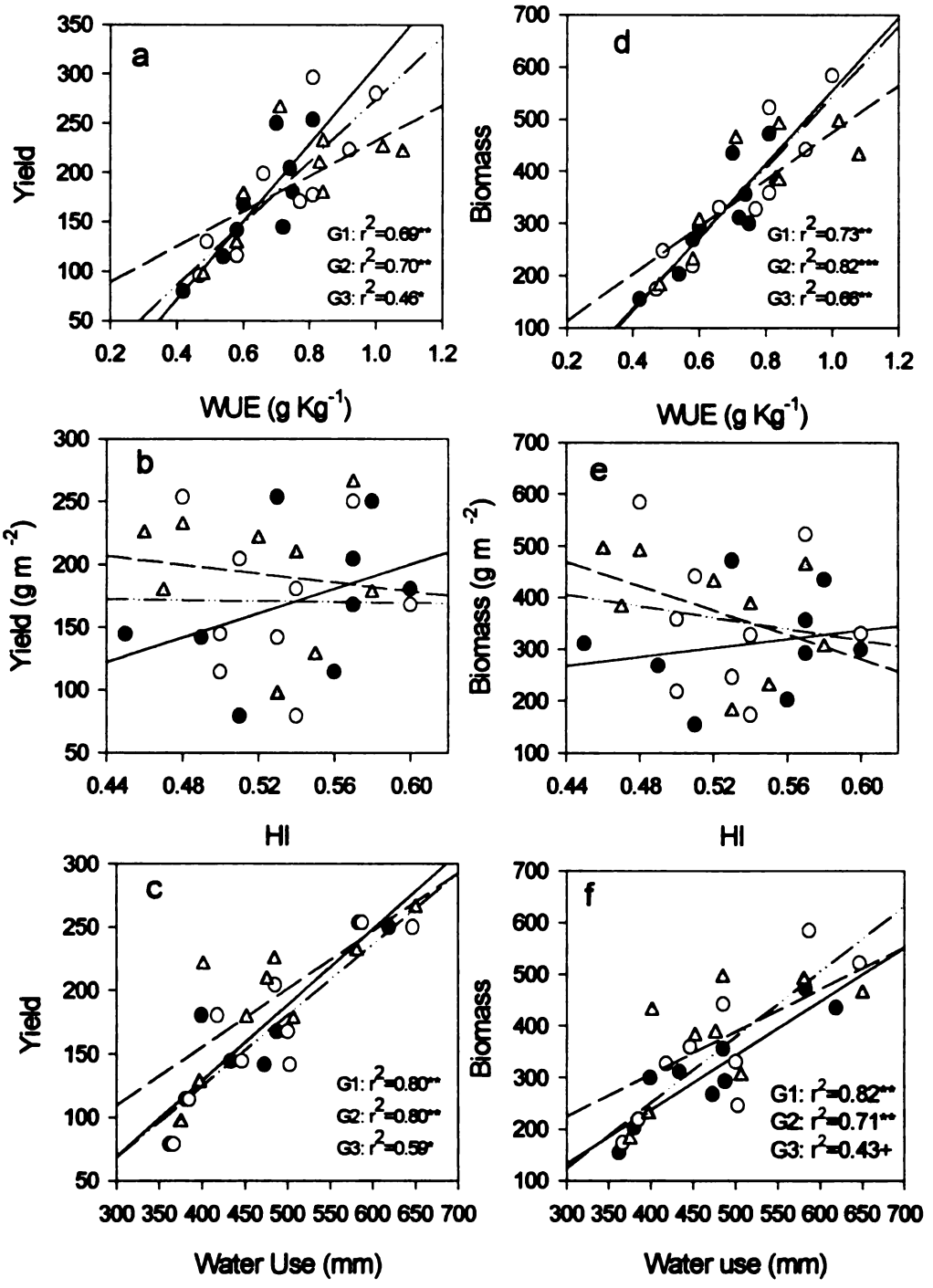

Figure 5. The relationships of water use efficiency (WUE), harvest index (HI), and water use with yield and biomass of three common bean genotypes grown under different water stress treatments at Hickory Corners, MI 1991-1993. Data are the pooled water by year means.

efficiency (WUE) and yield or biomass (Figure 5-a,d). This relationship was least strong for the resistant BAT 477 and strongest for LEF2RB. No significant correlations were found for any of the three genotypes between harvest index and yield or biomass (Figure 5-b,e).

On the other hand all genotypes had positive associations between water use and yield or biomass (Figure 5-c,f). Similar relationships were reported for water use and yield (Barros and Hanks, 1993).

Correlation analysis using the treatment means as well as any other second degree interactions did not indicate any negative cross correlations among the yield determinants (data not presented). Musick et al. (1994) analyzed 178 crop-year data for wheat and concluded a linear relationship between evapotranspiration and yield but a curvilinear relationship between yield and water use efficiency with no cross correlations found. Strong positive correlations were also reported in faba beans (Vicia faba L.)between seed yield and post anthesis water use (Mwanamwenge et al.,1998; Loss et al., 1997).

Since different genotypes do not respond equally to the same quantity of drought some researchers classify them as follows (Fernandez, 1993): high yielding resistant, high yielding susceptible, low yielding resistant, and low yielding susceptible. In this study, Seafarer was susceptible and BAT 477 resistant under the terminal reproductive moisture stress (Table 4) but they yielded equally well under control. LEF2RB, on the other hand, seems to be high yielding susceptible as it reduced its yield more than Seafarer but still yielded similarly to the resistant BAT 477 under the terminal reproductive stress.

CONCLUSIONS

The yield determinants of the three genotypes in this study behaved similarly. It is concluded that the yield determinants model as proposed for cereal crops seems to be applicable to beans but that the harvest index is more stable and higher in beans (Table 1 ; Figure 3-b, e; Pilbeam, 1996; Fernandez, 1981). Harvest index was the one factor that did not strongly correlate with yield but it is an important research objective for many breeders. This study does not encourage the use of harvest index as screening tool for yield improvement in beans and confirms similar findings by Pilbeam (1996) and Fernandez (1981). On the other hand, the other yield determining factors such as water use and water use efficiency seem to be more drought stress responsive and better predictors of yield under stress for beans. It follows that any screening trait that can be linked to these yield determinants may improve yield.

REFERENCES

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

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

Acosta-Gallegos, J.A., E. Acosta-Diaz, S. Padilla-Ramirez, E. Lopez-Salinas, R.A. Salinas-Perez, N. Mayek-Perez, and J.D. Kelly. 1998. Seed yield of dry bean cultivars under drought stress. Bean Improvement Cooperative 41:151-152.

Adams, M. W. 1996. An historical perspective on significant accomplishments in dry bean research- the first 100 years. Bean Improvement Cooperative 39:32-45.

Barros, L. C. G., and R. J. Hanks. 1993. Evapotranspiration and yield of beans as affected by mulch and irrigation. Agron. J. 85:692-697.

Castellanos, J. Z., J. J. Peña-Cabriales, and J. A. Acosta-gallegos. 1996. ^{15}N-determined dinitrogen fixation capacity of common bean (*Phaseolus vulgaris*) cultivars under water stress. J. Agric. Sci. 126:327-333.

Clarke, J. M., and T. F. Townley-Smith. 1984. Screening and selection techniques for improving drought resistance. In: P.B. Vose and S. G. Blixt. Crop breeding: a contemporary basis. Pergamon Press, New York.

De Costa, W. A. J. M., M. D. Dennett, U. Ratnaweera, K. Nyalemegbe. 1997. Effects of different water regimes on field-grown determinate and indeterminate faba bean (*Vicia faba* L.). II. Yield, yield components and harvest index. Field Crops Research 52:169-178.

Doorenbos, J., and A. H. Kassam. 1979. Yield response to water. FAO Irrigation and Drainage Paper no. 33, Rome.

Dubetz, S., and P. S. Mahalle. 1969. Effect of soil water stress on bush beans Phaseolus vulgaris L. at three stages of growth. J. Am. Soc. Hort. Sci. 94:479-481.

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

Fernandez, G.C.J. 1993. Effective selection criteria for assessing plant stress tolerance. In: C. G. Kuo (ed.) Adaptation of food crops to temperature and water stress. Proc. International Symposium, Taiwan 13-18 August 1992, AVRDC.

Fernandez, J.A.I. 1981. The effect of accumulation and remobilization of carbon assimilate and nitrogen on abscission, seed development, and yield of common bean (Phaseolus vulgaris L.) With different artichectural forms. Ph.D. diss. Michigan State University., East Lansing.

Laing, D. R., P. G. Jones, and J. H. C. Davis. 1984. Common Bean (Phaseolus vulgaris L.). In: P. R. Goldsworthy and N. M. Fisher. The physiology of tropical field crops. John Wiley & Sons, New York.

Loss, S.P., K.H.M. Siddique, and D. Tennant. 1997. Adaptation of faba bean (*Vicia faba* L.)to dryland mdeiterranean-type environments. III. Water use and water-use efficieency. Field Crops Research 54:153-162.

Ludlow, M. M., and R. C. Muchow. 1990. A critical evaluation of traits for improving crop yields in water-limited environments. Adv. Agron. 43:107-153.

Mackay, D. C., and C. A. Eaves. 1962. The Influence of irrigation treatments on yields and on fertilizer utilization by sweet corn and snap beans. Can. J. Plant Sci. 42:219-228.

Mahalakshmi, V., F.R. Bidinger, and G.D.P. Rao. 1990. Line-source vs. Irrigated/nonirrigated treatments for evaluation of genotype drought response. Agron. J. 82:841-844.

Martin, E. C., J. T. Ritchie, S. M. Reese, T. L. Loudon, and B. Knezek. 1988. A large-area, light-weight rainshelter with programmable control. Trans. ASAE 31:1440-1444.

Menéndez, C., and A. E. Hall. 1996. Heritability of carbon-isotope discrimination and correllations with harvest index in cowpea. Crop Sci. 36:233-238.

Miller, D. E., and D. W. Burke. 1983. Response of dry beans to daily deficit sprinkler irrigation. Agron. J. 75:775-778.

Musick, J.T., O.R. Jones, B.A. Stewart, and D.A. Dusek. 1994. Water-yield relations for irrigated and dryland wheat in the U.S. southern plains. Agron. J. 86:980-986.

Mwanamwenge, J., S. P. Loss, K.H.M. Siddique, and P.S. Cooks. 1998. Growth, seed yield and water use of faba beans (*Vicia faba* L.) In a short -season mediterranean-type environment. Aust. J. Exp. Agric. 38:171-180.

Nuland, D.S., and H. F. Schwartz. 1989. A visual description of the common bean plant: Four major groth periods. Bean Improvement cooperative 32:16-17.

Pannu, R.K., and D.P. Singh. 1993. Effect of Irrigation on water use, water-use efficiency,

growth and yield of mungbean. Field Crops Research 31:87-100.

Passioura, J. B. 1977. Grain yield, harvest index, and water use of wheat. Aust. Inst. Agric. Sci. 43:107-153.

Passioura, J.B. 1994. The yield of crops in relation to drought. In: K. J. Boote (ed.). Physiology and determination of crop yield. ASA-CSSA-SSSA, Madison, Wisconsin.

Pilbeam, C. J. 1996. Variation in harvest index of maize (*Zea mays*) and common bean (*Phaseolus vulgaris*)grown in a marginal rainfall area of Kenya. J. of Agric. Science 126:1-6.

Raeini-Sarjaz, M., and N. N. Barthakur. 1997. Water use efficiency and totaldry matter production of bush bean under plastic covers. Agric. and Forest Meteor. 87:75-84.

Robins, J. S., and C. E. Domingo. 1956. Moisture deficits in relation to the growth and development of dry beans. Agron. J. 48:67-70.

Samper, C. 1984. Effect of water stress imposed at mid-pod filling upon yield and dry matter partitioning in dry beans (*Phaseolus vulgaris* L.). M.S. Thesis. Michigan State University. East Lansing.

SAS Institute. 1993. SAS/STAT guide for personal computer, version 6, 4th ed. SAS Inst., Cary, NC.

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

Scully, B. T., and D. H. Wallace. 1990. Variation in relationship of biomass, growth rate, harvest index, and phenology to yield of common bean. J. Amer. Hort. Sci. 115(2):218-225.

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

Sponchiado, B. N., J. W. White, J. A. Castillo, and P.G. Jones. 1989. Root growth of four common bean cultivars in relation to drought tolerance in environments with contrasting soil types. Expl. Agric. 25:249-257.

Stanhill, G. 1986. Water use efficiency. Adv. Agron. 39:53-83.

Stegman, E. C., and H. M. Olson .1976. Water management relationships for irrigated pinto beans. N. D. Res. Rep. No. 61.

Stoker, R. 1974. Effect on dwarf beans of water stress at different phases of growth. N. Z. J. Expl. Agric., 2:13-15.

Walker G. K., and J. L. Hatfield. 1979. Test of the stress-degree -day concept using multiple planting dates of red kidney beans. Agron. J 71:967-971.

Walton, D. C., E. Galson, and M. A. Harris. 1977. The relationship between stomatal resistance and absicic acid levels in leaves of water-stressed bean plants. Planta 133:145-148.

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

CHAPTER 3

CARBON ISOTOPE DISCRIMINATION AND COMMON BEAN ADAPTATION TO DROUGHT

ABSTRACT

Carbon Isotope Discrimination (CID) has been suggested as a screening tool for drought resistance, but its relationship with common bean (*Phaseolus vulgaris* L.) productivity under moisture stress is not clear. This study was conducted to assess the effect of moisture deficit on CID and on the relationship of CID to yield, water use, water use efficiency (WUE) and harvest index (HI). Two drought resistant (BAT 477 and LEF2RB) and one drought susceptible (Seafarer) genotypes were grown under well watered conditions and under vegetative (intermittent) and reproductive (terminal) moisture stress from 1991 to 1993 in a rain shelter at the Kellogg Biological Station in Hickory Corners, MI. Reproductive moisture stress significantly reduced CID by 9% in comparison to the 5% reduction that occurred under intermittent stress. Seafarer had a significantly higher CID than both BAT 477 and LEF2RB. Positive correlations were found between CID and yield, WUE, and water use for each genotype or water treatment but not across genotypes or water treatments except for water use and pods per m^{-2}. Relative biomass and WUE reductions strongly and positively correlated with relative CID reduction. It is concluded that CID can be a useful screening tool for drought resistant genotypes but needs further testing with additional genotypes.

INTRODUCTION

Breeding for drought resistance in drought prone environments requires the existence of a heritable genotypic variation as well as a screening trait that is visible to the breeder (Clarke and Townley-Smith, 1984). Carbon isotope discrimination (CID or Δ) has been proposed as one such screening trait (Ludlow and Muchow, 1990), since a strong correlation was found between Δ and water use efficiency (WUE) in many crops, including legumes like peanuts (Hubick, 1990; Condon et al., 1987). Water use efficiency is considered to be the link between the drought factor and drought resistance (Stanhill, 1986).

Among the models that relate Δ to photosynthesis and water use efficiency, that of Farquhar et al. (1982) is considered to be the most developed and tested (Ehleringer et al., 1991). Seemann and Critchley (1985) reported that common beans, like other C_3 plants, followed the theoretically expected relationship between Δ and intercellular carbon dioxide concentration as proposed by Farquhar et al. (1982).

Genotypic differences among common bean cultivars for Δ have been reported (White et al., 1990; Ehleringer et al., 1991). However, results linking Δ to yield under drought or to any other drought adaptation trait by common bean were not conclusive (White et al., 1990). Carbon isotope discrimination (Δ) may be a useful screening tool if it can be related to long term water use efficiency or to other determinants of yield in a dry environment such as water use and harvest index. This should be true also if CID could separate known drought resistant and susceptible genotypes. This study investigated the effect of an intermittent and a terminal water stress on a drought susceptible (Seafarer)

and two drought resistant bean genotypes (BAT 477 and LEF2RB). The objective was to assess if Δ is associated with yield and yield determinants and whether Δ can be used as a predictor of drought resistance in common bean.

MATERIALS AND METHODS

Three common bean (*Phaseolus vulgaris* L.) Genotypes were planted on a Kalamazoo soil (Fine-loamy, mixed, mesic Typic Hapludalfs) under a rain shelter (Martin et al., 1988) at the Michigan State University Kellogg Biological Station at Hickory Corners, Michigan on 18 June 1991, 14 June 1992, and 4 June 1993. BAT 477 was reported in the literature as a drought resistant genotype based upon extensive germplasm evaluations in CIAT where it was developed (Singh, 1995). LEF2RB was evaluated in previous studies on the basis of yielding ability under drought (Schneider et al., 1997) and Seafarer is a commercial check widely grown and developed in Michigan (Adams, 1996). Based on soil tests about 98 kg ha^{-1} of urea (46% N) was applied as a starter fertilizer each year, together with soil rhizobium to avoid any existing non-uniformity of rhizobium in the soil. The automatic rain shelter was set to close as soon as it started raining. The maximum and minimum temperatures for each year are shown in Figure 1.

The experimental design was a split plot with water as the main plot, genotypes as the sub-plot, and three (1991) or four (1992 and 1993) replications. The main plot was 6 m by 3.5 m which was subdivided into 3 sub-plots each 2 m long. The three water treatments were a well-watered control, vegetative or intermittent stress: no irrigation from V5 (five leaf stage) to R1 (first open flower), and a reproductive terminal stress: no moisture from R1 to harvest. The amounts and times of irrigation are presented in Table 1

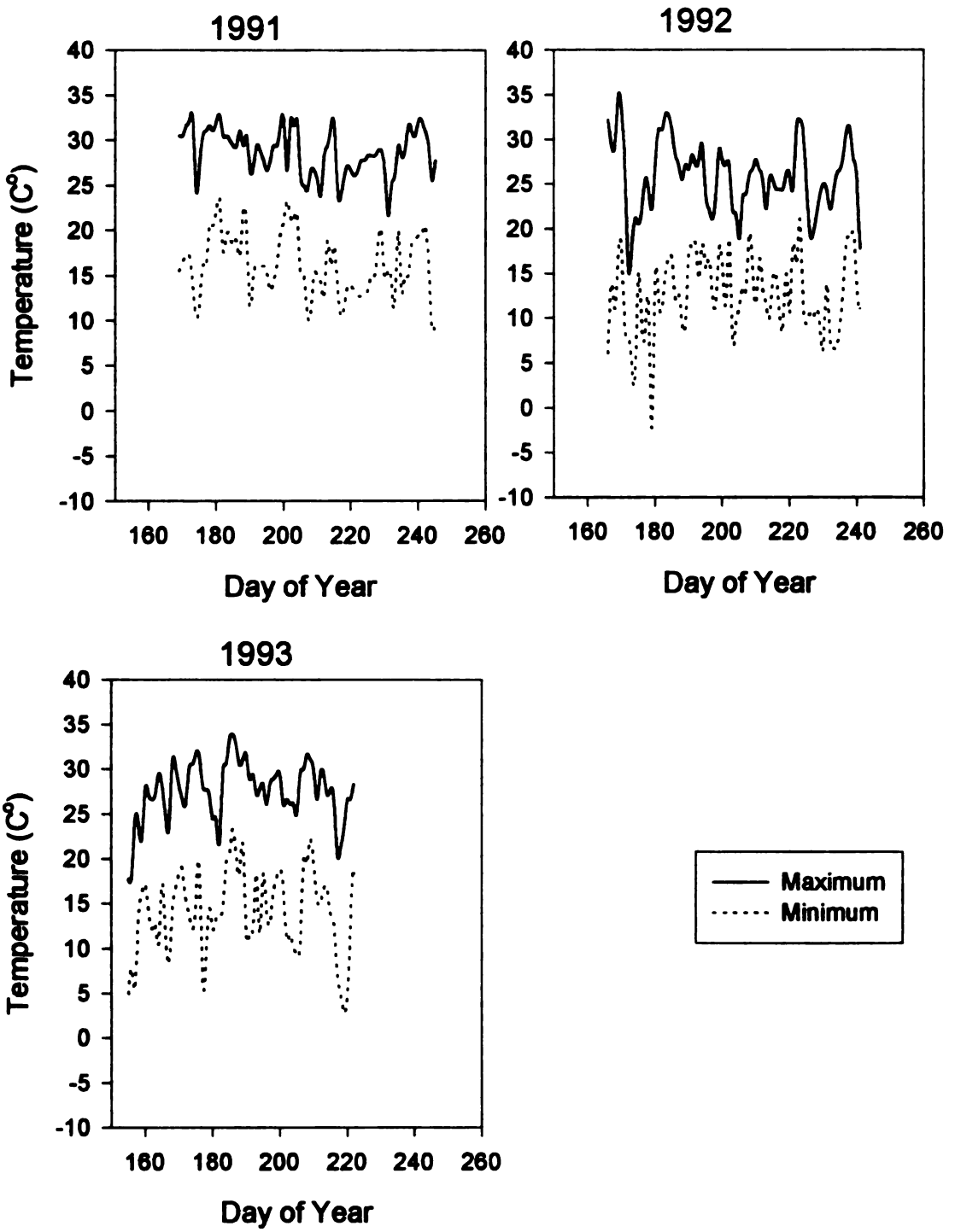

Figure 1. Maximum and minimum air temperatures for the growing season at experimental site 1991-1993.

