



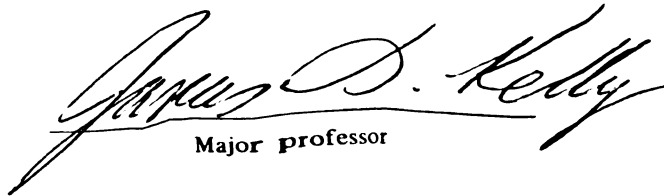
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**EFFECTS OF TERMINAL DROUGHT STRESS ON BLACK BEANS**

**By**

**Mark Aaron Frahm**

**A THESIS**

**Submitted to  
Michigan State University  
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## ABSTRACT

### EFFECTS OF TERMINAL DROUGHT STRESS ON BLACK BEANS

By

Mark Aaron Frahm

Terminal drought stress severely restricts bean production in Honduras during the dry season, known as "la Postrera". Genetic improvement of common bean (*Phaseolus vulgaris* L.) provides a means to assist farmers in production areas affected by drought. The objectives of this study were to i) identify drought resistant genotypes in two black bean Recombinant Inbred Line (RIL) populations, ii) evaluate bean root characteristics for their ability to predict yield performance under stress, and iii) evaluate the effectiveness of RAPD markers previously associated to drought resistance in pinto bean.

Two black bean populations segregating for drought resistance were evaluated for yield under moisture stress (Yd) and non-stress (Yp) conditions in Zamorano, Honduras. Sixteen RILs out-yielded all checks and parents and were identified as drought resistant based on the geometric mean (GM) of the two treatments. Adaptation of resistant and susceptible RILs was tested in Michigan. Despite the low drought stress in Michigan in 2001, GM was moderately correlated between locations,  $r = 0.63^*$ .

Root length and root architecture were calculated using a pouch method and the WinRhizo™ program. Fine roots and fractal dimension were negatively correlated to Yd,  $r = -0.13^*$ , whereas taproots were positively correlated to Yp,  $r = 0.19^{**}$ . Markers, F06<sub>970</sub> and IO3<sub>1130</sub>, explained 5% of the variation in Yp in both populations. Root traits combined with markers accounted for more variation than any one trait alone.

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## KEY OF ABBREVIATIONS

$\Delta$	Carbon Isotope Discrimination [(Ra/Rp-1)]
100 sw	100 Seed Weight
a	Altitude
AM	Arithmetic Mean
ANOVA	Analysis of Variance
ASB	Ashy Stem Blight
BGMV	Bean Golden Mosaic Virus
cb	Centibars
CBB	Common Bacterial Blight
CIAT	International Center for Tropical Agriculture
cM	Centi-Morgan
CRD	Completely Randomized Design
CV	Coefficient of Variation
dap	days after planting
DI	Disease Intensity
DII	Drought Intensity Index [1-(Xd/Xp)]
DS	Desirability Score
DSI	Drought Susceptibility Index [(1-(Yd/Yp))/DII]
Fsp	<i>Fusarium solani</i> pv. <i>phaseoli</i>
GCA	General Combining Ability
GM	Geometric Mean
HI	Harvest Index
LG	Linkage Group
LSD	Least Significant Difference
MAS	Marker Assisted Selection
masl	Meters Above Sea Level
MI	Michigan
NSL	Negro San Luis
PCR	Polymerase Chain Reaction
p <sub>e</sub>	Exterior Path Length
PIF	Programa de Investigaciónes en Frijol
PM	Physiological Maturity
ppi	pre-plant incorporation
QTL	Quantitative Trait Loci
r	Pearson Correlation Coefficient
R <sup>2</sup>	Coefficient of Determination
Ra	Ratio of Carbon in the Atmosphere
RAPD	Randomly Amplified Polymorphic DNA
RCBD	Randomized Complete Block Design
RIL	Recombinant Inbred Line
Rp	Ratio of Carbon in the Plant

<b>SCA</b>	<b>Specific Combining Ability</b>
<b>SCAR</b>	<b>Sequence Characterized Amplified Region</b>
<b>SSD</b>	<b>Single Seed Descent</b>
<b>Xd</b>	<b>Mean yield under moisture stress conditions</b>
<b>Xp</b>	<b>Mean yield under non-stress conditions</b>
<b>Yd</b>	<b>Yield under moisture stress conditions</b>
<b>Yp</b>	<b>Yield under non-stress conditions</b>