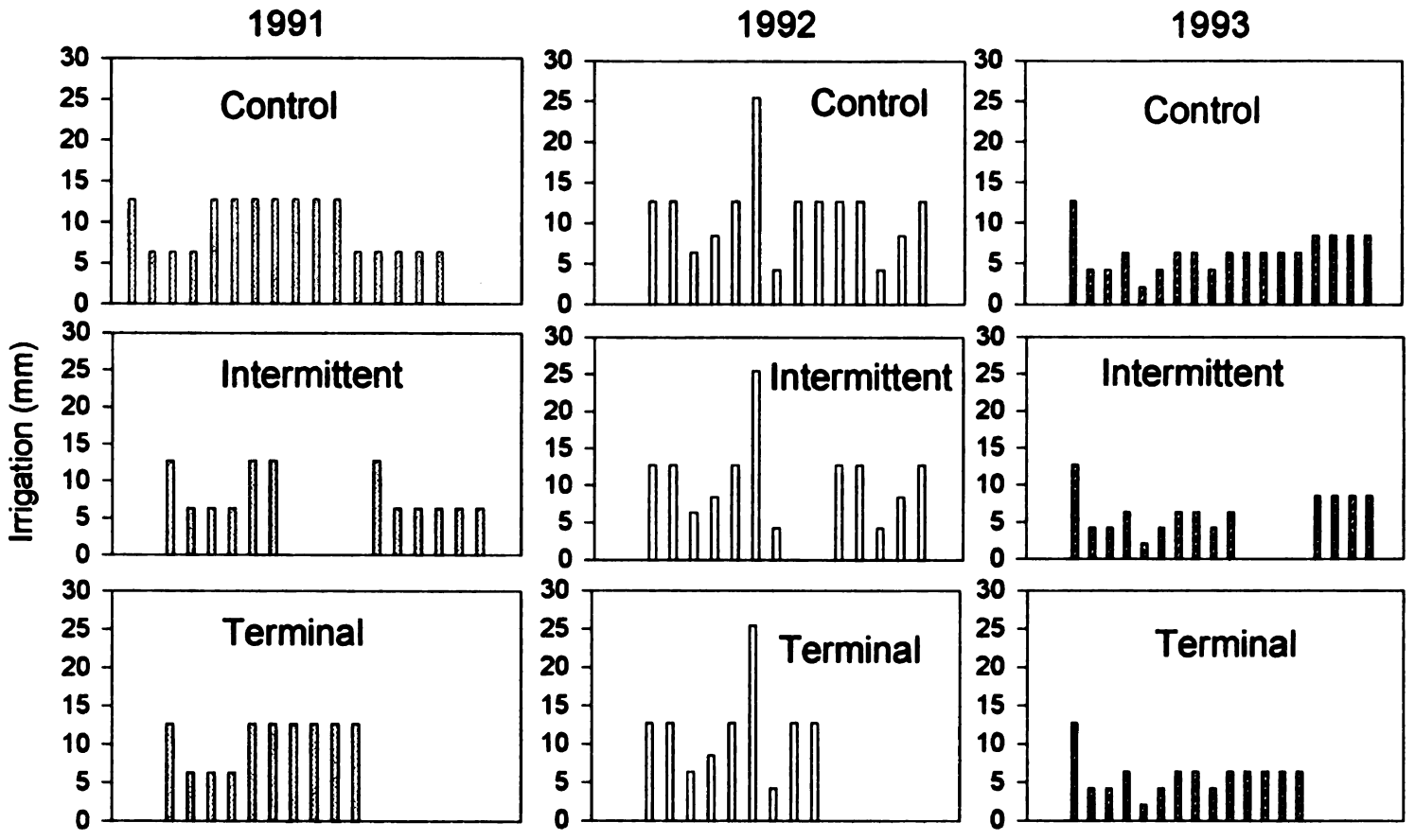

Figure 2. Different irrigation treatments for three common bean genotypes grown in a rain shelter at Hickory Corners, Michigan 1991-1993.

Table 1. Irrigation treatment and application for three common bean genotypes grown in a rain shelter at Hickory Corners, Michigan 1991-1993.

Year	Irrigation Treatment	Growth Stages: Planting to 5-leaf	5-leaf to R1†	R1 to Harvest	Sum	% of Control
		mm				
1991	Control (W1)	114	102	89	305	100
	Stress (W2)	114	0	89	203	67
	Stress (W3)	114	102	0	216	71
1992	Control (W1)	152	51	90	294	100
	Stress (W2)	152	0	90	242	83
	Stress (W3)	152	51	0	203	69
1993	Control (W1)	114	51	85	250	100
	Stress (W2)	114	0	85	199	80
	Stress (W3)	114	51	0	165	66

†R1=First open flower in any node.

and in Figure 2.

Phenology

Water stress treatments were matched with phenological growth stages to elucidate the differential effect of moisture deficit on physiological and morphological traits. Phenological stages were measured by counting leaf number during vegetative growth. The first flower was considered as R1 and the details of other growth stages were based on the procedure of Nuland and Schwartz (1989).

CID, Water use, and WUE

Carbon isotope discrimination was measured by sampling 5 fully open, expanding trifoliates per plot at mid-pod filling time (R7). These were then bulked per plot, dried in the oven for 48 hours at 70 °C and ground before sending for $^{13}C/^{12}C$ analysis. Leaf samples were analyzed by mass spectrometry at the University of Utah (1991) or by Isotope Services Inc., Los Alamos (1992, 1993). Laboratory results are usually expressed in negative values representing isotopic compositions of $\delta^{13}C$ since plant materials have usually lower ^{13}C than the standard Pee Dee Belemnite (PDB). These negative $\delta^{13}C$ values are converted to CID or Δ as follows:

$$CID(\Delta) = (\delta^{13}C_{air} - \delta^{13}C_{plant})/(1+\delta^{13}C_{plant})$$ (Farquhar et al., 1989).

Water use was calculated from neutron probe readings before and after the water treatment. The access tube for each plot was placed on one of the two center rows of each plot. The count ratios of the neutron probe were calibrated with gravimetrically measured soil moisture for each depth. Water use efficiency (WUE) was measured from the ratio of biomass yield and seasonal cumulative water use.

Grain Yield, Yield Components, and Relative Biomass

Grain yield, yield components and the above ground dry weight (biomass) data were recorded from the harvested samples of 1.5 m of the two center rows. Relative water use is defined as (control-stress)/control. It was determined by using a neutron probe to assess water extraction from a 1.20 m depth. Drought resistance is defined as relative yield reduction in response to drought: (control-stress)/control (Sinha, 1986; Doorenbos and Kassam, 1979).

The data were analyzed by the SAS General Linear Models (GLM) including the analysis of variance (ANOVA) and regression (SAS Institute, 1993). Further analysis of the differences between genotypes under specific water treatments was done by analyzing the data as a randomized complete block design when there was no water by genotype interaction. Correlation analysis was done to examine any associations among parameters. A combined analysis over all the years was done whenever the single year results were similar or in addition to individual yearly analysis to show the general trend. Means from different treatments were compared by using Fisher's ANOVA protected Least Significant Difference (LSD).

RESULTS AND DISCUSSION

CID and Water Stress

Intermittent vegetative water stress reduced CID in 1991 while terminal reproductive stress reduced CID relative to the control in 1992 and 1993 (Table 2). Across water treatments, Seafarer had a significantly higher CID than both BAT 477 and LEF2RB in 1992 and 1993. The latter two genotypes were not significantly different

Table 2. Carbon Isotope Discrimination (CID) of three common bean genotypes grown under well watered control (W1), vegetative intermittent (W2), and reproductive terminal (W3) water stress regimes at Hickory Corners, MI in 1991-1993.

	1991	1992	1993	3-years
Treatment	----------	$\Delta \times 10^{-3}$	----------	
Control (W1)	19.60	20.44	18.07	19.34
Stress (W2)	18.58	19.73	17.90	18.75
Stress (W3)	19.24	18.63	16.51	18.03
LSD (0.05)	NS	1.15	0.88	0.67
LSD (0.10)	0.74	0.91	0.70	
Genotype				
Seafarer	19.14	20.53	18.60	19.95
LEF2RB	19.05	19.05	16.80	18.23
BAT 477	19.23	19.22	17.07	18.44
LSD (0.05)	NS	0.52	0.61	0.67

Table 3. Carbon isotope discrimination as affected by Moisture stress at different growth stages for three common bean genotypes grown in a rain shelter in Hickory Corners Michigan in 1991-1993.

		Water Stress Treatment			LSD
Year	Genotype	Control (W1)	Stress (W2)	Stress (W3)	(0.05)
		----------$\Delta \times 10^{-3}$----------			
1991	Seafarer	19.39	18.96	19.06	NS
	LEF2RB	19.77	18.01	19.38	0.76
	BAT 477	19.63	18.78	19.28	NS
	LSD (0.05)	NS	NS	NS	
1992	Seafarer	21.36	20.52	19.72	0.56
	LEF2RB	20.04	18.84	18.26	NS
	BAT 477	19.91	19.82	17.92	1.56
	LSD (0.05)	NS	1.06	0.72	
1993	Seafarer	19.08	18.93	17.79	0.42
	LEF2RB	17.65	17.02	15.74	1.31
	BAT 477	17.47	17.73	15.99	NS
	LSD (0.05)	NS	1.22	0.90	
3-yrs	Seafarer	19.99	19.52	18.84	0.41
	LEF2RB	19.10	17.95	17.65	0.77
	BAT 477	18.94	18.78	17.59	0.86
	LSD (0.05)	0.66	0.59	0.63	

(Table 2). No interactions between water treatments and genotypes were found at any year. The lack of genotype by water interaction suggested that the genotypes were similar in their reduction of CID values due to water stress. A similar lack of such interactions in the presence of treatment and genotypic differences were reported for cool season grasses (Johnson and Bassett, 1991).

For any given year, there was no significant difference in CID among the genotypes when they were grown under well watered conditions (Table 3), although BAT 477 had a significantly lower CID than Seafarer when data were combined across years. Thus, no confounding effects from preexisting varietal differences in CID potential was noted, which if found, could affect subsequent genotypic evaluations.

Intermittent stress significantly reduced CID for LEF2RB (1991) and Seafarer (1992) and the same was true for Seafarer when the data for the intermittent stress treatment were combined across years (Table 3). Under the intermittent water stress, Seafarer had a significantly higher CID than LEF2RB (1992 and 1993). There was no significant difference between BAT 477 and the other two genotypes in any year under the intermittent water stress treatment (Table 3). The ranking of the three genotypes was consistent for all three years under this treatment, unlike the variable rankings under the terminal stress treatment.

Terminal water stress reduced CID relative to the control for all genotypes when the data were combined across years (Tables 2). This was true for Seafarer (1992, 1993), LEF2RB (1991,1993) and BAT 477 (1992)(Table 3) . Terminal stress reduced CID relative to the intermittent stress only for Seafarer (1992 and 1993) and for Seafarer

and BAT 477 when data were pooled across years. This may be an indication that the susceptible genotype was discriminating against the heavier isotope more than the resistant genotypes under this stress. Since there was no significant difference between Seafarer and BAT 477 under the intermittent stress within each year (Table 3), their differences under the terminal stress may be due to differential response to drought at later growth stages. The effect of growth stage on CID determination in common bean was studied by Ehleringer (1990) who concluded that the ranking of the genotypes did not change when CID was sampled at different growth stages within a season. Carbon isotope discrimination was sampled only at the pod filling period (R7) in this study, so results cannot be compared to those of Ehleringer (1990). On the other hand, the ranking of the genotypes across water treatments appeared stable for 1992-93 (Table 2). In contrast, the genotypic ranking varied among water treatments (Table 3).

All three genotypes had positive correlations (Seafarer:$r=0.92$ $p< 0.001$ $n=11$; LEF2RB: $r=0.82^{**}$ $p<0.01$ $n=11$; BAT 477:$r=0.53+$ $p<0.10$ $n=11$) when the control treatments were compared to the vegetative stress treatments. Similar positive correlations were found between the control and reproductive stress treatments ($r=0.76^{**}$ $p<0.01$ $n=11$ for Seafarer; $r=0.64^{*}$ $p<0.05$ $n=11$ for LEF2RB; and $r=0.63^{*}$ $p<0.05$ $n=11$ for BAT 477). Ehleringer et al. (1991) found differences among cultivars based on the geographic location for which the common beans were developed when they compared irrigated and stressed treatments.

The differential reduction in CID by the genotypes due to the terminal stress might indicate that the resistant check BAT 477 was more stable than the others since it reduced

Table 4. Relative reductions- (control-stress)/control- in CID, yield, WUE, HI and pod numbers for three common bean genotypes subjected to an intermittent vegetative (W2) and a terminal reproductive(W3) moisture stress in Hickory Corners, Michigan (1991-1993).

			Relative Reductions= (Control-Stress)/Stress					
Stress Treatment	DS†	Genotype	CID	Yield	WUE	HI	Pod s Plant^{-1}	Pods m^{-2}
Stress (W2)	0.82	Seafarer	0.02ab	0.22	0.003	-0.03	0.20a	0.12ab
	0.79	LEF2RB	0.06a	0.34	0.09	0.05	0.14ab	0.19a
	0.79	BAT 477	0.005b	0.09	-0.29	0.05	0.03b	0.01b
		LSD (0.1)	0.04	NS	NS	NS	0.13	0.15
Stress (W3)		Seafarer	0.060	0.45a	0.18ab	0.13a	0.26a	0.27a
	0.82	LEF2RB	0.08	0.48a	0.29a	0.04b	0.26a	0.30a
	0.80	BAT 477	0.07	0.29b	0.002b	0.05b	0.08b	0.14b
	0.77	LSD (0.1)	NS	0.15	0.19	0.07	0.12	0.10

†Drought Stress (DS)= (control-stress)/control) in water use during treatment period.

its CID in only one year compared to the others. This stability in CID reduction from stress could be a useful indicator of drought resistance if it could be correlated to similar stability in yield reduction under drought.

CID and Drought Resistance

Drought resistance has been defined in this study as the reduction in yield or its determinants in response to soil moisture deficit relative to the well watered treatment. The resistant genotype BAT 477 had a significantly smaller relative CID reduction than LEF2RB under intermittent water stress (Table 4). In addition, BAT 477 had smaller relative reductions in pods plant^{-1} and pods m^{-2} than Seafarer and LEF2RB, respectively. There were no significant differences in relative reduction among the genotypes in yield , WUE, or HI under the intermittent stress (Table 4). Across genotypes, relative CID reduction correlated positively with relative reductions in biomass, yield, and WUE but did not correlate with reductions in HI or water use (Table 5).

The three genotypes did not differ significantly in their relative reductions for CID under terminal moisture stress (Table 4). This could indicate a similarity in their response to CID reductions at this growth stage. As expected BAT 477 was more drought resistant than Seafarer under the terminal stress with lower relative reductions in yield, HI and pod number. Surprisingly, LEF2RB was as drought susceptible as Seafarer except for HI (Table 4). Similar to the intermittent stress, relative reductions in CID positively correlated with relative reductions in biomass and WUE. Carbon isotope discrimination positively correlated with water use but not with yield under this stress. White et al. (1990) found similar results when they compared biomass and seed yield to reductions in

Table 5. The relationships of relative CID reduction-(control-stress)/control- to relative reductions in biomass, yield and yield determinants for three common bean genotypes grown under an intermittent vegetative (W2) and terminal reproductive (W3) moisture stress at Hickory Corners, Michigan 1991-1993.

Stress Treatment	Relative Reduction in	Relative Reduction in CID			
		1991	1992	1993	pooled
Stress (W2)	Biomass	0.53	0.75**	0.92***	0.76***
	Yield	0.68*	0.50+	0.60***	0.74***
	Water Use Efficiency	0.62+	0.71**	0.91***	0.72***
	Harvest Index	0.22	-0.30	0.45	0.09
	Water Use	-0.22	0.02	0.47	0.02
Stress (W3)	Biomass	0.49	0.58*	0.17	0.35*
	Yield	0.59+	0.31	0.13	0.25
	Water Use Efficiency	0.60+	0.57+	0.16	0.38*
	Harvest Index	0.43	-0.37	-0.37	-0.16
	Water Use	0.41	0.58*	0.21	0.48**

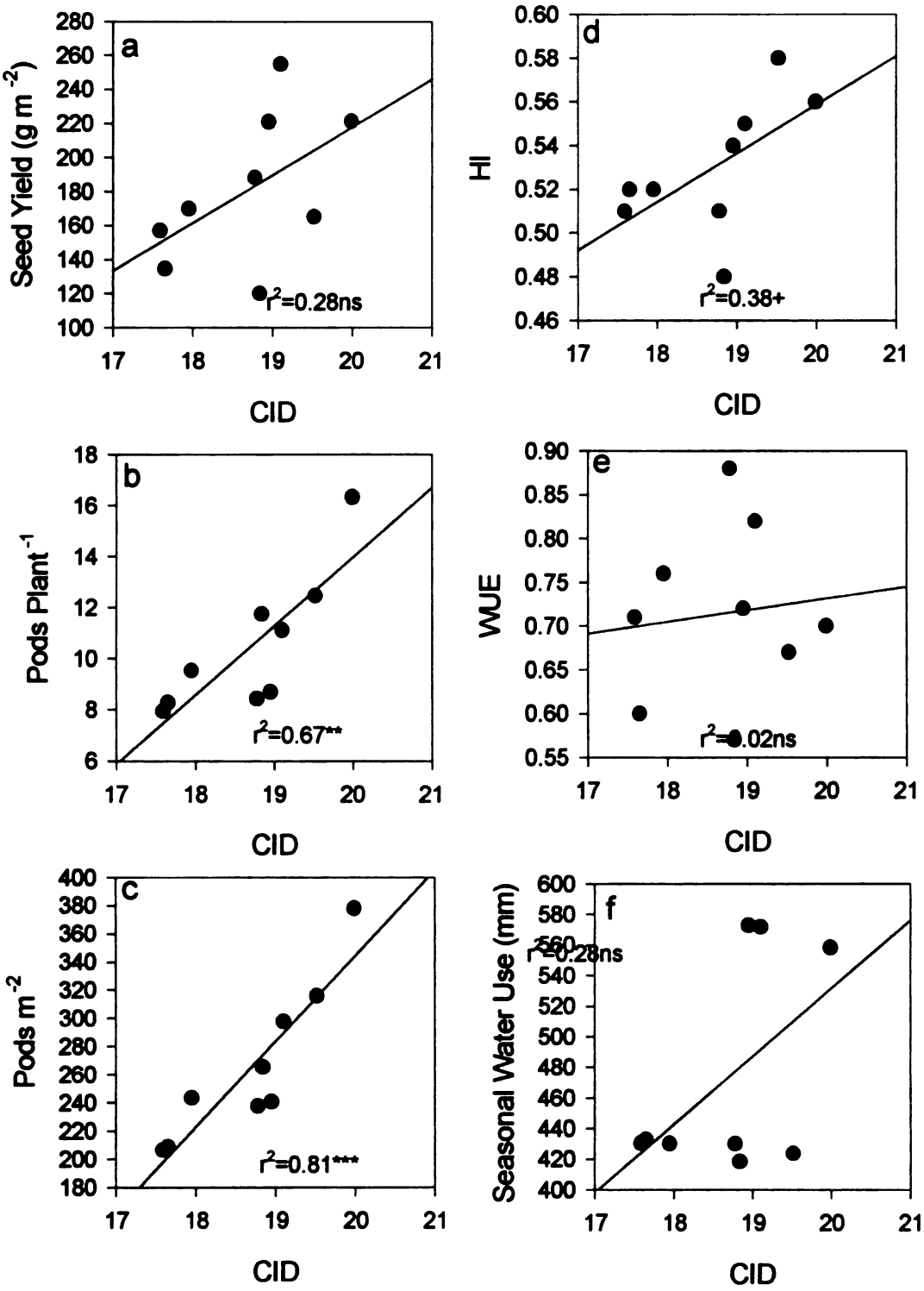

Figure 3. Relationship of carbon isotope discrimination (CID) to yield, pod number, and yield determinants when averaged across treatments for common bean grown at Hickory Corners, Michigan 1991-1993.