## INTRODUCTION

Drought is the second major constraint after disease to negatively affect yield of common bean, *Phaseolus vulgaris* L. Approximately 60 % of the bean crop in the developing world is produced under drought stress (Graham and Ranalli, 1997). An example of bean production under stress occurs in the lowland tropical areas of Central America. In Honduras, the bimodal pattern of rainfall permits two seasons of crop production. The first season, la Primera, is known as the rainy season because 54 % of the annual rainfall occurs (Cotty et al., 2001). Following la Primera (May-Aug.), less frequent rainfall and diminishing soil moisture create the terminal drought stress in the second production season known as, la Postrera (Sept.-Dec.). The short life-cycle of common bean makes it an ideal crop to grow at the end of la Primera. More than 60 % of the area cultivated to bean in Honduras is planted in la Postrera under a relay system after corn (*Zea mays*) has reached physiological maturity or after the corn has been harvested (Rosas et al., 1991). The bean production area in Honduras increases three-fold during la Postrera despite an overall yield reduction of 50 % due to terminal drought (Cotty et al., 2001). Since adequate irrigation schemes are unrealistic due to socio-economic constraints, genetic improvement for drought resistance offers a long-term improvement of bean productivity under drought stress in Honduras.

The genetic improvement of drought resistance in common bean has been previously documented (Acosta-Gallegos and Shibata, 1989; Acosta-Gallegos and Adams, 1991; White et al., 1994a; Singh, 1995; Schneider et al., 1997b; Abebe et al., 1998; Ramirez-Vallejo and Kelly, 1998; Rosales-Serna et al., 2000; Terán and Singh,

2002). Drought resistance can be compared to the evolutionary success of plant adaptation. Plant adaptation is defined as the relative ability of plants to survive and produce more biomass and progeny (seed) compared with other plants growing in the same environment (Hall, 1993). Drought resistance is based on relative yield of a genotype compared with other genotypes subjected to the same drought and where drought escape is not a major factor (Hall, 1993). Yield in common bean has been reduced by 58 % due to water stress (Acosta-Gallegos and Adams, 1991). Each yield component, pods per plant, seeds per pod and seed weight per 100 seeds, has shown varying negative responses to water stress (Acosta-Gallegos and Shibata, 1989; Acosta-Gallegos and Adams, 1991; Ramirez-Vallejo and Kelly, 1998). Pods per plant is the one yield component most affected by water stress ( $r = 0.56$ ; Acosta-Gallegos and Adams, 1991). Seeds per pod and 100 seed weight are reduced by water stress but to a lesser extent than pods per plant. Yield is measured under moisture stress ( $Y_d$ ) and non-stress conditions ( $Y_p$ ) to calculate drought resistance of individual genotypes. Non-stress conditions maintained by irrigation reveal the yield potential ( $Y_p$ ) of genotypes. Both yield variables are commonly combined in different equations to identify genotypes stable across diverse environmental conditions.

The measurement of drought resistance in common bean has been a topic of discussion among breeders for many years. Yield differential ( $Y_p - Y_d$ ) was commonly used as a selection criterion for drought resistance, yet it was shown to be counterproductive due to the likelihood of selecting a low yielding genotype with a relatively small yield differential due to drought (Samper, 1984). Arithmetic mean of stress and non-stress treatments ( $AM = (Y_p + Y_d)/2$ ) was suggested as selection criteria,



based on theoretical experiments (Rosielle and Hamblin, 1981). However, selection for drought resistance based on AM could be confounded due to genotypes with high yield potential and low yield under stress.

The variation in yield potential between genotypes can be determined by the drought susceptibility index (DSI) (Fischer and Maurer, 1978). DSI is a dimensionless slope calculated from the following formula:  $DSI = (1 - (Y_d/Y_p))/DII$  where DII is the drought intensity index of the experiment. DII is calculated by  $DII = 1 - (X_d/X_p)$ ,  $X_d$  and  $X_p$  being the mean yield of the drought and irrigated treatments, respectively. Since the DII of the experiment is considered, individual genotypes can be compared across locations using DSI. Geometric mean,  $GM = (Y_p * Y_d)^{1/2}$ , was introduced as a calculation that takes into account yield data from both treatments and represented an actual yield measurement of the genotype. Geometric mean differs from arithmetic mean by moderating inflated or diminished means resulting from extreme values between treatments.

Four different selection criteria including yield differential, AM, DSI and GM were compared for their potential to evaluate drought resistance in common bean genotypes (Samper and Adams, 1985). Twenty-two bean genotypes of diverse origin were ranked according to each criterion. Genotypes were ranked similarly based on yield differential and DSI. Rankings based on AM and GM were similar, yet genotypic rankings based on GM were drastically different than DSI rankings. A possible explanation is that the DSI identified low-yielding genotypes that could tolerate drought well, whereas GM better reflected the actual yield potential of the genotype. The most effective approach in selection for drought resistance in common bean is based on

sequential selection for high GM yield, followed by high Yd yield, low to moderate DSI and harvest index (HI) values (Schneider et al., 1997b; Ramirez-Vallejo and Kelly, 1998).

The genetic makeup of populations created for drought resistance is an important factor to consider. Interracial populations have been suggested as the most effective way to combine high yield with drought resistance among different races of common bean (Singh et al., 1991; Singh, 1995; Terán and Singh, 2002). Genotypes from the Durango race showed higher yields, larger seed weights and earlier maturity than genotypes from the Jalisco race (Terán and Singh, 2002). Durango genotypes have an indeterminate type III growth habit and a life cycle less than 120 days while Jalisco genotypes exhibit a climbing type IV growth habit and a life cycle greater than 150 days. For these reasons, the Durango race is preferred by breeders in interracial crosses to the Mesoamerican race to improve drought resistance.

Interspecific hybridizations between common bean and tepary bean (*Phaseolus acutifolius* A. Gray) have also been suggested to improve drought resistance since tepary bean has exhibited high levels of drought tolerance (Thomas et al., 1983; Rosas et al., 1991). Obtaining viable offspring from interspecific crosses is impossible without embryo rescue. Over 1500 plants were generated in a *P. vulgaris* x *P. acutifolius* hybridization where embryo rescue was employed (Mejía-Jiménez et al., 1994). Recurrent and congruity backcrossing was implemented to overcome any incompatibility barriers. Tepary beans, highly resistant to common bacterial blight, were successfully introgressed into common bean germplasm (Singh and Munoz, 1999), yet the impact of interspecific hybridizations to enhance common bean germplasm for drought resistance has been limited.

Breeding for drought resistance is more difficult due to the quantitative nature of inheritance. Expression of quantitative traits result from independent segregation of many genes that have small effects and are more affected by environmental variation (Paterson et al., 1990). Drought resistance exhibits continuous variation and heritability estimates have generally been low. Reported values for heritability of drought resistance in common bean range from 0.09 to 0.80 (White et al., 1994a; Singh, 1995; Schneider et al., 1997b; Ramirez-Vallejo and Kelly, 1998). The wide range of heritabilities results from differences in genetic variability among populations and different intensities of stress. General combining ability (GCA) and specific combining ability (SCA) were calculated for yield under drought stress from a nine bean diallel grown in tropical mid-elevation regions (altitude 800-1600m) and semi-arid highlands (1700-2400m) (White et al., 1994a). GCA for yield was consistently significant and larger than SCA in both environments. These results suggested the importance of additive gene effects for yield and 100 seed weight of bean grown under stress. Parental genotypes adapted to both environments were used in the diallel crosses. At the highland location in Durango, Mexico, parental genotypes adapted to the mid-elevation environment showed negative GCA values while all highland genotypes were positive. Reciprocal results occurred in the mid-elevation location where mid-elevation parents showed positive GCA values while highland parents had negative GCA values. These location effects underscore the importance of identifying the target environment before choosing parents to improve drought resistance in common bean.

The expression of drought resistance or the adaptation to stress is more clearly illustrated when individual genotypes are compared between locations. Two RIL

populations of the Durango race were evaluated for drought resistance under two locations in Michigan, two locations in Zacatecas, Mexico and three in Durango, Mexico (Schneider et al., 1997b). Yield calculations were made using data from all seven locations (Schneider et al., 1997b) and from the three locations in Durango (Rosales-Serna et al., 2000). Different RILs ranked in the top five based on GM for each experiment. These differences can be explained by the limited ability of the Durango race to adapt to different environments and the evasive nature of drought resistance.

Drought stress occurs in two contrasting moisture environments (intermittent and terminal) of the semiarid tropics (Ludlow and Muchow, 1990). Intermittent drought is due to climatic patterns of sporadic rainfall that causes intervals of drought. The nature of this rainfall is unpredictable and leads to marginal yields in potentially valuable land. This rainfall pattern is chronic and endemic to the semiarid highlands (1800 masl) of Mexico (Singh, 1995). Terminal drought occurs when plants suffer from a lack of water only at later stages of growth or when crops are planted in a dry season. This farming practice predisposes the crop to a terminal drought in two very important phases of its life cycle; flowering and pod-fill. Terminal drought characteristically occurs in lowland tropical areas when the bean crop is planted at the end of the rainy season.

Different growth habits in common bean offer unique adaptive advantages to the different types of drought. The type II growth habit is characterized by an indeterminate, upright plant structure with reduced branching angle whereas the type III habit is typical of an indeterminate prostrate sprawling plant structure (Brothers and Kelly, 1993). The desired growth habit for resistance to intermittent drought in the Mexican highlands is a type III plant. The prostrate canopy has an opportunistic growth pattern when moisture is

available which helps retain moisture in the soil by shading whereas erect growth habits allow soil moisture to be lost during hot and windy days. Type III genotypes can be planted at lower densities to reduce inter-plant competition since they have a sprawling superficial root system that is able to utilize soil moisture in a wider zone than deeper, narrow taproots of type II growth habit. The type III growth habit also produces many root meristems and basal roots to access soil moisture in a wider superficial zone (Lynch and van Beem, 1993).