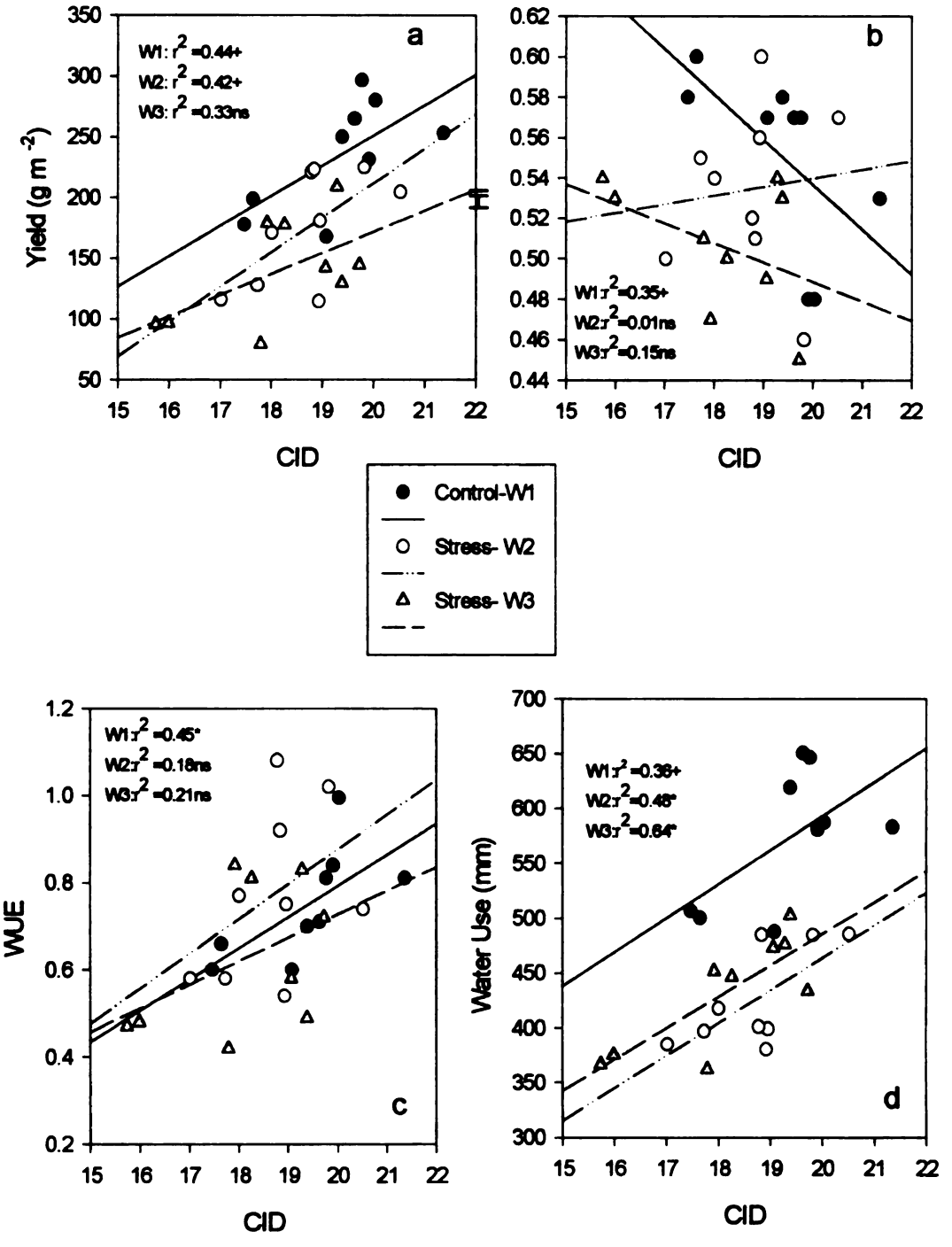

Figure 4. The relationships between CID and yield or yield determinants for three moisture deficit treatments across three common bean genotypes at Hickory Corners, Michigan 1991-1993. (Data are the pooled genotype by year means).

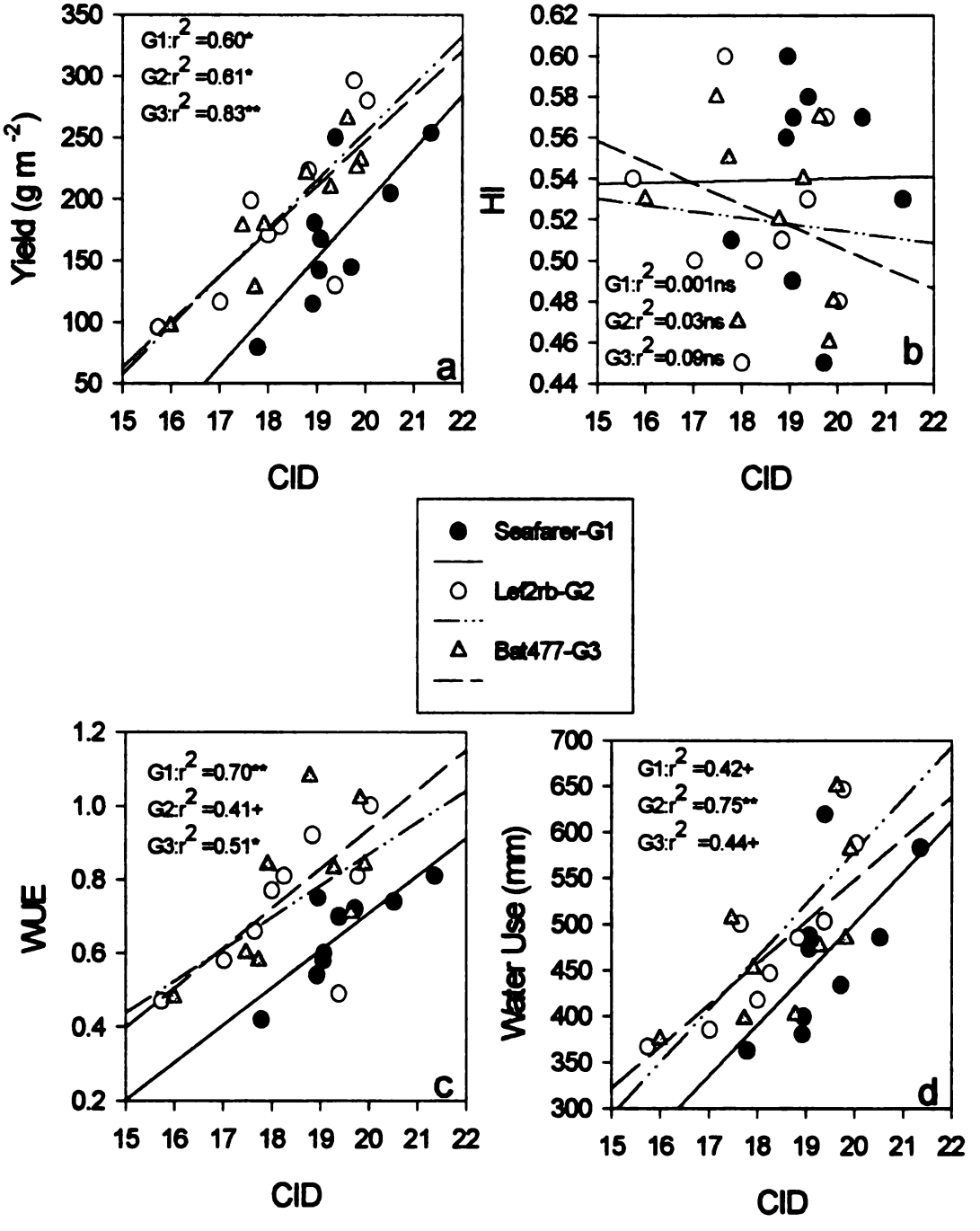

Figure 5. The relationships between CID and yield or yield determinants for three common bean genotypes grown under different moisture deficit treatments at Hickory Corners, MI 1991-1993. The data are the pooled water by year means.

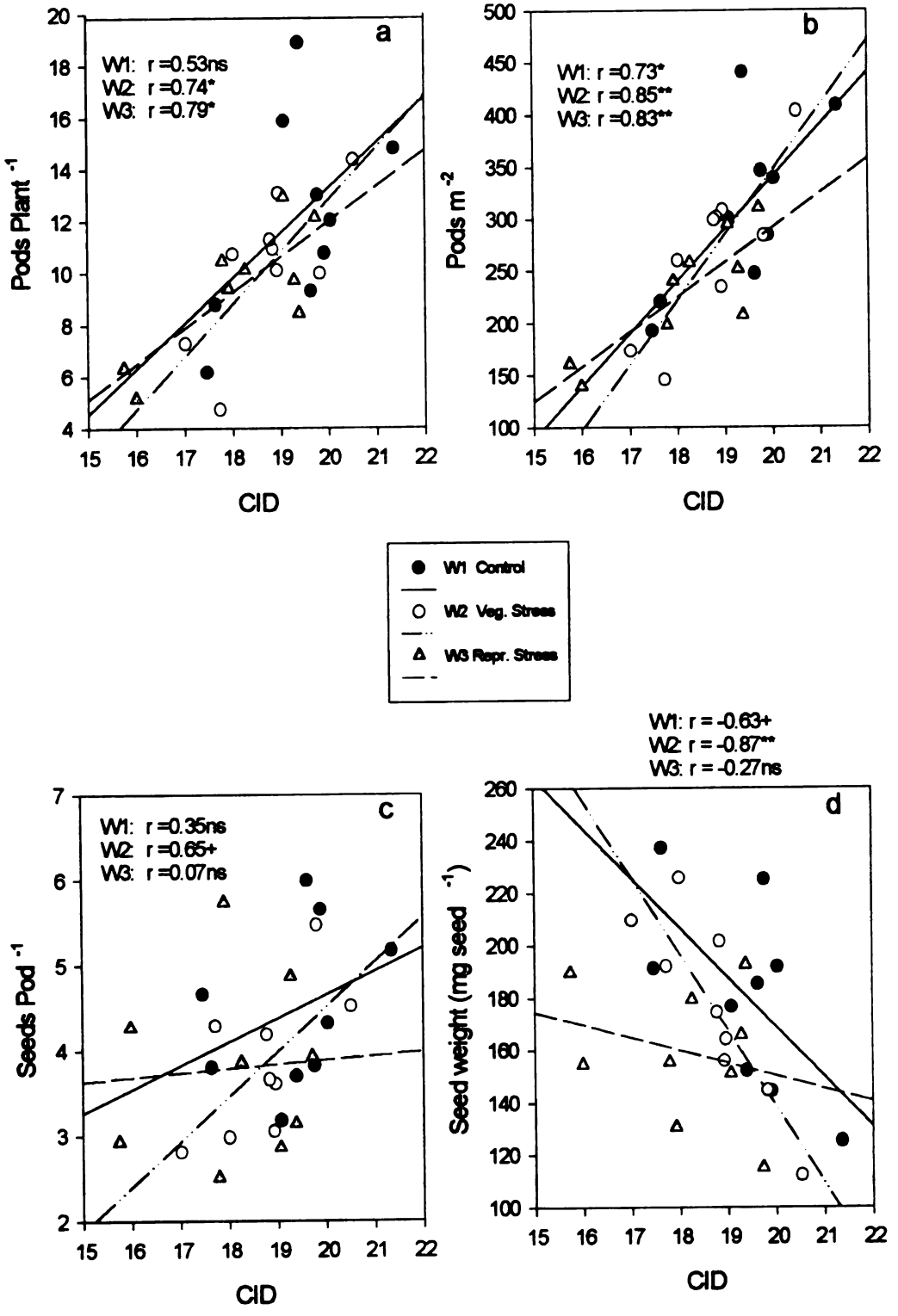

Figure 6. Correlations between CID and yield components under three water treatments across three common bean genotypes at Hickory Corners, Michigan 1991-1993. (Data are the pooled genoype by year means).

CID from control to stress.

The results presented for relative reductions indicate that these three genotypes reacted differently when subjected to similar amounts of drought stress (DS) of around 80% soil moisture depletion (Table 4). Seafarer reduced its harvest index (HI) while LEF2RB reduced its WUE more (Data not presented). Both of these genotypes reduced their pod numbers compared to the resistant genotype.

CID and Yield

There was no significant linear correlation between CID and seed yield across genotypes, water treatments, and years (Figure 3-a). There was a weak association between CID and yield under different water treatments when the data were pooled across genotypes and years (Figure 3-a). Seed yield correlated weakly with CID under the well watered and vegetative stress treatments, but not under the reproductive moisture stress treatment (Figure 4-a).

There was a strong positive correlation between seed yield and CID for each of the three genotypes when data were combined across water treatments and years (Figure 5-a). BAT 477, the resistant genotype, had a stronger association between its CID and yield than the other two genotypes. White et al. (1990) reported a lack of correlation between CID and yield in common bean and reports from other crops were inconsistent (Menendez and Hall, 1996; Matus et al., 1997; Matus et al., 1995; Condon et al., 1987).

CID and WUE

There was no significant linear correlation between CID and WUE when the data were combined across water treatments, genotypes, and years (Figure 3-e). These results

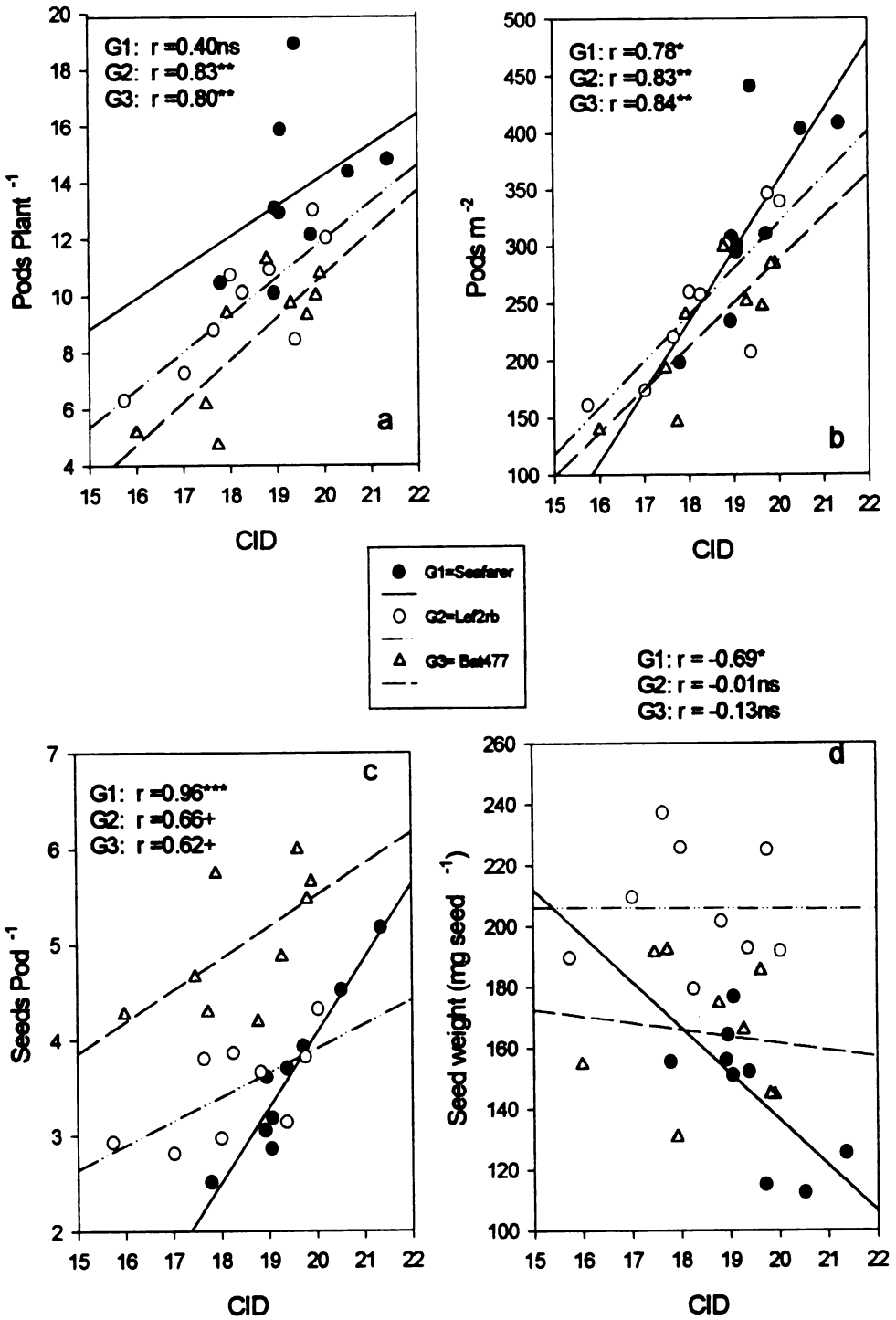

Figure 7. Correlation between CID and the yield components of three common bean genotypes grown under three moisture treatments at Hickory Corners, Michigan 1991-1993. (Data are the pooled water by year means)

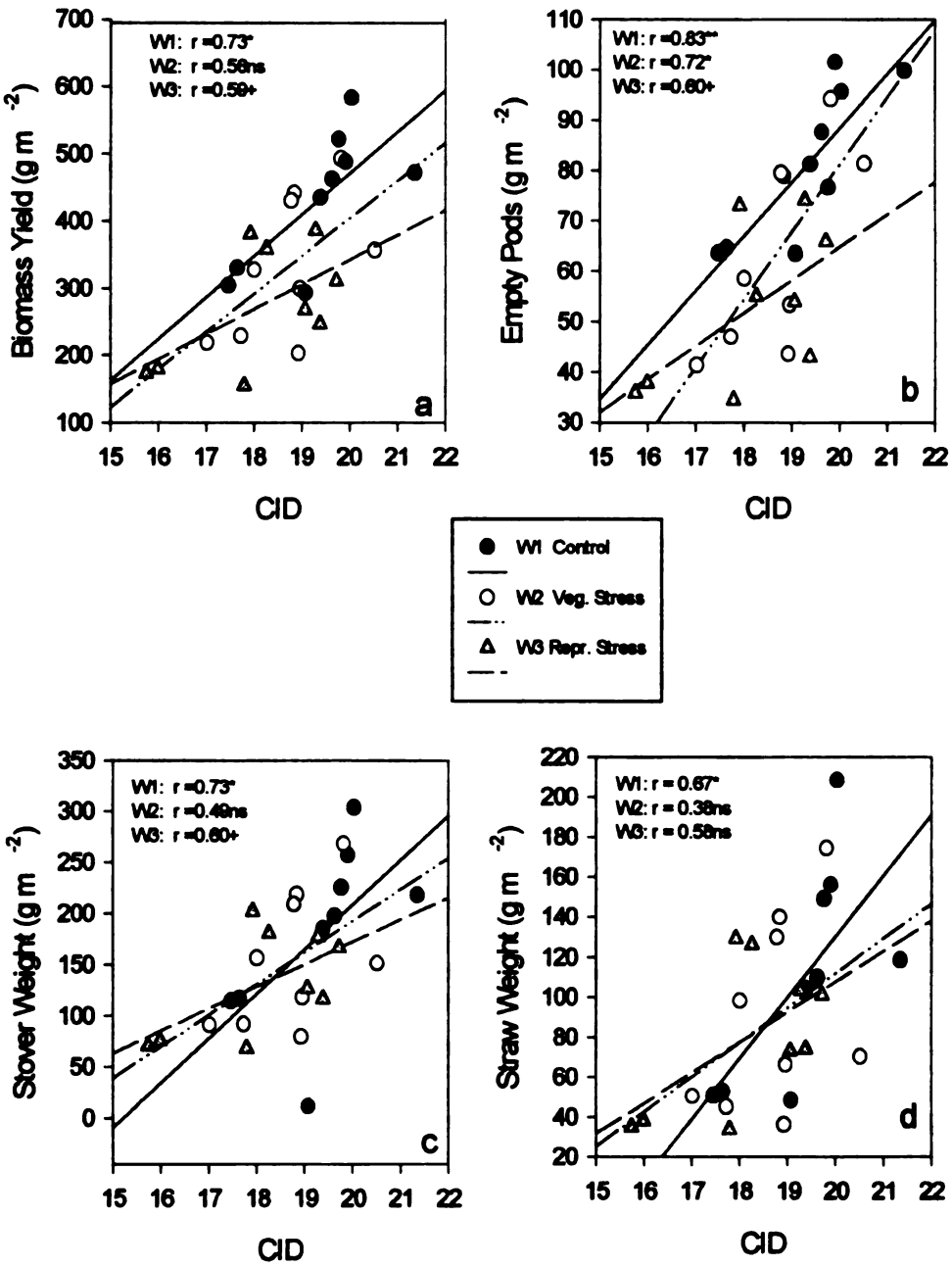

Figure 8. Relationship between CID and biomass components of three common bean genotypes as affected by different moisture treatments at Hickory Corners, Michigan 1991-1993. Data are the pooled genotype by year means.

disagree with those of Saranga et al. (1998) in cotton. There was a significant association between CID and WUE under the well watered treatment (Figure 4-c). In contrast, CID did not correlate with WUE under any stress treatment (Figure 4-c).

CID correlated positively with WUE for all three genotypes (Figure 5-c) . However, Seafarer and BAT 477 had stronger relationship between CID and WUE than LEF2RB (Figure 5-c). Ehleringer et al., (1991) found differences in the slopes of the WUE and CID correlations based on the geographical area for which genotypes were developed (North/South American). We did not find any such difference between Seafarer (North American) and BAT 477 (South American) using Chi square ($p<0.05$ $n=11$). Positive correlations between CID and WUE were reported for wheat (Farquhar and Richards, 1984), but negative correlations were found between CID and WUE in peanuts (Craufurd et al., 1999) and in coffee (Meinzer et al., 1990).

CID and HI

There was a weak linear correlation between CID and HI (Figure 3-d) when the data were combined across water treatments, genotypes, and years. This weak association was lost when data were separated by water treatment (Figure 4-b). Similarly, there was no significant linear association between CID and HI for any of the three genotypes (Figure 5-b). This differs from the low to moderate correlation between CID and HI that Menendez and Hall (1996) reported in cowpea, and the significant and positive correlation that Matus et al. (1995) reported in lentil.

CID and Water Use

There was no significant linear correlation between CID and water use (Figure 3-f) when the data were combined across water treatments, genotypes, and years. There was a positive linear association between CID and water use under specific water treatments. This association was weakest under the well watered conditions and strongest under the terminal moisture stress treatment (Figure 4-d). Carbon Isotope Discrimination correlated positively with water use for all three genotypes but LEF2RB had a stronger correlation than the other genotypes (Figure 5-d).

CID and Yield Components

There was a highly significant positive correlation between CID and pods plant^{-1} or pods m^{-2} (Figure 3-b and c). CID did not correlate with other yield components like seeds per pod and seed weight across treatments (Data not presented). Similar results were found for biomass components other than seed yield except that empty pod weights correlated weakly with CID (r^2=0.37, $p<0.10$, n=11).

Seed yield components indicated contrasting relationships with CID under different water treatments. Under well-watered conditions, CID strongly and positively correlated with pods m^{-2}, and weakly but negatively with seed weight (Figure 6-b,d). Under vegetative moisture stress, CID correlated positively with pods plant^{-1}, pods m^{-2}, and seeds pod $^{-1}$ but had a strong negative correlation with seed weight (Figure 6-a,b,c,d). Additionally, the correlations with pod number were much stronger than the correlations with seed number. Under the reproductive moisture stress treatment, CID strongly and positively associated with pods plant^{-1}and pods m^{-2} but did not correlate with the other

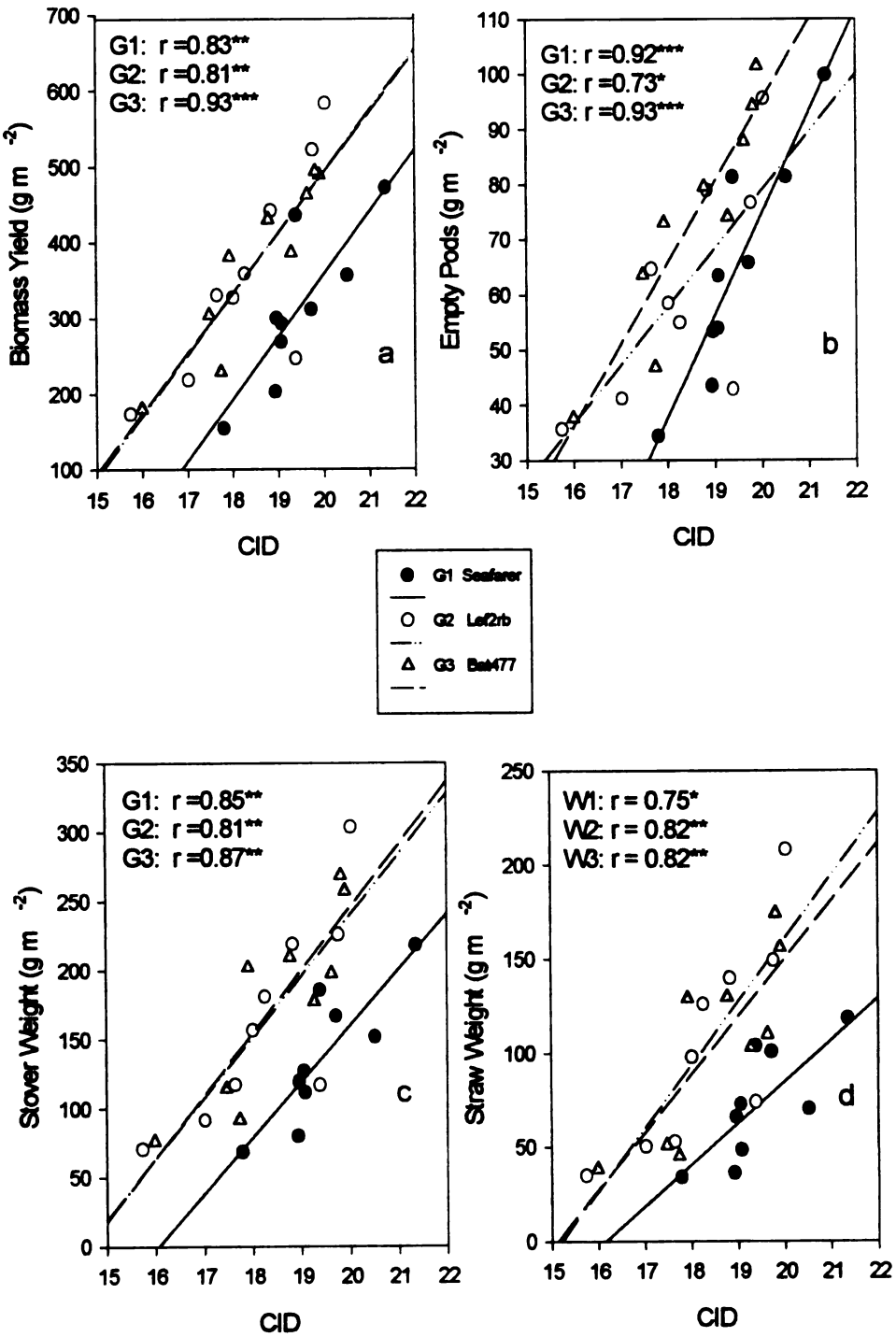

Figure 9. Correlation between CID and biomass components of three common bean genotypes grown at Hickory Corners, Michigan 1991-1993. Data are the pooled water by year means.

seed yield components (Figure 6).

CID did not correlate significantly with pods plant^{-1} for Seafarer but strongly did so for the other genotypes (Figure 7-a), yet CID strongly correlated with pods m^{-2} for all three genotypes (Figure 7-b). Seafarer had the strongest correlation between CID and seeds pod^{-1} (figure 7-c), and only Seafarer had a strong negative correlation with seed weight (Figure 7-d). This contrasting relationship of CID with seed yield components due to genotype or water treatment may have masked the overall relationship between CID and seed yield across genotypes and water treatments (Figure 3-a).

CID, Biomass and Biomass Components

There was a strong positive correlation between CID and biomass under the well watered conditions (Figure 8-a). In contrast, there was no significant correlation between CID and biomass under the vegetative moisture stress (Figure 8-a). Positive correlations between CID and biomass were reported in wheat (Condon et al., 1987) while negative correlations were found in cool season grasses (Johnson and Bassett, 1991). In common bean, White et al., (1990) reported positive CID and biomass correlations in one of three sites but no negative relationship was noted.

CID strongly and positively correlated with biomass in each of the genotypes (Figure 9-a). The susceptible genotype (Seafarer) could be identified from the resistant genotypes by its lower line indicating that it had a higher CID per biomass.

There was a positive linear relationship between CID and empty pod weight under all water treatments (Figure 8-b). This relationship was stronger under the control and vegetative stress treatments and was weaker under the terminal reproductive stress

(Figure 8-b). A strong and positive correlation was found between CID and empty pod weight for all three genotypes (Figure 9-b).

Similarly, there was a strong positive correlation between CID and stover yield under the well watered conditions (Figure 8c). But this relationship was either non existent or weaker under the stress treatments (Figure 8-c). CID strongly and positively correlated with stover yield for all three genotypes (Figure 9-c).

Straw yield correlated with CID only under the intermittent water stress treatment (8-d). Across water and years, however, a strong and positive correlation was found between CID and straw yield for all three genotypes (Figure 9-d).

CONCLUSION

It has been proposed that, in drought prone environments, yield is the product of water use efficiency (WUE), harvest index (HI), and water used by the plants (Passioura, 1977; Menendez and Hall 1996). Carbon isotope discrimination could potentially serve as useful screening tool for yield or its determinants in drought prone environments. The theoretical models relating CID to assimilation rate and hence productivity also predict a negative relationship with water use efficiency (Farquhar et al., 1982; Farquhar et al., 1989; Ehleringer et al., 1991).

Contrasting correlations for CID with water use efficiency, water use, and productivity have been reported for different crops. These include reports about the relationships between CID and biomass (Johnson and Bassett, 1991; Condon et al., 1987), CID and yield (White et al., 1990; Matus et al., 1997), and CID and WUE (Johnson and Bassett, 1991; Saranga et al., 1998; Meinzer et al., 1990; Craufurd et al., 1999).

Our results generally confirm those of other bean researchers who reported positive associations between CID and productivity (White et al., 1990; Ehleringer, 1990; Ehleringer et al., 1991) and other crops (Saranga et al.,1998; Matus et al.,1995). In contrast, we did not find negative correlations for CID and WUE or water use as reported for beans (Ehleringer et al., 1991), cowpeas (Ismail and Hall, 1992), peanuts (Brown and Byrd, 1996; Hubick, 1990), barley (Hubick and Farquhar, 1989) and tomatoes (Martin and Thorstenson, 1988). Departures from theory for CID specially in field grown crops had been discussed before (Farquhar et al.,1989; Matus et al., 1997) and might have contributed to the positive relationship between CID and water use efficiency in our study.

In conclusion, our results directly relate relative CID reductions to genotypic drought resistance defined as relative reduction in productivity due to moisture deficit and to relative reductions in water use and water use efficiency as well (Tables 4 and 5). No such relationships were found for harvest index (HI). Our results need further verification with larger number of genotypes and environments but do clearly indicate that CID can be used as screening tool when selecting for drought resistant genotypes.

REFERENCES

Adams, M. W. 1996. An historical perspective on significant accomplishments in dry bean research- the first 100 years. Bean Improvement Cooperative Annual Report 39:32-45.

Brown, R.H., and G. T. Byrd. 1996. Transpiration efficiency, specific leaf weight, and mineral concentration in peanut and pearl millet. Crop Science 36:457-480

Clarke, J. M., and T. F. Townley-Smith. 1984. Screening and selection techniques for improving drought resistance. *In* P. B. Vose and S. G. Blixt. Crop breeding: a contemporary basis. Pergamon Press, New York.

Condon, A. G., R. A. Richards, and G. D. Farquhar. 1987. Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field-grown wheat. Crop Science 27:996-1001.

Craufurd, P. Q., T. R. Wheeler, R.H. Ellis, R. J. Summerfield, and J. H. Williams. 1999. Effect of temperature and water deficit on water-use efficiency, carbon isotope discrimination, and specific leaf area in peanut. Crop Sci. 39:136-142.

Doorenbos, J. and A. H. Kassam. 1979. Yield response to water. FAO Irrigation and Drainage Paper no. 33, Rome.

Ehleringer, J. R. 1990. Correlations between carbon isotope discrimination and leaf water conductance to water vapor in common beans. Plant Physiol. 93:1422-1425.

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

Farquhar, G. D., J. R. Ehleringer, and K. T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40:503-537.

Farquhar, G. D., M. H. O'Leary, and J. A. Berry. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Aust. J. Plant Physiol 9:121-137.

Hubick, K. T. 1990. Effects of nitrogen source and water limitation on growth, transpiration efficiency and carbon-isotope discrimination in peanut cultivars. Aust. J. Plant. Physiol. 17:413-430.

Hubick, K. T. and G. D. Farquhar. 1989. Carbon isotope discrimination and the ratio of carbon gained to water lost in barley cultivars. Plant, Cell and Environment

12:795-804.

Ismail, A. M. and A. E. Hall. 1992. Correlation between water- use efficiency and carbon isotope discrimination in diverse cowpea genotypes and isogenic lines. Crop Sci. 32:7-12.

Johnson, R. C., and L. M. Bassett. 1991. Carbon isotope discrimination and water use efficiency in four cool-season grasses. Crop Sci. 31:157-162.

Ludlow, M. M., and R. C. Muchow. 1990. A critical evaluation of traits for improving crop yields in water-limited environments. Adv. Agron. 43:107-153.

Martin, B. and Y. R. Thorstenson. 1988. Stable carbon isotope discrimination, water use efficiency, and biomass productivity of Lycopersicon esculentum, Lycopersicon penelli, and the F1 hybrid. Plant Physiol. 88:213-217.

Martin, E. C., J. T. Ritchie, S. M. Reese, T. L. Loudon, and B. Knezek. 1988. A large-area, light-weight rainshelter with programmable control. Trans. ASAE 31:1440-1444.

Matus, A., A. E. Slinkard, and C. van Kessel. 1995. Carbon isotope discrimination and indirect selection for seed yield in lentil. Crop Sci. 35:679-684.

Matus, A., A. E. Slinkard, and C. van Kessel. 1997. Genotype x Environment interaction for carbon isotope discrimination in spring wheat. Crop Sci. 37:97-102.

Menendez, C., and A. E. Hall. 1996. Heritability of carbon-isotope discrimination and correlations with harvest index in cowpea. Crop Sci. 36:233-238.

Meinzer, F. C., G. Goldstein, and D. A. Grantz. 1990. Carbon Isotope discrimination in coffee genotypes grown under limited water supply. Plant Physiol. 92:130-135.

Nuland, D.S., and H. F. Schwartz. 1989. A visual description of the common bean plant: Four major growth periods. Bean Improvement cooperative 32:16-17.

Passioura, J. B. 1977. Grain yield, harvest index, and water use of wheat. J. Aust. Inst. Agric. Sci. 43:117-121.

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

SAS Institute. 1993. SAS/STAT guide for personal computer, version 6, 4th ed. SAS Inst., Cary, NC.

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

Seemann, J. R., and C. Critchley. 1985. Effects of salt stress on the growth, ion content, stomatal behavior and photosynthetic capacity of a salt-sensitive species, Phaseolus vulgaris L. Planta 164:151-62.

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

Sinha, S. K. 1986. Drought resistance in crop plants: a physiological and biochemical analysis. *In* V. L. Chopra and R. S. Paroda (eds.) Approaches for incorporating drought and salinity resistance in crop plants. Oxford & IBH Publishing Co., New Delhi.

Stanhill, G. 1986. Water use efficiency. Adv. Agron. 39:53-83.

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

CHAPTER 4

OSMOTIC ADJUSTMENT IN COMMON BEAN SUBJECTED TO WATER STRESS

ABSTRACT

The role of osmotic adjustment due to moisture stress and its contribution to yield or yield determinants in common bean (*Phaseolus vulgaris* L.) have not been well documented. This study was conducted to assess genotypic differences in osmotic adjustment and its relationship to yield, harvest index, water use efficiency, rooting depth and carbon isotope discrimination. Three common bean genotypes with similar yield potential but differing in drought resistance were grown in a rain shelter at Hickory Corners, MI in 1991 - 1993 in a split plot design. Treatments were a control, intermittent vegetative stress, and a terminal reproductive stress. Water potential and osmotic potential were significantly reduced (15 to 59%) while solute accumulation increased (21 to 34%) under the stress treatments. Genotypic differences in osmotic adjustment were found only under terminal moisture stress. Osmotic adjustment ($r = -0.91$ $p \leq 0.05$ $n=6$) and solute accumulation ($r = -0.91$ $p \leq 0.05$ $n=6$) both strongly and negatively correlated with yield. Similarly, significant negative correlations of osmotic adjustment with water use efficiency, harvest index and water use were found across water treatments at the reproductive growth stage. Solute accumulation or osmotic adjustment may be indicative of susceptibility to moisture deficit rather than resistance in common bean.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important protein source in subsistence agriculture worldwide, and common bean consumption in the United States has been increasing (Holden and Haytowitz, 1998). Terminal moisture stress which occurs after flowering and intermittent moisture stress which occurs before flowering both have been shown to reduce yield in common bean (Acosta-Gallegos et al., 1998). The development of drought resistant genotypes has been hampered by the lack of screening techniques that easily distinguish between resistant and susceptible genotypes.