In the terminal drought environment, a deep penetrating root is needed to maintain relative water content in the bean plant during the ever-intensifying dry period. The ideal growth habit for this stress would be a type II. A striking feature of this growth habit is its deep penetrating root system. The root system of the type II growth habit has a herringbone structure which characteristically goes deep into the soil profile to extract moisture (Lynch and van Beem, 1993). The erect architecture of the type II shoot allows continued transpiration to be sustained by deep penetrating roots, so that the plant can deliver an acceptable yield under terminal drought.

Root architecture is associated to shoot architecture. Shoot height was used as a selection criterion to predict root depth in soybean (*Glycine max*) (Mayaki et al., 1976). Water stressed and non-stressed treatments were used to measure differences in shoot height and root depth in the field. Root depth was correlated to shoot height ( $R^2 = 0.99$ ) (Mayaki et al., 1976). The root depth:shoot height ratio was 2:1 from six node stage to pod fill stage in stressed plots. In non-stressed plots, the 2:1 ratio decreased to 1.4:1 during pod initiation. This research offered a quick and non-destructive method of predicting rooting depth in the field. Although root growth has been correlated to yield

under drought stress, its use as an efficient screening technique in common bean has been limited, due to the cumbersome nature of measurements.

The intensity of the moisture stress can be detrimental to most physiological functions. Nitrogen fixation has been studied under differing degrees of drought stress (Acosta-Gallegos, 1988; Foster et al., 1995). In an experiment where DII = 0.41, N partitioning from the leaf to the seed in common bean was not impaired (Foster et al., 1995). N-remobilization was severely affected by a more severe stress, DII = 0.92, and has been suggested as an important drought adaptation strategy under moderate or intermittent moisture deficits (Foster et al., 1995). N partitioned to seed also decreased with terminal drought in other leguminous species (Chapman and Muchow, 1985).

Above-ground biomass is one physiological trait that correlates well to yield under water stress, ( $r = 0.79$ ), despite the severity of moisture stress (Acosta-Gallegos, 1988). Since it accounts for the total nutrients fixed in vegetative growth and seed production, increased biomass is often associated with late maturity in common bean. Harvest index (HI = plot yield/ biomass) accounts for the efficiency of plant partitioning the nutrients to seed production. HI must be combined with biomass when selecting for performance under stress.

Three mechanisms that plants use to respond to water stress are escape, avoidance and tolerance (Ludlow, 1989). Desert annuals and short season, annual crops use the escape mechanism during water stress. In Honduras, landraces such as Cuarenteño, Cincuentaño and Chingo that reach maturity within 65 days are planted by farmers to escape drought (Rosas et al., 1991). Although earliness is popular among farmers, the trait is negatively correlated to yield. Drought resistance must combine avoidance and/or

tolerance mechanisms but not escape mechanisms (Fischer and Maurer, 1978). Breeding lines with improved yield potential under non-stress must be combined with avoidance and tolerance traits to increase drought resistance.

The mechanisms of avoidance and tolerance are not mutually exclusive in all drought resistant traits but their definitions are unique. Plants that avoid drought must do so because they have tissues that are sensitive to dehydration (Ludlow, 1989). These plants respond to drought stress by maximizing water uptake and minimizing water loss. Drought tolerant plants are insensitive to dehydration (Ludlow, 1989). They are characterized as having a high osmotic adjustment. Since different mechanisms operate in plants, numerous physiological mechanisms have been evaluated as screening techniques for yield under drought stress.

Drought tolerance mechanisms involving leaf gas exchange affected by water stress were studied in common bean (Farquhar et al., 1989; Ehleringer et al., 1990; White et al., 1990; Ehleringer et al., 1991; White et al., 1994b). Carbon isotope discrimination ( $\Delta$ ) was used as an indicator of water use efficiency and adaptation to water deficits in common bean (White et al., 1994b). Carbon isotope discrimination is defined as  $\Delta = (R_a/R_p - 1)$  where  $R_a$  and  $R_p$  are the  $^{13}\text{C}/^{12}\text{C}$  ratios of carbon in the atmosphere and plant, respectively (White et al., 1990).  $\Delta$  is directly proportional to the intercellular  $\text{CO}_2$  concentration. With this measurement, higher photosynthetic rates can be derived from higher  $\Delta$  values. The  $\Delta$  measurement has only been significantly correlated to biomass and not to yield. Although  $\Delta$  is unsuitable as a screening technique for yield under drought stress, it could be used to identify different adaptation mechanisms present in common bean (White et al., 1994b).

Roots are recognized as playing an important role in drought avoidance in common bean. Greater root growth supports yield in common bean through drought avoidance by extracting more soil moisture at greater depths. Roots of drought resistant bean genotypes reach greater depths than those in non-stress soils and were hypothesized to be an important drought avoidance mechanism (Sponchiado et al., 1989).

The root and shoot characteristics of common bean genotypes under water stress were compared for their association to yield under drought stress in fertile and acidic soils (White and Castillo, 1989; White and Castillo, 1992). Root and shoot genotypes of drought resistant and susceptible genotypes were combined through grafting. The resulting plants were transplanted to the field for evaluation under drought conditions. When the root of drought resistant genotype, BAT 477, was grafted onto the shoots of BAT 477 and drought susceptible genotype BAT 1224, the plants yielded 600 and 840 kg/ha, respectively under drought stress. In the reciprocal graft using BAT 1224 as the root genotype, the shoots of BAT 477 and BAT 1224 yielded 160 and 30 kg/ha respectively, compared with the normal yields (700 and 40 kg/ha) of BAT 477 and BAT 1224 grown under water stress. This data suggests that the bean root genotype is more important in drought resistance than the shoot genotype. In both, fertile and acidic soils, the root genotype had a large and significant effect on yield while the shoot genotype had no effect. The root systems of four food legumes were compared for their response to drought (Pandey et al., 1984c). Peanut (*Arachis hypogaea* L.) with the most extensive root system when compared to the other three legumes, showed greater yield (Pandey et al., 1984a) and cooler canopy temperatures (Pandey et al., 1984b) under water stress suggesting the important role that roots play in drought tolerance.



Root reaction of plants to water stress affects stomatal response. In wheat and sunflower, roots that detect the soil drying consequently sent a message to the leaves, which induces the stomates to close (Gollan et al., 1986). This signal was reproduced and shown to be related to the metabolism of cytokinins (Schulze, 1986). In common bean, roots under moisture-stressed conditions produced a signal that was transported to the leaves causing a continuous decline in stomatal conductance (Aguirre-Medina et al., 1998). Shoot responses to moisture stress detected by roots is also observed as paraheliotropic leaf movements in common bean (Kao et al., 1994). This paraheliotropic movement of the shoot decreases the incidence of solar radiation and ultimately minimizes water loss.

Screening techniques for drought resistance are important since improving crops in tropical environments by selecting solely on grain yield is problematic because of the variability in amount and annual distribution of rainfall (Ludlow and Muchow, 1990). Breeding for high yield would be more efficient if traits correlated to yield under water stress were identified and could be used in selection. Screening techniques for drought resistance would be valuable to plant breeders to reduce variety development time and resource expenditures.

Many physiological measurements have been suggested as an indirect screen for yield in early generations following hybridization. These traits including 100 seed weight, leaf area of primary leaves, stem and total dry weight as well as hypocotyl diameter have been significantly correlated to seed yield in bean (Acosta-Diaz, 1998). The response of leaf angles to sunlight was suggested as a valuable trait for selection in drought environments due to its correlation in water use efficiency of the plant (Kao et al., 1994).

Although, these physiological measurements indirectly relate to drought tolerance, their application as a screening technique can be laborious and time-consuming.

The most recent method in which traits are being indirectly selected is based on molecular markers linked to the trait of interest. As a screening technique, molecular markers can be used to screen large numbers of individuals in a relatively short amount of time. Molecular markers have been associated with qualitative and quantitative traits in common bean (Kelly et al., 2002, in review). Markers linked to single genes for disease resistance in anthracnose (Young and Kelly, 1996), bean common mosaic virus (Haley et al., 1994), bean golden mosaic virus (Urrea et al., 1996), and bean rust (Miklas et al., 1993) have been developed. Markers have been useful in the identification of single genes masked by epistatic effects and the building of gene pyramids in common bean (Kelly et al., 1995). Breeders unable to phenotypically screen for disease resistance can use markers as a selection criterion. Marker Assisted Selection (MAS) allows selection of traits in early generations. With MAS, breeders can reduce the number of breeding lines depending on the presence of the marker and few individuals need to be screened to identify superior genotypes.