Osmotic adjustment, the ability of plants to actively accumulate solutes to maintain cell turgor and volume (Radin, 1983; Guei and Wassom, 1993), has been suggested as a criterion for selection of drought resistant genotypes (Ludlow and Muchow, 1990; Morgan, 1983; Sloane et al., 1990; Rodriguez et al., 1992; Kumar and Singh, 1998) and has, in some cases, been shown to be controlled by a single gene (Morgan, 1991). Compared to other crops, few studies have reported osmotic adjustment or water relations for common bean (Parsons and Howe, 1984; Wullschleger and Oosterhuis, 1991; Raggi, 1994), and all three studies were conducted in a controlled environment.

The transferability of findings from a controlled environment to a field situation has not been consistent (Morgan et al., 1991; Oosterhuis and Wullschleger, 1987; Turner et al., 1987). Similarly, there has been no consensus on the benefits of osmotic adjustment to yield under drought (Morgan et al., 1991; Cortes and Sinclair, 1986;Li et al., 1993; Guei and Wassom, 1993; Kumar and Singh, 1998; Blum, 1989; Rodriguez et al., 1992). It is critical to obtain this information since any selection criterion that has no direct

relationship with yield or its determinants may not be useful in drought-prone environments (Passioura, 1994; Menéndez and Hall, 1996; Passioura, 1977).

This study investigated the relationship of osmotic adjustment, relative water content (RWC), and water potential components to yield, harvest index, and water use efficiency in three common bean genotypes that differed in yielding ability under drought but which were similar in yield potential. The objectives were (1) to assess osmotic adjustment in common bean grown in a field environment, (2) to assess genotypic differences in osmotic adjustment or water relations, and (3) to determine the relationship of osmotic adjustment and other water relations variables to yield, harvest index, water use efficiency, rooting depth, and carbon isotope discrimination.

MATERIALS AND METHODS

Three common bean genotypes were planted on a Kalamazoo soil (Fine-loamy, mixed, mesic Typic Hapludalfs) under a rain shelter (Martin et al., 1988) at the Michigan State University Kellogg Biological Station at Hickory Corners, Michigan on 18 June 1991, 14 June 1992, and 4 June 1993 and harvested in September each year. Based on soil test results, approximately 98 kg ha^{-1} of urea (46% N) was applied as a starter fertilizer each year together with *Rhizobium phaseoli* to avoid any existing non-uniformity of rhizobium in the soil. Agronomic practices were similar each year and reflected those recommended for commercially grown crops. The automatic rain shelter was set to close as soon as it accumulated 15 mm of rainfall. Maximum and minimum temperatures are presented in Figure 1 for the three seasons.

The experimental design was a split plot with water as the main plot, genotype as

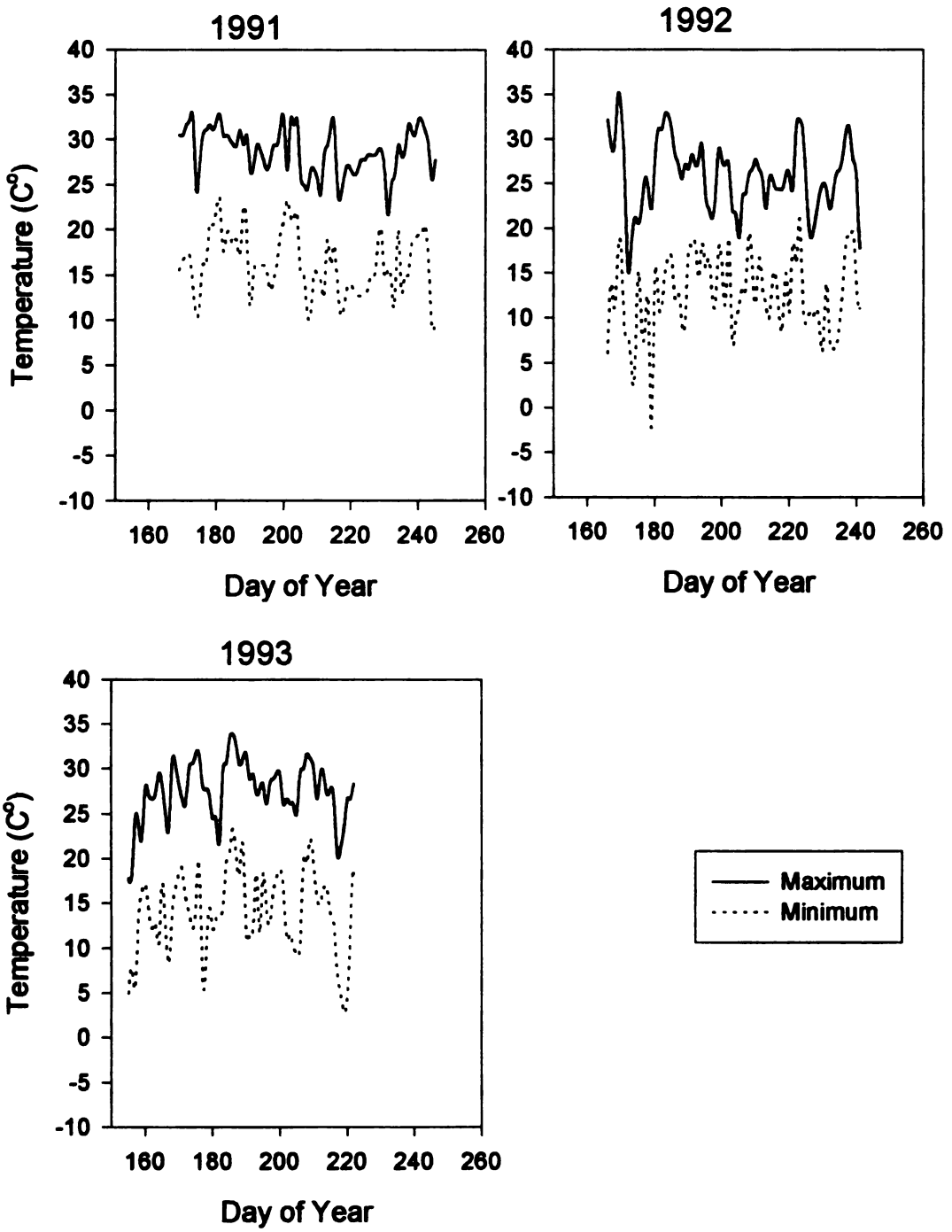

Figure 1. Maximum and minimum air temperatures for the growing season at experimental site 1991-1993.

the subplot, and three (1991) or four (1992 and 1993) replications. The main plot was 6 m by 3.5 m and was sub-divided into 3 sub-plots, each 2 m long. The three genotypes in this experiment were a drought susceptible Seafarer (G1)(Adams, 1996) and two drought resistant LEF2RB (G2) (Schneider et al., 1997) and BAT 477 (G3) (Singh, 1995) genotypes. The three water treatments were a well-watered control (W1); intermittent vegetative stress (W2)with no irrigation from 5-leaf stage (V5) to first open flower (R1); and terminal reproductive stress (W3) with no moisture from R1 to harvest. Water stress treatments were closely matched with phenological growth stages using the method described by Nuland and Schwartz (1989). The amounts and times of irrigations are presented in Table 1 and in Figure 2.

Osmotic Adjustment and Leaf Water Relations

Water potential and its components were measured using end-window thermocouple psychrometers (Merrill Specialty Equipment, 84-series, Logan, Utah). Both the calibration and equilibration methods closely followed those described by Oosterhuis and Wullschleger (1987). Each Psychrometer, representing one plot was calibrated with NaCl solutions of known water potential (Lang, 1967). A close fit was found between actual psychrometer output and those predicted by Brown and Bartos (1982) for the 15 second cool time at 25 ^{0}C temperature such that one equation was developed by linear regression from the predictive data published by Brown and Bartos (1982) to convert microvolts of field data to water potential in megapascal.

Osmotic adjustment (OA) was calculated as follows:

$OA=\pi_p-\pi_s$ where $\pi_p=(\pi_1 \times RWC_1)/RWC_2$ (Note: negative values mean $\pi_p<\pi_s$).

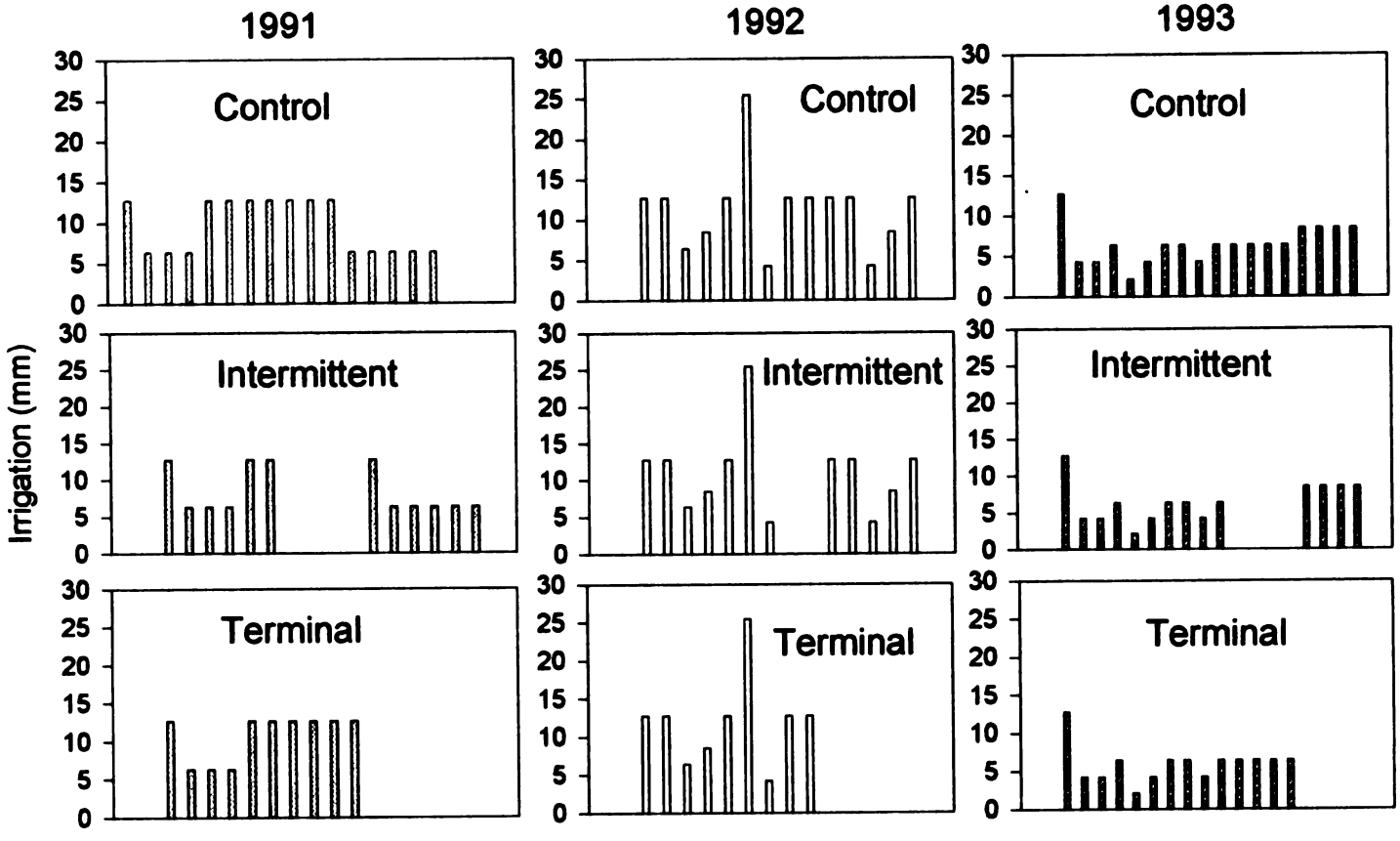

Figure 2. Different irrigation treatments for three common bean genotypes grown in a rain shelter at Hickory Corners, Michigan 1991-1993.

Table 1. Irrigation treatment and application for three common bean genotypes grown in a rain shelter at Hickory Corners, Michigan 1991-1993.

Year	Irrigation Treatment	Growth Stages: Planting to 5-leaf	5-leaf to R1†	R1 to Harvest	Sum	% of Control
		----------mm----------				
1991	Control (W1)	114	102	89	305	100
	Stress (W2)	114	0	89	203	67
	Stress (W3)	114	102	0	216	71
1992	Control (W1)	152	51	90	294	100
	Stress (W2)	152	0	90	242	83
	Stress (W3)	152	51	0	203	69
1993	Control (W1)	114	51	85	250	100
	Stress (W2)	114	0	85	199	80
	Stress (W3)	114	51	0	165	66

†R1=First open flower in any node.

π_p=Predicted Osmotic potential (Salisbury and Ross, 1985).

π_a=Actual osmotic potential measured at end of stress

π_1=Osmotic potential before stress

RWC_1=Relative water content before stress

RWC_2=Relative Water Content after stress

Solute accumulation (n)was calculated from the equation of Morgan (1991)with some modification as follows:

$n=-(\pi*RWC)/RT$

n=Number of solute molecules

R=The gas constant

T=absolute temperature (leaf temperature taken at sampling time)

π=Osmotic potential

RWC=Relative Water Content

Relative Water Content

Relative water content was measured from 3 cm by 0.9 cm leaf strips cut from one side of the midrib of the same trifoliate that was used for water potential measurements. The strips were immediately sealed in glass vials and placed in a cooler with ice to reduce photosynthesis. Each strip was weighed for fresh weight within 1 hour and returned to the vial, filled with deionized water and capped. To minimize respiration, the vials were kept in a dark room for 24 hours at 4 ^{0} C to ensure full turgidity (Pardossi et al., 1992; Lecoeur et al., 1992; Rodriguez-Maribona et al., 1992), dried with paper towel, and their turgid weight taken. Each leaf strip was then returned to its vial and oven dried at 70 ^{0} C for 48

hours. Relative water content was calculated as per Turner (1981). The 24 hour time for turgidity in this study differs from the 4 hours usually reported by other authors because it is species or genotype dependent (Turner, 1981; Yabba and Foster, 1996) and because preliminary tests found that all the leaves were not fully saturated at shorter periods for Seafarer.

Water Use and WUE

Water use was monitored using neutron probe measurements that were calibrated against soil moisture using gravimetric measurements. The water holding capacity of this soil type was found to be about 100 cm per 1.20 m profile which was the maximum depth sampled. Water use was calculated from the difference of neutron probe readings upon initiation and conclusion of the water treatment. The access tube for each plot was placed on one of the two center rows of each plot. The count ratios of the neutron probe were calibrated with gravimetrically measured soil moisture for each depth. Water use efficiency (WUE) was measured from the ratio of biomass yield and seasonal cumulative water use.

Seed Yield

Seed yield was recorded from the harvested plants of 1.5 m of the two center rows. The seed was oven dried and is reported on a 0% moisture basis.

Carbon Isotope Discrimination

Carbon isotope discrimination was measured by sampling 5 fully open, expanding leaves (trifoliates) per plot at mid pod filling time (R7). These were then bulked per plot, dried in the oven for 48 hours at 70 °C and ground before sending for analysis. Leaf

samples were analyzed by mass spectrometry at University of Utah (1991) or by Isotope Services Inc., Los Alamos (1992, 1993).

Rooting Depth

Minirhizotron tubes were placed at a 45° angle in one of the two center rows of each plot to record root growth by a video camera. Root depth was recorded by visually monitoring the depth of common bean roots in each plot through each minirhizotron tube before and after each treatment.

The data were analyzed by the SAS General Linear Model (GLM) including the analysis of variance (ANOVA) and regression (SAS Institute, 1993). Correlation analysis was done to examine associations among parameters. After checking for compliance with the assumptions for equal variance and normality, a combined analysis over all years was done whenever the single year results were similar or as an addition to yearly analysis to show the general trend. Means from different treatments were compared by using Fisher's ANOVA protected least significant difference (LSD).

RESULTS AND DISCUSSIONS

Osmotic Adjustment

Intermittent water stress did not affect osmotic adjustment across genotypes (Table 2). In contrast, there were significant differences in osmotic adjustment between the control and terminal water stress treatments (Table 2). The few studies reporting osmotic adjustment in common bean did not include the reproductive growth stages . This finding confirms that common bean is capable of osmotic adjustment but differs from earlier reports that found osmotic adjustment at the vegetative stage (Wullschleger and

Table 2. Osmotic Adjustment and other water relations variables of common bean grown at Hickory Corners Michigan under different moisture treatments, 1991-1993.

Treatment	Variable	Units	Control	Stress	LSD
		Sampled at first flower (R1)			
Intermittent Stress	Osmotic Adjustment†‡	MPa	-0.01	0.33	NS
	Solute Accumulation	Moles $Kg^{-1}H_2O$	27.72b	33.44a	*
	Water potential	Mpa	-1.22a	-1.51b	*
	Osmotic Potential	MPa	-1.45a	-1.67b	*
	Turgor Potential	Mpa	0.23	0.16	NS
	RWC	%	47.76	50.04	NS
		Sampled at mid pod (R8)			
Terminal Stress	Osmotic Adjustment	MPa	-0.04b	0.32a	***
	Solute Accumulation	Moles $Kg^{-1}H_2O$	27.31b	36.54a	***
	Water potential	Mpa	-0.86a	-1.37b	***
	Osmotic Potential	MPa	-1.15a	-1.53b	***
	Turgor Potential	Mpa	0.29a	0.16b	*
	RWC	%	59.18	59.86	NS

Note: +=p-value < 0.10 *=p-value < 0.05 **=p-value < 0.01 NS=Not Significant
Note: Same letters in a row indicate no significant differences between means.
† data for that row are from two years 1991-1992 only . The rest of the data are from all 3 years.
‡Negative osmotic adjustment means predicted osmotic potential is less than the actual.

Oosterhuis , 1991; Raggi, 1994). The different results at the vegetative stage may be due to the differences in the methods. Both of the above studies were conducted in a controlled environment and both rewatered the plants, to more than 88% of the relative water content (RWC) in the case of Wullschleger and Oosterhuis (1991), before sampling for osmotic adjustment. In this study the plants were field grown and were not rewatered similar to other field studies (Bolaños and Edmeades,1991).

The three genotypes responded differently to the intermittent vegetative stress but similarly to terminal reproductive stress. Both Seafarer and BAT 477 osmotically adjusted under the vegetative stress but LEF2RB did not (Table 3). In contrast, all three genotypes had osmotic adjustment under the reproductive stress (Table 4). Genotypic differences in osmotic adjustment were found only under terminal moisture stress where Seafarer had a higher osmotic adjustment than BAT 477 and LEF2RB and comparisons across water treatment indicated that Seafarer had a higher osmotic adjustment than the other genotypes (Tables 5 and 6).

Osmotic adjustment had a significant negative correlation with water potential and osmotic potential only at the reproductive stage sampling (Figure 3-a and b) but did not correlate with turgor potential at any growth stage sampled (Figures 3-c).

Solute Accumulation

There was a significant difference in solute accumulation between the control and stress treatments across genotypes in both growth stages (Table 2). Other studies have also reported increases in solute accumulation due to stress (Raggi, 1994; Zhang and Archbold, 1993; Dingkuhn et al., 1991). The specific solutes that accumulate appear to

Table 3. Osmotic Adjustment and other water relations variables of common bean grown at Hickory Corners Michigan under intermittent water stress, 1991-1993.

Genotype	Variable	Units	Control	Stress	LSD
Seafarer	Osmotic Adjustment	MPa	0.08b	0.39a	0.29*
	Solute Accumulation	Moles $Kg^{-1}H_2O$	29.59b	36.33a	6.51*
	Water potential	Mpa	-1.25a	-1.72b	0.41*
	Osmotic Potential	MPa	-1.53a	-1.81b	0.22*
	Turgor Potential	Mpa	0.28	0.09	NS
	RWC	%	48.44	50.36	NS
LEF2RB	Osmotic Adjustment	MPa	0.19	0.17	NS
	Solute Accumulation	Moles $Kg^{-1}H_2O$	27.64b	33.75a	5.38*
	Water potential	Mpa	-1.22a	-1.48b	0.14**
	Osmotic Potential	MPa	-1.43a	-1.67b	0.19+
	Turgor Potential	Mpa	0.21	0.19	NS
	RWC	%	48.23b	50.61a	2.21+
BAT 477	Osmotic Adjustment‡	MPa	-0.30b	0.43a	0.65+
	Solute Accumulation	Moles $Kg^{-1}H_2O$	25.92	30.23	NS
	Water potential	Mpa	-1.18	-1.32	NS
	Osmotic Potential	MPa	-1.39	-1.53	NS
	Turgor Potential	Mpa	0.21	0.21	NS
	RWC	%	46.61	49.14	NS

Note: +=p-value < 0.10 *=p-value < 0.05 **=p-value < 0.01 NS=Not Significant
Note: Same letters in a row indicate no significant differences between means. Data sampled at first flower (R1).
‡Negative osmotic adjustment means predicted osmotic potential is less than the actual.

Table 4. Osmotic Adjustment and other water relations variables of common bean grown at Hickory Corners Michigan under terminal water stress, 1991-1993.

Genotype	Variable	Units	Control	Stress	LSD
Seafarer	Osmotic Adjustment	MPa	0.04b	0.53a	0.29**
	Solute Accumulation	Moles $Kg^{-1}H_2O$	30.79b	41.03a	4.04***
	Water potential	Mpa	-0.86a	-1.42b	0.24***
	Osmotic Potential	MPa	-1.25a	-1.65b	0.18***
	Turgor Potential	Mpa	0.39	0.22	NS
	RWC	%	60.94	62.39	NS
LEF2RB	Osmotic Adjustment ‡	MPa	-0.06b	0.27a	0.24*
	Solute Accumulation	Moles $Kg^{-1}H_2O$	26.15b	35.81a	8.54*
	Water potential	Mpa	-0.84a	-1.41b	0.26**
	Osmotic Potential	MPa	-1.10a	-1.50b	0.33*
	Turgor Potential	Mpa	0.26a	0.09b	0.18*
	RWC	%	60.08	60.31	NS
BAT 477	Osmotic Adjustment	MPa	-0.09b	0.16a	0.24*
	Solute Accumulation	Moles $Kg^{-1}H_2O$	24.97b	32.79a	6.71*
	Water potential	Mpa	-0.89a	-1.27b	0.27*
	Osmotic Potential	MPa	-1.09a	-1.45b	0.25*
	Turgor Potential	Mpa	0.20	0.17	NS
	RWC	%	56.53	56.87	NS

Note: +=p-value < 0.10 *=p-value < 0.05 **=p-value < 0.01 NS=Not Significant
Note: Same letters in a row indicate no significant differences between means. Data sampled at mid pod (R8). ‡Negative osmotic adjustment means predicted osmotic potential is less than the actual.

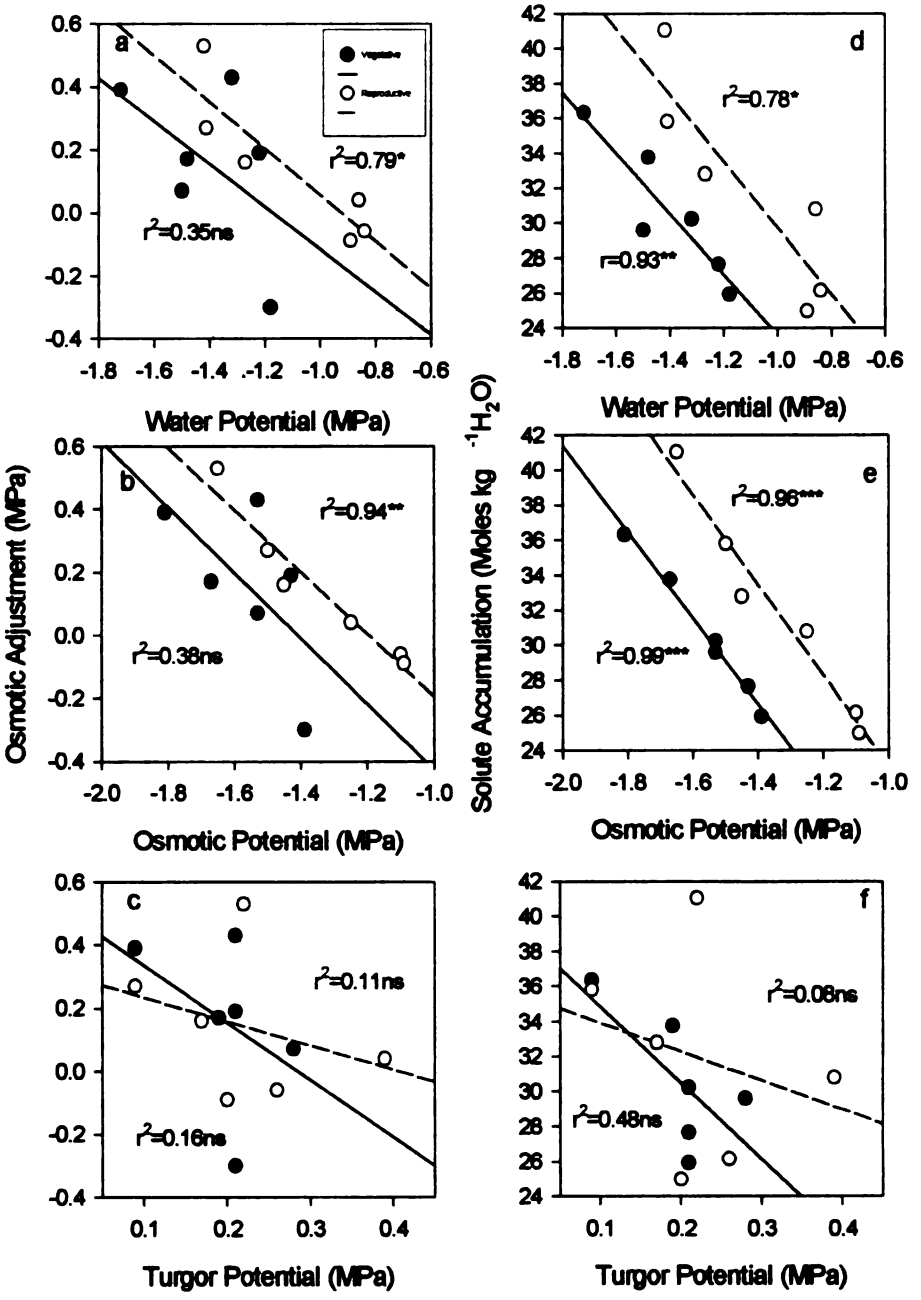

Figure 3. Osmotic adjustment and solute accumulation as related to water potential components at two growth stages for three common bean genotypes grown under differential irrigationat Hickory Corners, Michigan 1991-1993.

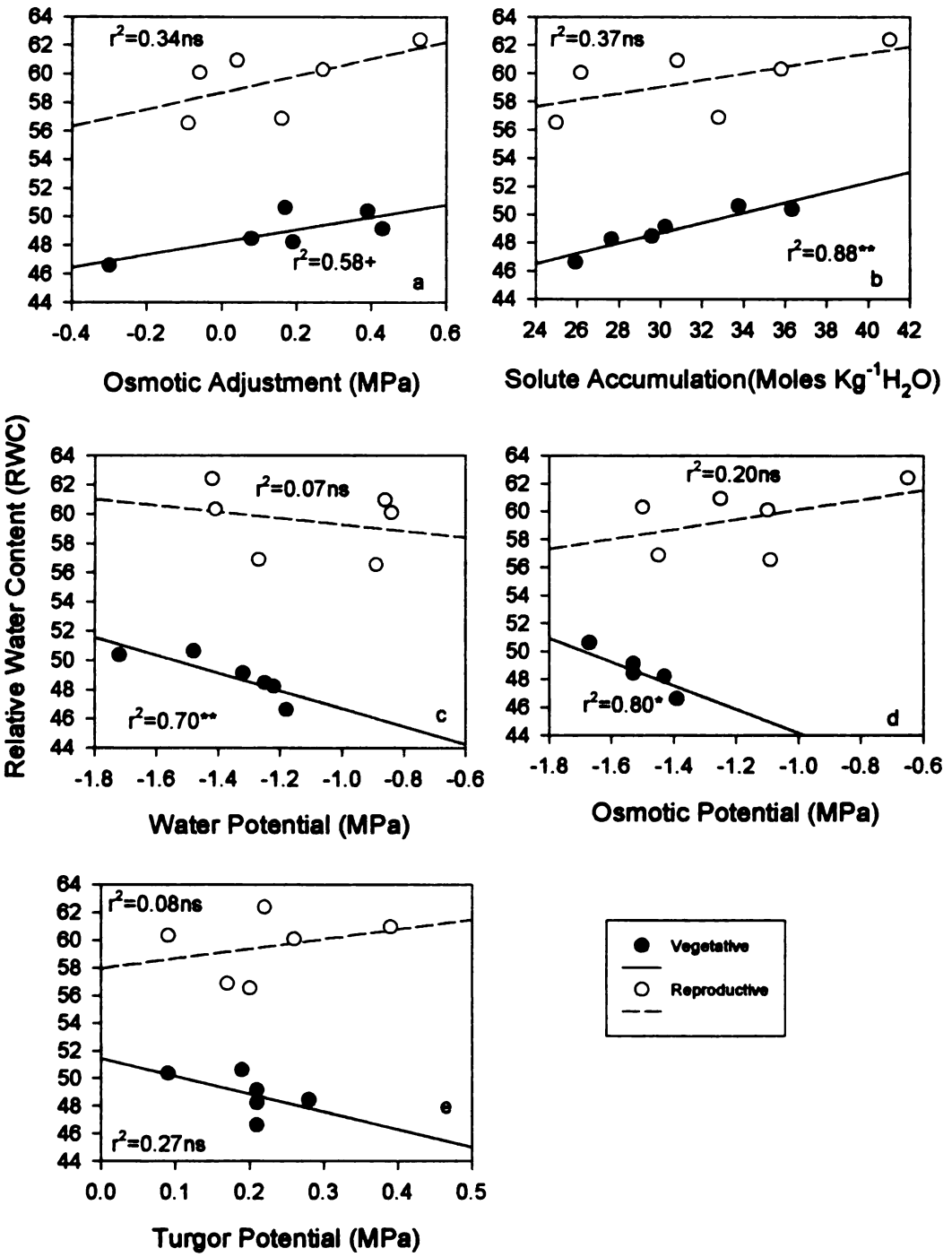

Figure 4. Relative water content (RWC) as related to osmotic adjustment, solute accumulation, and water potential components at two growth stages for three common bean genotypes grown under differential irrigation at Hickory Corners, Michigan 1991-1993.

differ among crop species. Raggi (1994) found free amino acids like proline in common bean. Proline was found in rice (*Oryza sativa*)(Dingkuhn et al.,1991) but not in *Fragaria* spp which accumulated glucose, fructose and non-proline amino acids instead. Furthermore, organic acids were found in chickpea (Lecoeur et al., 1992). It is possible that specific accumulated solutes may have differing effects on source-sink relationships, translocation, and yield.

Across years within genotypes, both Seafarer and LEF2RB increased their solute accumulation under intermittent stress ($P \leq 0.05$) while Bat 477 did not (Table 3). In addition, terminal stress increased solute accumulation in all genotypes (Table 4). Among genotypes, BAT 477 had a lower solute accumulation than Seafarer under both moisture stress treatments (Tables 5 and 6). Across water treatments, Seafarer had significantly a higher solute accumulation than LEF2RB under the reproductive growth stage (Table 6).

Solute accumulation strongly but negatively correlated with water and osmotic potentials at both growth stages, but there was no association with turgor potential (Figure 3-d, e, f)).

Relative Water Content

Although, there were no significant differences between stress and control treatments for RWC across genotypes at either growth stage (Table 2), there was a tendency for RWC to increase numerically under stress treatment. In fact, under intermittent stress, RWC increased in LEF2RB (Table 3). While significant only at the 10% probability level, the LEF2RB response indicates this tendency and agrees with Raggi (1994) who found significantly higher RWC in stressed plants but disagrees with

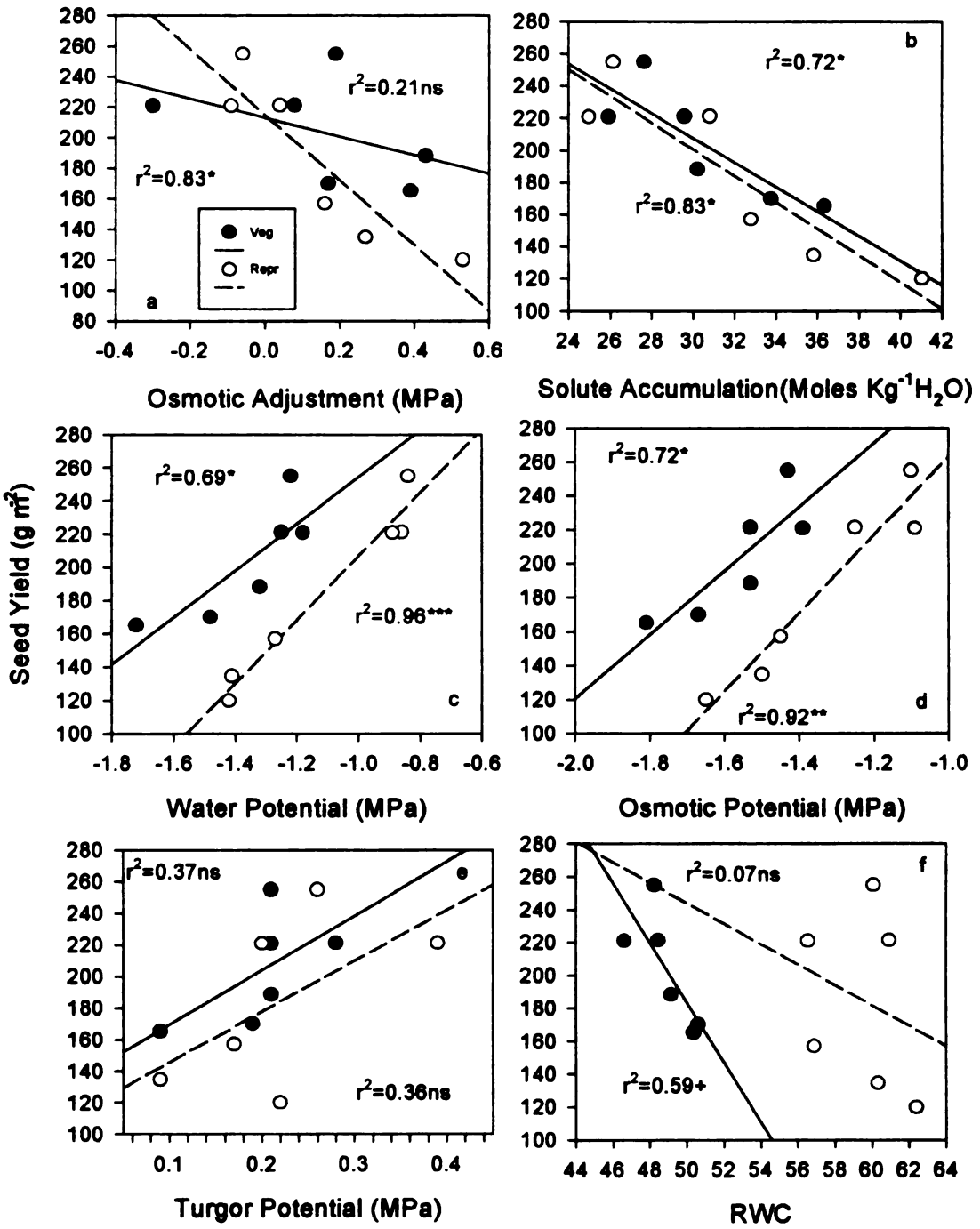

Figure 5. Seed yield as related to osmotic adjustment, solute accumulation, relative water content, and water potential components at two growth stages for three common bean genotypes grown under differential irrigation at Hickory Corners, Michigan 1991-1993.

Table 5. Osmotic adjustment and leaf water relations of common bean genotypes grown at Hickory Corners Michigan under intermittent moisture stress 1991-1993.

Treatment	Genotype	Osmotic†‡ Adjustment	Solute Accumulation	Water potential	Osmotic Potential	Turgor Potential	RWC
		(MPa)	(Moles Kg^{-1} H_2O)	(MPa)	(MPa)	(MPa)	(%)
Control	**Seafarer**	0.08	29.59	-1.25	-1.53	0.28	48.44
	LEF2RB	0.19	27.64	-1.22	-1.43	0.21	48.23
	BAT 477	-0.30	25.92	-1.18	-1.39	0.21	46.61
	LSD	NS	NS	NS	NS	NS	NS
Stress	**Seafarer**	0.39	36.33a	-1.72b	-1.81b	0.09	50.36
	LEF2RB	0.17	33.75ab	-1.48ab	-1.67ab	0.19	50.61
	BAT 477	0.43	30.23b	-1.32a	-1.53a	0.21	49.14
	LSD	NS	4.16+	0.27+	0.19*	NS	NS
Pooled	**Seafarer**	0.24	32.96a	-1.48b	-1.67b	0.18	49.40
	LEF2RB	0.18	30.69ab	-1.35ab	-1.55ab	0.20	49.42
	BAT 477	0.06	28.08b	-1.25a	-1.46a	0.21	47.88
	LSD	NS	3.34*	0.15+	0.14*	NS	NS

Note: different letters in a column indicate significant differences.
Note: + = p-value <0.10 *= p-value <0.05 ** = p-value <0.01 NS=Not Significant.
†Data for this column is from 2 years only (1991-1992) while the rest include 1993 as well.
‡Negative osmotic adjustment means predicted osmotic potential is less than the actual.

Table 6. Osmotic adjustment and leaf water relations of common bean genotypes grown at Hickory Corners Michigan under terminal moisture stress 1991-1993.

Treatment	Genotype	Osmotic ‡ Adjustment (MPa)	Solute Accumulation (Moles $Kg^{-1}H_2O$)	Water potential (MPa)	Osmotic Potential (MPa)	Turgor Potential (MPa)	RWC (%)
Control	**Seafarer**	0.04	30.79a	-0.86	-1.25	0.39a	60.94a
	LEF2RB	-0.06	26.15b	-0.84	-1.10	0.26ab	60.08ab
	BAT 477	-0.09	24.97b	-0.89	-1.09	0.20b	56.53b
	LSD	NS	3.67+	NS	NS	0.13+	3.67+
Stress	**Seafarer**	0.53a	41.03a	-1.42b	-1.65	0.22	62.39a
	LEF2RB	0.27b	35.81ab	-1.41b	-1.50	0.09	60.31a
	BAT 477	0.16b	32.79b	-1.27a	-1.45	0.17	56.87b
	LSD	0.19**	6.07*	0.12+	NS	NS	3.32**
Pooled	**Seafarer**	0.29a	35.91a	-1.14	-1.45b	0.31	61.66a
	LEF2RB	0.10b	30.98b	-1.12	-1.30a	0.18	60.19a
	BAT 477	0.04b	28.88b	-1.08	-1.27a	0.19	56.70b
	LSD	0.16**	3.62**	NS	0.14*	NS	2.67***

Note: different letters in a column indicate significant differences.
Note: + = p-value <0.10 *= p-value <0.05 ** = p-value <0.01 NS=Not Significant.
‡Negative osmotic adjustment means predicted osmotic potential is less than the actual.