Quantitative traits linked to a simply inherited genetic marker were first observed in common bean with the co-segregation of seed size and seed coat color (Sax, 1923). More recently markers associated to quantitative trait loci (QTL) in common bean have been identified for resistance to ashy stem blight (Miklas et al., 1998), common bacterial blight (Nodari et al., 1993), BGMV (Miklas et al., 1996), web blight (Jung et al., 1996), white mold (Miklas et al., 2001), root rot (Schneider et al., 2001), and drought (Schneider et al., 1997a). Success of QTL analysis has centered on the identification of a few major

loci controlling quantitative trait expression. The discovery of major QTLs explaining large percentages of genetic variation of quantitative traits has encouraged the use of MAS. The effectiveness of MAS for quantitative traits is inversely proportional to the heritability of the trait being selected (Lande and Thompson, 1990). Markers for QTLs associated with drought resistance have been detected in common bean (Schneider et al., 1997a), rice (*Oryza sativa*) (Champoux et al., 1995), sorghum (*Sorghum bicolor* L. Moench) (Kebede et al., 2001), soybean (Specht et al., 2001), maize (Ribaut et al., 1997), and barley (*Hordeum vulgare* L.) (Teulat et al., 1998). In soybean, a major QTL accounted for 33 to 38 % of the phenotypic variation in yield under various irrigation regimes (Specht et al., 2001).

In common bean, RAPD markers associated with drought resistance were identified and used in MAS (Schneider et al., 1997a). Seventy polymorphic primers were screened across two RIL populations. Nine linkage groups were identified in one population and ten in the other. A linkage group from each population was significantly associated with Yd, Yp and/or GM. One linkage group explained 8-14 % of the genetic variation combined across all locations while the other explained 10-16 %. These linkage groups were used in MAS. Yield under stress among genotypes selected by MAS in one population was improved by 10 g/m<sup>2</sup> despite a severe drought stress (DII = 0.76) imposed in Michigan. When these same genotypes were grown in two Mexican locations, significant differences were not detected between drought resistant and susceptible genotypes. Only significant differences were detected for the second population in the Mexican locations. MAS was more effective than conventional selection in one of the populations where heritability estimates for yield were lower (Schneider et al., 1997b).

Recently, researchers have used molecular markers to identify and characterize root morphology traits in rice (Champoux et al., 1995; Lilley et al., 1996; Zheng et al., 2000). Drought resistance is an important trait for rice breeders as 40 % of the area planted to rice worldwide experiences water stress. Subsistence farmers grow rice without irrigation in lowland and highland environments. Root traits perform an important mechanism in avoiding drought in rice. Researchers generated a mapping population to study drought resistance in rice by crossing a *japonica* cultivar, Moroberekan, to an *indica* cultivar, Co39 (Champoux et al., 1995). Moroberekan is a drought resistant cultivar grown in the highlands and is known to possess a deep, thick root system. Co39 is a lowland cultivar susceptible to drought with a shallow root system yet possessing high dehydration tolerance traits. Root thickness, root/shoot ratio and root dry weight per tiller were recorded for 203 RILs in 3 different greenhouse experiments. Plant response to drought stress was recorded visually by the degree of leaf rolling. This drought avoidance trait was associated with three root traits mapped to various locations on 10 different chromosomes. Most of the QTL identified for root characteristics clustered around chromosomal regions conferring drought avoidance. Markers associated with these root traits would facilitate selection for otherwise hard-to-score root traits. The linkage map was used as a basis to add additional drought-related traits in subsequent studies (Lilley et al., 1996). Osmotic adjustment at 70 % relative water content and lethal osmotic potential which are characterized as drought tolerant traits were added (Lilley et al., 1996). Three of the five QTL associated with drought tolerance were mapped to the same chromosomal regions as were the root traits. Since the drought tolerance and drought avoidance traits were inherited from separate parents the drought tolerant traits were negatively associated

with the root traits that aid in drought avoidance. Linked markers should facilitate the process of breaking the negative linkage between the avoidance and tolerance traits.

Technological advances have improved our understanding of roots. Historically, the line intersect method provided the first easy way to estimate the total root length in plants (Newman, 1966). The excised root is placed in an area with randomly spaced lines. For each root sample only the number (N) of intersections between root and the straight lines is recorded. The total length of the straight lines (H) and the observing area (A) remain constant among experiments. The proposed equation of  $R = \pi NA(2H)^{-1}$ , allows a fast estimation of root length. The line intersect method can be used to measure 3.43 meters of root in 24 minutes with a coefficient of variation of 4.3 % while direct measurement took 67 minutes (Newman, 1966). This method was later revised to replace the randomly oriented lines with a grid and a length conversion factor for A and H (Tennant, 1975).

The next advance was to study the root system *in vivo*. All previous methods involved excavation of roots from the soil. In Georgia, an underground laboratory called a Rhizotron was built (Box, 1996). Angled glass acted as the ceiling of this laboratory. Roots would grow next to the glass and the roots could be monitored as they grew (Taylor et al., 1970). The initial investment is too high for the data collected using Rhizotron technology. This level of technology benefitted the development of the understanding of root physiology. It is unlikely that information generated through the Rhizotron technology will aid in breeding since large numbers of genotypes need to be evaluated.