Parsons and Howe (1984) who reported higher RWC in stressed plants. In this study, RWC was maintained by all genotypes under the terminal stress (Table 4). Genotypes did not differ in RWC at the initiation of flowering under any water treatment (Table 5). However, BAT 477 had a significantly lower RWC than the other two genotypes under stress and across water treatments at the R7 growth stage (Table 5). Relative water content correlated weakly with osmotic adjustment at the vegetative stage (Figure 4-a). On other hand, relative water content strongly and positively correlated with solute accumulation and negatively correlated with water and osmotic potential at the vegetative stage (Figure 4-b, c, d). RWC did not correlate with turgor potential at any growth stage (Figure 4-e). No correlations were significant between RWC and the other water relations parameters at the reproductive stage. These results may indicate that relative water content was maintained at reproductive stage when osmotic adjustment occurred.

Water Potential and Its Components

Water and osmotic potential were reduced by water stress at both growth stages, while turgor potential was reduced by the terminal water stress (Table 2). Similar results were reported by other researchers for common bean (Parsons and Howe,1984; Wullschleger and Oosterhuis,1991; Raggi, 1994) and for other crops (Premachandra et al., 1992; Li et al., 1993; Sloane et al.,1990; Oosterhuis and Wullschleger, 1987).

Intermittent water stress reduced water and osmotic potential for Seafarer and LEF2RB but not BAT 477 (Table 3). This stress did not reduce turgor potential relative to control for any genotype (Table 3). In contrast, reproductive terminal stress significantly reduced water and osmotic potential for all genotypes, while turgor potential

was reduced for LEF2RB only (Table 4). No genotypic differences were found for any of the water relations variables under well watered conditions during the vegetative growth stage but BAT 477 had significantly higher (less negative) water and osmotic potentials than Seafarer under the stress treatment (Table 5). On the other hand, BAT 477 had a lower turgor potential than Seafarer under well watered conditions at R7 stage (Table 6). This difference was only significant at the 10 % level but may indicate that the resistant genotype may have lower thresholds for moisture deficit than the susceptible genotype. In addition, BAT 477 maintained a higher water potential than the other two genotypes under terminal stress measurd at the R7 stage (Table 6). Across water treatments at R7, Seafarer had a lower (more negative) osmotic potential than the other two genotypes, but there were no significant differences in water and turgor potential among the genotypes (Table 6).

Seed Yield and Water Relations

Osmotic adjustment correlated strongly and negatively with seed yield (Figure 5-a) under terminal stress when the data was pooled across years. Wullschleger and Oosterhuis (1991), while not reporting yield data, did not find any association between osmotic adjustment and leaf growth in common bean. Moreover, growth was reduced in grape even as turgor was maintained (Schultz and Mathews, 1993). Similarly, Guei and Wassom (1993) found non significant correlations between osmotic adjustment and yield in maize (*Zea mays*). Blum (1989) found a non-linear negative correlation between osmotic adjustment and percent reduction in barley (*Hordeum vulgare*) yield. Kumar and Singh (1998) reported a positive correlation between osmotic adjustment and seed yield in

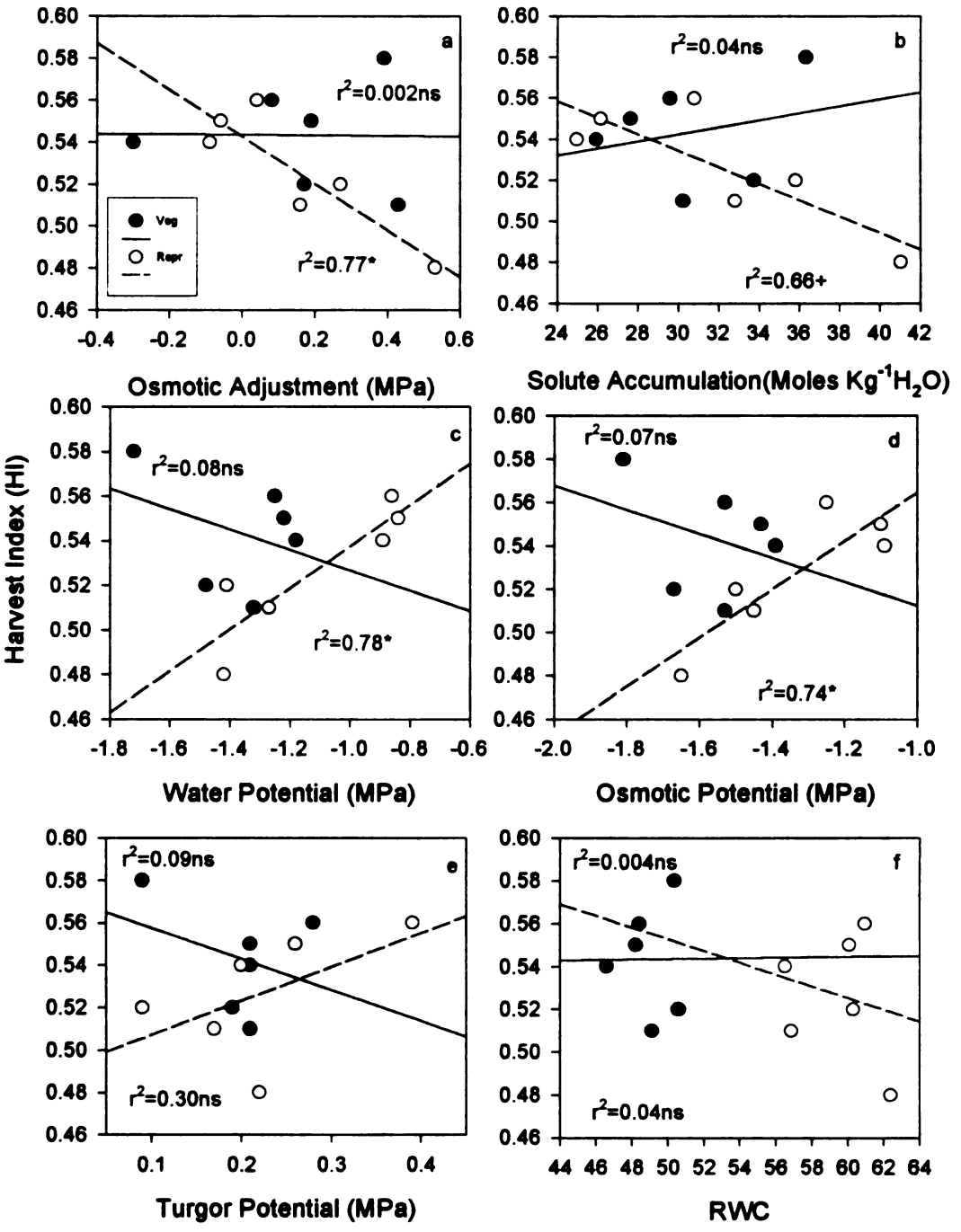

Figure 6. Harvest index (HI) as related to osmotic adjustment, solute accumulation, relative water content, and water potential components at two growth stages for three common bean genotypes grown under differential irrigation at Hickory Corners, Michigan 1991-1993.

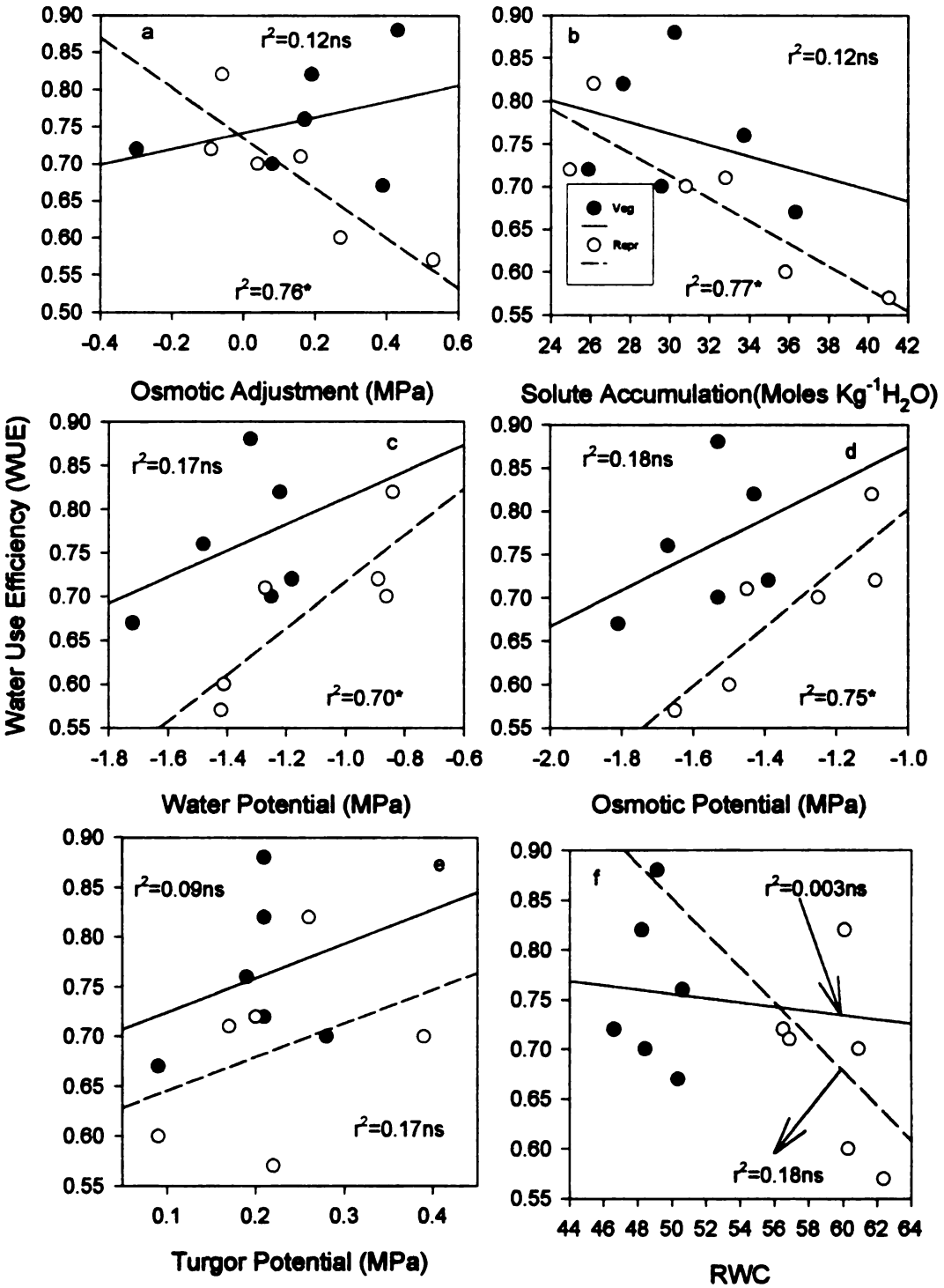

Figure 7. Water Use Efficiency (WUE) as related to osmotic adjustment, solute accumulation, relative water content, and water potential components at two growth stages for three common bean genotypes grown under differential irrigation at Hickory Corners, Michigan 1991-1993.

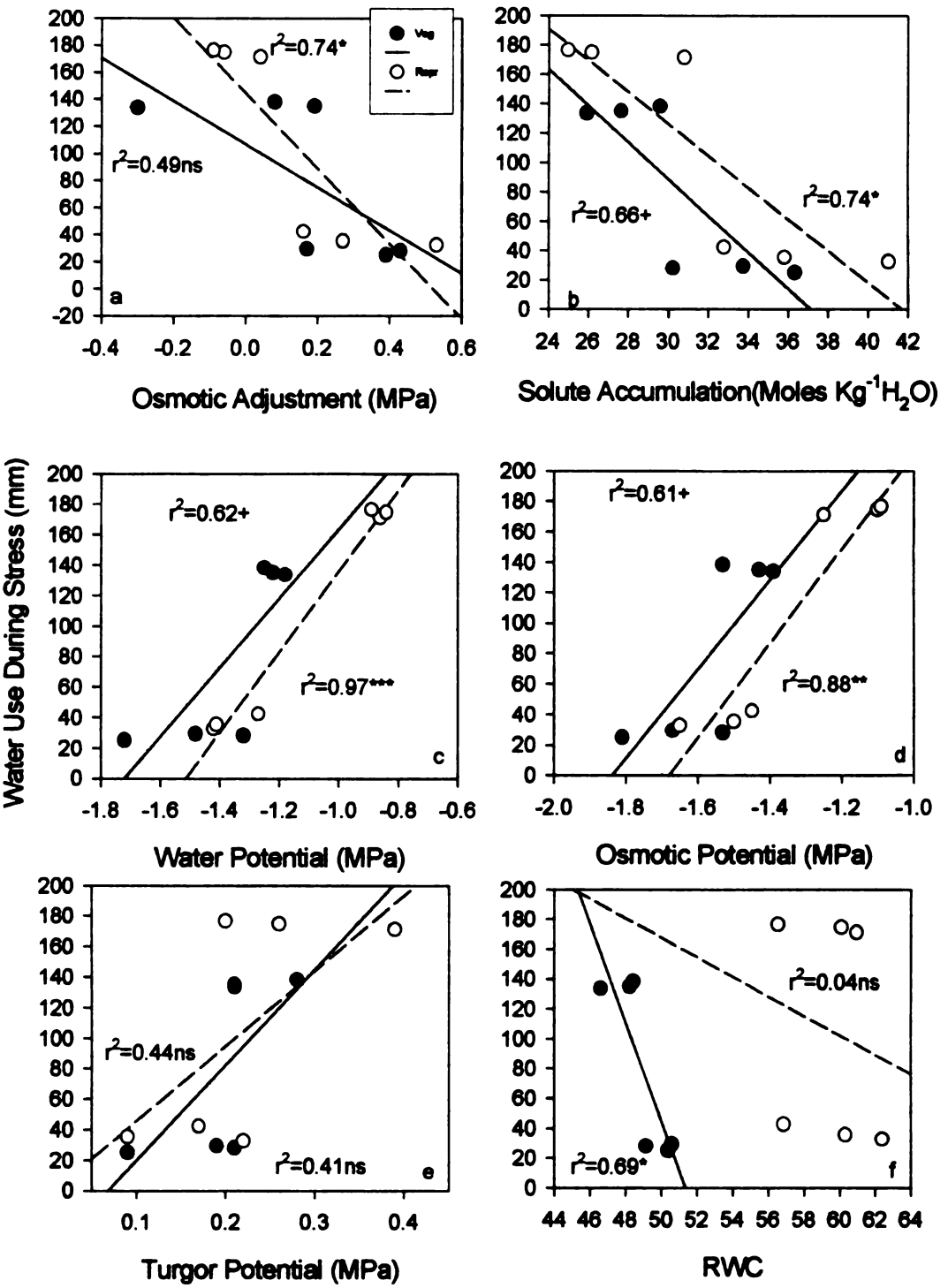

Figure 8. Water use at duration of stress as related to osmotic adjustment, solute accumulation, relative water content, and water potential components at two growth stages for three common bean genotypes grown under differential irrigation at Hickory Corners, Michigan 1991-1993.

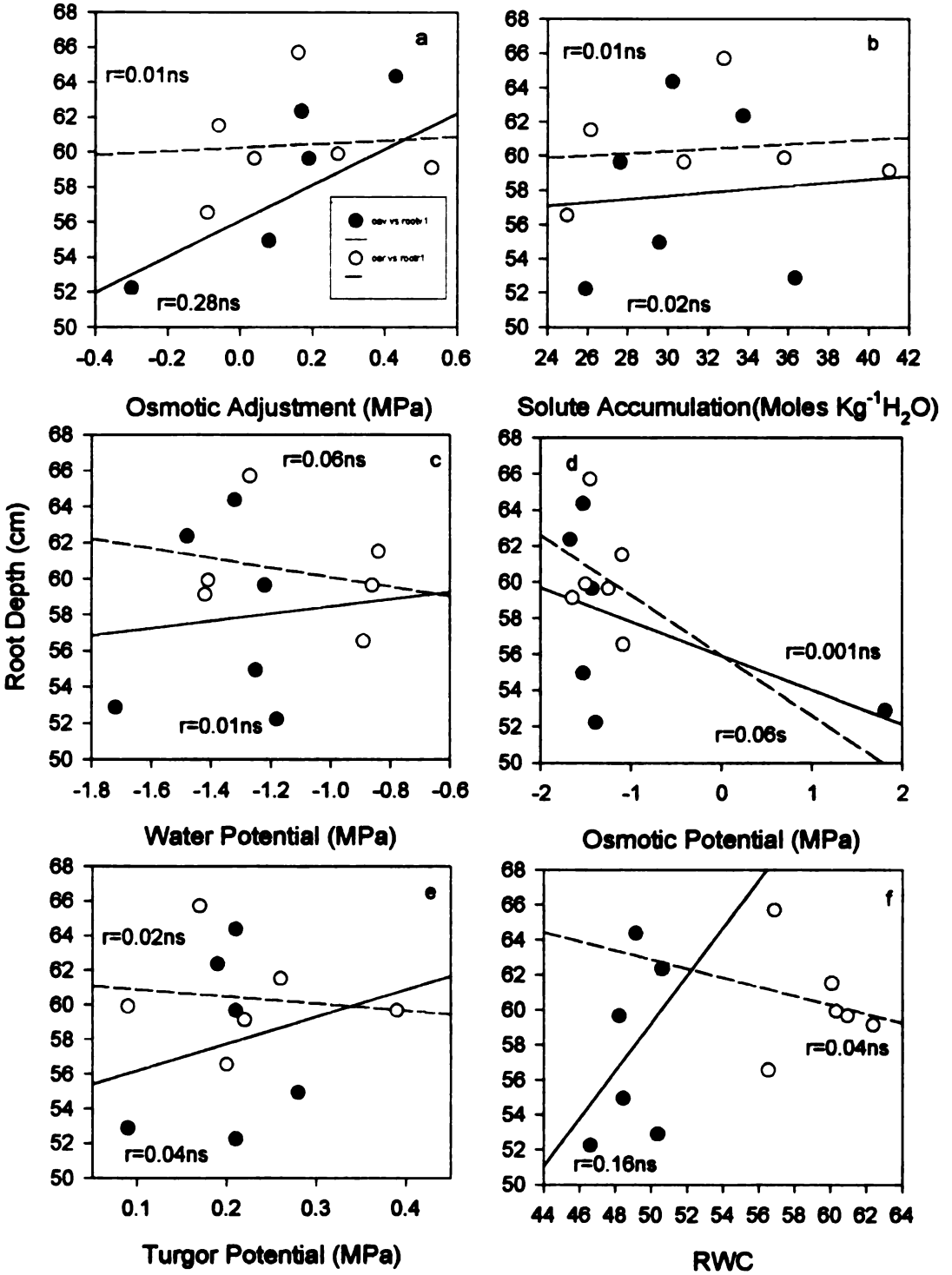

Figure 9. Rooting depth as related to osmotic adjustment, solute accumulation, relative water content, and water potential components at two growth stages for three common bean genotype grown under differential irrigation at Hickory Corners, Michigan 1991-1993.

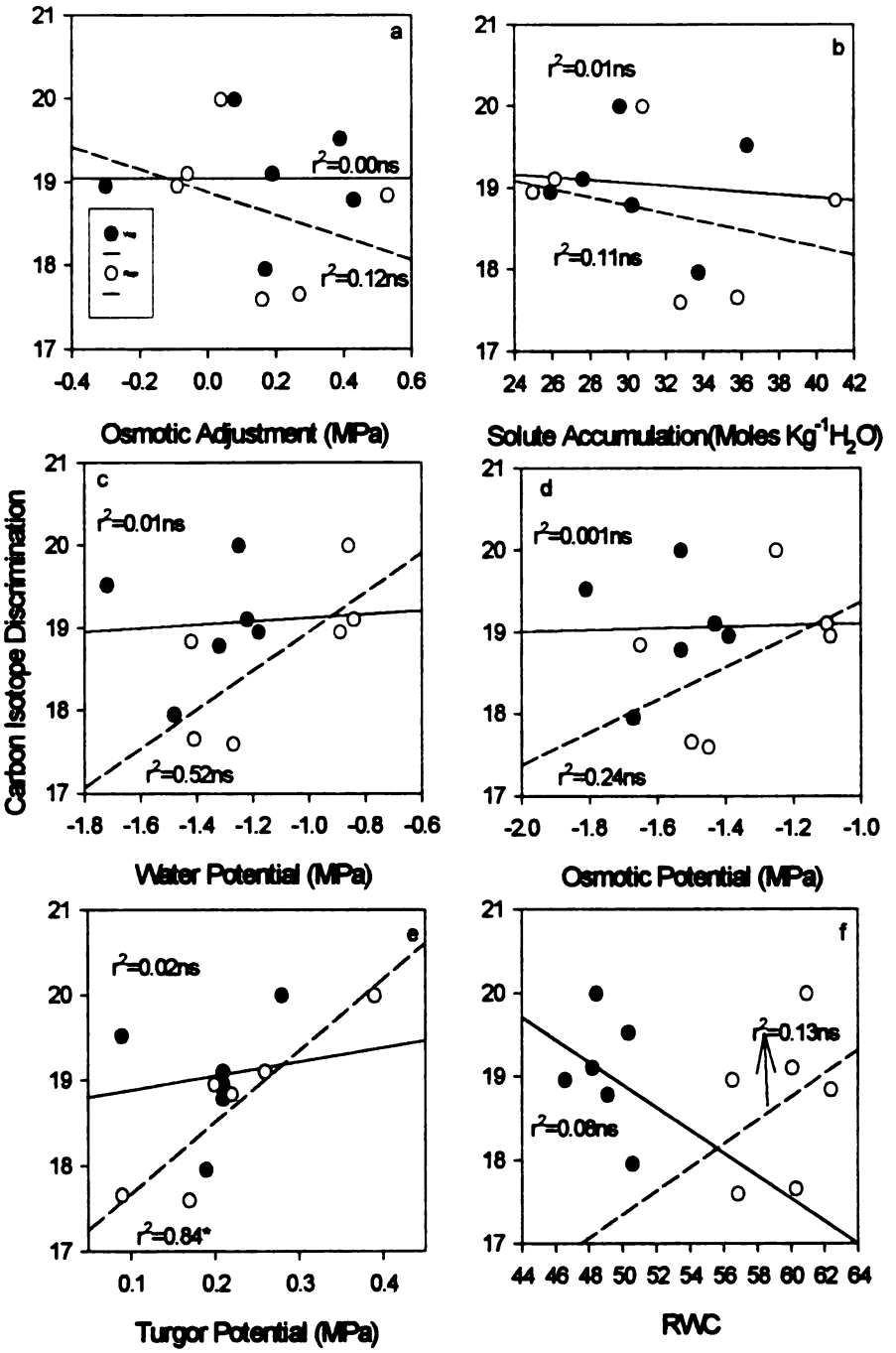

Figure 10. Carbon Isotope Discrimination (CID) as related to osmotic adjustment, solute accumulation, relative water content, and water potential components at two growth stages for three common bean genotypes grown under differential irrigation at Hickory Corners, Michigan 1991-1993.

different brassica spp. They calculated osmotic adjustment as the reciprocal of the slope of the linear regression of log transformed relative water content on osmotic potential. Likewise, Rodriguez et al. (1992) reported positive osmotic adjustment associations with yield in chickpea (*Cicer arietinum*) when osmotic adjustment was expressed as the relative water content at -1.5 MPa osmotic potential. Our findings support other work suggesting that osmotic adjustment has a physiological cost to the plant (Munns, 1988; Li et al., 1993) and may actually reduce yield under drought (Grumet et al., 1987).

Solute accumulation strongly and negatively correlated with seed yield at both growth stages (Figure 5-b)when the data is pooled across years. In contrast, genotypic differences in osmoregulation measured in a controlled environment were consistent with yield in the field for wheat (*Triticum aestivum*) and chickpea (Morgan, 1983; Morgan et al., 1991).

Water and osmotic potential positively correlated with seed yield at both growth stages while turgor potential did not (Figure 5-c, d, e). In addition, relative water content at the initial flower stage weakly and negatively correlated with yield (Figure 5-f).

Harvest index

Osmotic adjustment correlated strongly and negatively with harvest index (Figure 6-a) at mid podfill stage when the data was pooled across years but solute accumulation correlated weakly with harvest index (Figures 6-b) at the same growth stage. Further more, water and osmotic potentials both correlated positively and strongly with harvest index at the reproductive growth stage only (Figures 6-c,d). There was no correlation between turgor potential and relative water content with harvest index at either of the two

growth stages (Figure 6-e,f).

Water use and Water use efficiency

Osmotic adjustment and solute accumulation correlated negatively and strongly with water use efficiency during reproductive stage across water treatments (Figure 7-a, b). Similar results were found for the amount of water used during the stress period (Figure 8-a, b).

Water and osmotic potentials positively correlated with WUE at mid-podfill (R7) but not at flowering(R1) but turgor potential did (Figures 7-c,d,e). In addition, there was positive relationship between water and osmotic potentials and water use but the relationship was stronger at R7 growth stage (Table 8-c, d). In contrast, turgor did not correlate with water use at any growth stage (Figure 8-e).

Relative Water Content did not correlate with WUE at either of the two growth stages but did correlate negatively with the water used during the intermittent stress period (establishment up to R1 growth stage) (Figures 7-f, and 8-f).

Rooting Depth

Osmotic adjustment did not correlate with rooting depth at any growth stage (Figure 9-a), contrary to Wullschleger and Oosterhuis's (1991) report of osmotic adjustment in common bean roots. Across years and genotypes, solute accumulation did not correlate with rooting depth at either of the two growth stages (Figure 9-b). None of the water potential components correlated with rooting depth across treatments (Figure 9-c, d, e). However, when data was analyzed as water by year, only LEF2RB had a significant positive correlation between turgor potential and rooting depth at R8 growth

stage (r = 0.88 p ≤ 0.01 n=9). Similarly, relative water content did not correlate with rooting depth at any growth stage across treatments (Figure 9-f). Water stress reduced root osmotic potentials in a study reported by Wullschleger and Oosterhuis (1991).

Carbon Isotope Discrimination

Osmotic adjustment and solute accumulation did not correlate with CID at either growth stage (Figure 10-a, b). Across water treatment and over years, BAT 477 (r = -0.57 p≤ 0.01 n=33) and LEF2RB (r = -0.30 p≤ 0.10 n=33) showed a negative correlation of solute accumulation with CID at R7 sampling. The literature gives no indication of other studies that compared osmotic adjustment with carbon isotope discrimination in common bean or other crops.

Among the water potential components, only turgor strongly and positively correlated with CID and only at mid pod-fill (Figure 10-c,d,e). Likewise, relative water content did not correlate with CID at either of the two growth stages (Figure 10-f). Again, relative water content correlated negatively with CID only for BAT 477 when the data was analyzed across water treatments and years (r=-0.43 p≤ 0.05 n=33).

CONCLUSIONS

Osmotic adjustment was proposed as a screening tool for yield under stress or for yield determinants such as water use efficiency (Morgan, 1991; Ludlow and Muchow, 1990) even though in theory it could come as a cost to the plant (Munns, 1988). The few studies reporting osmotic adjustment in beans were conducted in controlled environments and did not relate it to yield or its determinants. In this study, osmotic adjustment was documented for field-grown common bean. Genotypic differences in osmotic adjustment,

solute accumulation and leaf water relations paralleled their differences in yield determinants. Terminal moisture stress increased osmotic adjustment across and within genotypes. The drought resistant line, BAT 477, had a lower osmotic adjustment as well as lower relative water content than the more drought susceptible Seafarer. These results support the theory that osmotic adjustment appears to have a physiological cost to the plants that may result in yield reduction (Munns, 1988; Li et al., 1993).

It is concluded that solute accumulation (osmoregulation) does not appear to be beneficial to yield or its determinants such as water use, water use efficiency, and harvest index in common bean. This study suggests that yield reduction may be predicted by solute accumulation. Genotypes with low solute accumulation may have other response mechanisms that impart drought resistance traits such as intrinsically lower relative water content, deep rooting or the ability to maintain higher water potential (Tables 5 and 6). Thus assessment of solute accumulation may be applicable for screening genotypes for drought resistance by direct selection or through the use molecular markers.

REFERENCES

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

Adams, M. W. 1996. An historical perspective on significant accomplishments in dry bean research- the first 100 years. Bean Improvement Cooperative 39:32-45.

Blum, A. 1989. Osmotic adjustment and growth of genotypes under drought. Crop Science 29:230-233.

Bolaños, J. and G.O. Edmeades. 1991. Value of selection for osmotic potential in tropical maize. Agronomy Journal 83:948-956.

Brown, R. W. and D. L. Bartos. 1982. A calibration model for screen-caged peltier thermocouple psychrometers. USDA For. Serv. Res. Pap. INT-239 Intermt. For. and Range Exp.Stn., Ogden, Utah.

Cortes, P.M. and T.R. Sinclair. 1986. Water relations of field -grown soybean under drought. Crop Science 26:993-998.

Dingkuhn, M., R.T. Cruz, J.C. O'Toole, N.C. Turner, and K. Doerffling. 1991. Responses of seven rice cultivars to water deficits. III Accumulation of abscisic acid and proline in relation to leaf water-potential and osmotic adjustment. Field Crops Research 27:103-117.

Grumet, R., A. J. Abrechtsen, and A.D. Hanson. 1987. Growth and yield of barley isopopulations differing in solute potential. Crop Science 27:991-995.

Guei, R.G and C.E. Wassom. 1993. Genetics of osmotic adjustment in breeding maize for drought tolerance. Heredity 71(4):436-441.

Holden, J. and D. Haytowitz. 1998. The nutritional value of beans. Bean Improvement Cooperative 41:41-44.

Kumar, A and D.P. Singh. 1998. Use of physiological indices as a screening technique for drought tolerance in oil seed brassica species. Ann. Bot. 81(3):413-420.

Lang, A. R. G. 1967. Osmotic coefficients and water potentials of sodium chloride solutions from 0 to 40° C. Aust. J. Chem. 20:2017-2023.

Lecoeur, J., J. Wery and O. Turc. 1992. Osmotic adjustment as a mechanism of dehydration postponement in chickpea (Cicer arietinum L.) leaves. Plant and Soil 144:177-189.

Li, X., Y. Feng and L. Boersma. 1993. Comparison of osmotic adjustment responses to water and temperature stresses in spring wheat and sudangrass. Ann. Bot. 71(4):303-310.

Ludlow, M.M., and R.C. Muchow. 1990. A critical evaluation of traits for improving crop yields in water-limited environments. Advances in Agronomy 43:107-153.

Martin, E. C., J. T. Ritchie, S. M. Reese, T. L. Loudon, and B. Knezek. 1988. A large-area, light-weight rainshelter with programmable control. Trans. ASAE 31:1440-1444.

Menéndez, C., and A. E. Hall. 1996. Heritability of carbon-isotope discrimination and correllations with harvest index in cowpea. Crop Sci. 36:233-238.

Morgan, J.M. 1983. Osmoregulation as a selection criterion for drought tolerance in wheat. Australian Journal Agricultural Research 34:607-614.

Morgan, J.M. 1991. A gene controlling differences in osmoregulation in wheat. Australian Journal Plant Physiology 18:249-257.

Morgan, J.M., B. Rodriguez-Maribona, and E.J. Knights. 1991. Adaptation to water-deficit in chickpea breeding lines by osmoregulation:relationship to grain-yields in the field. Field Crops Research 27:61-70.

Munns, R. 1988. Why measure osmotic adjustment?. Australian Journal Plant Physiology 15:717-726.

Nuland, D.S., and H. F. Schwartz. 1989. A visual description of the common bean plant: Four major growth periods. Bean Improvement cooperative 32:16-17.

Oosterhuis, D.M. and S.D. Wullschleger. 1987. Osmotic adjustment in cotton (Gossypium hirsutum L.) leaves and roots in response to water stress. Plant Physiology 84:1154-1157.

Pardossi, A., P. Vernieri, and F. Tognoni. 1992. Involvement of abscisic acid in regulating water status in *Phaseolus vulgaris* L. during chilling. Plant Physiology 100:1243-1250.

Parsons, L. R and T. K. Howe. 1984. Effects of water stress on the water relations of

Phaseolus vulgaris and the drought resistant *Phaseolus acutifolius*. Physiologia Plantarum 60:197-202.

Passioura, J.B. 1977. Grain yield, harvest index, and water use of wheat. Journal Australian Institute Agricultural Science 43:117-121.

Passioura, J.B. 1994. The yield of crops in relation to drought. In: K. J. Boote (ed.). Physiology and determination of crop yield. ASA-CSSA-SSSA, Madison, Wisconsin.

Premachandra, G.S.,H. Saneoka, K. Fujita and S. Ogata. 1992. Osmotic adjustment and stomatal response to water deficits in maize. Journal Experimental Botany 43(256):1451-1456.

Radin, J.W. 1983. Physiological consequences of cellular water deficits: osmotic Adjustment. In: Limitations to efficient water use in crop production.

Raggi, V. 1994. Changes in free amino acids and osmotic adjustment in leaves of water-stressed bean. Physiologia Plantarum 91:427-434.

Rodriguez-Maribona, B., J.L. Tenorio, J.R. Conde and L. Ayerbe. 1992. Correlations between yield and osmotic adjustment of peas (Pisum sativum L.) Under drought stress. Field Crop Research 29:15-22.

Salisbury, F. B., and C. W. Ross. 1985. Plant physiology. 3rd ed. Wadsworth Publishing Company, Belmont, CA.

SAS Institute. 1993. SAS/STAT guide for personal computer, version 6, 4th ed. SAS Inst., Cary, NC.

Schultz, H.R. and M. A. Mathews. 1993. Growth, Osmotic adjustment and cell-wall mechanics of expanding grape leaves during water deficits. Crop Science 33:287-294.

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

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

Sloane, R.J., R.P. Patterson and T.E. Carter. 1990. Field drought tolerance of a soybean plant introduction. Crop Science 30:118-123.

Turner, N.C. 1981. Techniques and experimental approaches for the measurement of plant water status. Plant and Soil 58:339-366.

Turner, N.C., W.R. Stern and P. Evans. 1987. Water relations and osmotic adjustment of leaves and roots of lupins in response to water deficits. Crop Science 27:977-983.

Wullschleger, S. D. and D. M. Oosterhuis. 1991. Osmotic adjustment and growth response of seven vegetable crops following water-deficit stress. HortScience 26(90:1210-1212.

Yabba, M.D., and E.F. Foster. 1996. Relative water content and carbon isotope discrimination of a susceptible and tolerant genotype of common bean. Bean Improvement Cooperative 39:172-173.

Zhang, B., and D.D. Archbold. 1993. Solute accumulation in leaves of a Fragaria chiloensis and a F. Virginiana selection responds to water deficit stress. Journal American Society Horticultural Science 118(2):280-285.

CHAPTER 5

SUMMARY AND CONCLUSIONS

SUMMARY

One strategy to increase yield of common bean in drought affected areas is to grow drought resistant genotypes. Breeding for drought resistance in drought-prone environments requires the existence of heritable genotypic variation as well as a visible screening trait that can be related to yield. Obviously, such a screening trait must be easier to obtain than yield in order to be useful. The existence of genotypic variation for drought resistance in common bean has been reported for small seeded black or cream colored types. But the search for a useful screening tool for drought resistance has remained elusive.

The current conceptual model for yield determination in moisture deficit environments was originally developed for determinate cereal crops and considers yield under stress as the product of water-use efficiency (WUE), harvest index (HI), and water use but its applicability to semi-determinate crops like common bean has not been verified. In addition, a number of physiological traits such as carbon isotope discrimination (CID), deeper rooting, relative water content, osmotic adjustment, and water potential components were proposed as screening tools for drought resistance but the role of some of the thermodynamically based water relations traits have been questioned. This study was conducted to evaluate the relationship of water use efficiency, harvest index and water use with yield for common bean growing under moisture deficit and to assess genotypic differences in osmotic adjustment and carbon isotope discrimination and their

relationship to yield, harvest index, water use efficiency, and rooting depth.

Three common bean genotypes with similar yield potential but with known difference in drought tolerance characteristics were grown in a rain shelter at Hickory Corners, MI in 1991 - 1993. Irrigation treatments included a well watered control, intermittent vegetative stress from V5 to R1, and a terminal reproductive stress from R1 to harvest. The three genotypes in this study were BAT 477 and LEF2RB two drought resistant genotypes, and Seafarer a commercial but drought susceptible genotype.

Vegetative stress reduced yield by 15 to 30% while reproductive stress reduced yield by 35 to 50%. Reproductive moisture stress significantly reduced CID by 9% in comparison to the 5% reduction that occurred under intermittent stress. Similarly, water potential and osmotic potential were significantly reduced (15 to 59%) while solute accumulation increased (21 to 34%) under intermittent vegetative and terminal reproductive moisture stress. Seafarer had a significantly higher CID than both BAT 477 and LEF2RB. Genotypic differences in yield and osmotic adjustment were found only under terminal moisture stress where Seafarer had a significantly lower yield and a significantly higher osmotic adjustment than BAT 477. Water use and water use efficiency correlated positively with yield and biomass and CID had positive correlations with yield, WUE, and water use for each genotype or water treatment but not across genotypes or water treatments. Relative biomass and WUE reductions strongly and positively correlated with relative CID reduction. Osmotic adjustment ($r = -0.91$ $p \leq 0.05$ $n=6$) and solute accumulation ($r = -0.91$ $p \leq 0.05$ $n=6$) both strongly and negatively correlated with yield. Similarly, osmotic adjustment and solute accumulation had

significant negative correlations with water use efficiency, harvest index and water use were found across water treatments.

CONCLUSIONS

Common beans seem to follow a simpler model relating water use and water use efficiency to yield in that harvest index is generally higher and more stable in common bean than in other crops. Harvest index was the one factor that did not strongly correlate with yield and this raises the question of whether or not breeders should use harvest index as a selection criterion for yield in common bean.

Carbon isotope discrimination (CID) appears to be a useful screening tool for drought resistant genotypes but further testing of CID response to drought is needed with additional genotypes and environments. In this study CID correlated positively with water use efficiency, a finding that conflicts with the findings of other common bean researchers and with theory.

Water and osmotic potential correlated strongly and positively with yield and with yield determinants at the reproductive growth stage. These findings support the continued use of these traits for drought stress research. Solute accumulation or osmotic adjustment may be indicative of susceptibility to moisture deficit rather than resistance in common bean. Genotypes with low osmotic adjustment should be selected for drought resistance rather than those with higher adjustment. The larger issue of why osmotic adjustment through solute accumulation correlates differently with yield in different crops needs to be addressed by further research.