Mini-rhizotrons were developed with the aid of miniature cameras. A glass tube penetrating the ground at an angle intersects the roots. A miniature video camera can

traverse the length of the tube and record the growth of the roots at different depths. In a drought stress experiment in corn, a mini-rhizotron showed that a short-term drought resulted in root losses near the soil surface and large increases in deep root growth (Box et al., 1989). Root growth over time and root morphology characteristics are measured *in vivo*. Root images are stored in a video format for a computer analysis.

The first computer program used to analyze root images was the DOS-based Delta-T Scan (Harris and Campbell, 1989). The program was used to measure root length, projected area and average diameter. Although the commands and print-out are difficult to understand, the program allowed the recording of more measurements in a shorter time. A window-based program, WinRhizo™, also measures multiple factors and has easy to use commands and an easy to understand print-out of the analysis. Roots are scanned into the computer and a digital image of the root is used to measure length based on pixel size. Resolution to distinguish root parts is very fine and a color analysis can be conducted to separate root parts based on color differences. Roots discolored by disease infection can be separated from healthy roots in WinRhizo™. Length separated by diameter and root morphology characteristics such as topological indices and fractal dimension can also be measured.

Topology is a method of mathematically describing the root system's branching structure. Root systems are, in large part, trivalent branching structures meaning that each node or vertex has three branches or links (Fitter, 1996). The number of links in a system can be separated into exterior links which end in a meristem and internal links which join other links. The magnitude of any individual link is the number of exterior links it serves. Other measurements such as the length of links, branching angle, distribution of branches

and relative diameter can describe the system in further detail. Plants with equal magnitude can vary in branching structure from herringbone at one extreme to dichotomous to the other (Figure 9). These branching patterns can be quantified using two parameters, altitude ( $a$ ), and the exterior path length ( $p_e$ ). Altitude is the number of links in the longest path connecting an exterior link to a base link. Exterior path length is the sum of the number of links in all such paths. In common bean, a type II growth habit exhibits a herringbone structure while a type I plant tends to have a dichotomous root structure (Lynch and van Beem, 1993).

Root growth and architecture may be associated with genotypic adaptation to water stress. Four different growth habits of common bean and three different root parts (taproot, taproot laterals, and basal roots) were evaluated for growth rates, dry weight and final root length (Lynch and van Beem, 1993). The taproot lengths were similar in all genotypes. No genetic differences were observed in specific root length (length/weight). Significant genotypic differences were observed in root branching patterns. The number of apical meristems was highest in type III than type I growth habits. Topological indices differed significantly between type II (herringbone) and type I (dichotomous). This research supports an association between root and shoot architecture. Topology and number of meristems were very descriptive of growth habit and root architecture. Root length, dry weight and fractal analysis were equal in usefulness. The utility of fractal dimension as a selection criterion requires further study in bean.

Fractal analysis has been related to plant root systems (Tatsumi et al., 1989). Various objects in nature such as clouds, mountains, coastlines and trees have been described by fractal geometry (Mandelbrot, 1977). The intricacy of shape of the root

systems is characterized by the slope of each line as an estimate of the fractal dimension, D. Methods to quantify root morphology, such as topology and fractal dimension have been developed but have not been widely applied.

Numerous methods have been used to collect bean root data. Field (Yan et al., 1995), pouch (McMichael et al., 1985; Yabba, 2001), split-root (Snapp et al., 1995; Aguirre-Medina et al., 1998), hydroponic (Gabelman et al., 1986; Checkai et al., 1987) and soil-filled PVC tube (Yabba, 2001) mediums have been used to collect root samples. For breeding purposes, a quick and efficient method of collection and analysis is desired. Soil-less mediums are less laborious and time consuming. The roots are free from soil or debris so that measurement is fast and efficient.

The objectives of this study was i) to identify drought resistant genotypes from two black bean RIL populations grown under moisture stress and non-stress conditions in Central and North America and ii) to evaluate root characteristics and previously reported RAPD markers associated with drought resistance for their ability to predict yield under stress in the two RIL populations.



## MATERIALS AND METHODS

### Field Study

#### Parents and Pedigrees

Three black bean genotypes were crossed to produce two RIL populations segregating for drought resistance. The drought resistant genotype, B98311, was originally derived from a cross between drought resistant breeding line, T-3016 and the Michigan cultivar, Raven. T-3016 is a non-commercial Durango race breeding line previously identified as the most drought resistant genotype based on GM from a cross of Sierra/AC1028 (Schneider et al., 1997b). T-3016 was previously evaluated for root length (Yabba and Foster, 1997) and RAPD markers associated with drought resistance (Schneider et al., 1997a). Raven is an early-season black bean with resistance to anthracnose and Bean Common Mosaic Virus (Kelly et al., 1994). During the 1998 drought in Michigan, B98311 was selected as the highest yielding genotype under stress (Kolkman and Kelly, 1999).

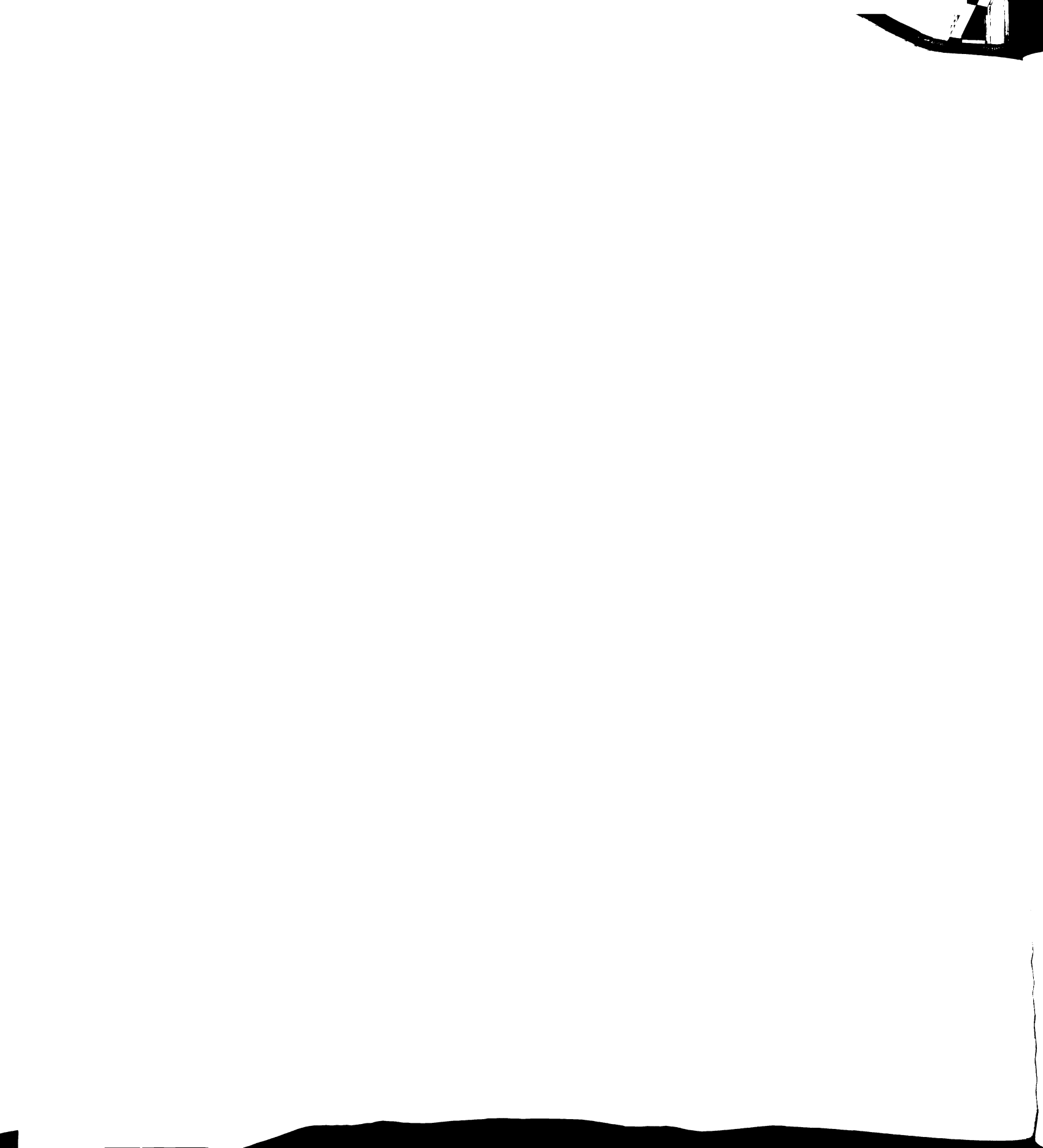
TLP 19 was developed for tolerance to low phosphorous at the International Center for Tropical Agriculture (CIAT). Phosphorus-efficient bean genotypes respond to phosphorus stress by developing a shallow root system (Liao et al., 2001). The contrasting root architecture of B98311 and TLP 19 was considered in parental selection to create genotypes with different root systems which could aid in drought resistance. Under terminal drought stress in Mexico, TLP 19 has shown resistance to *Macrophomina phaseolina* (Tassi) Goid., the causal fungus of ashy stem blight (ASB), a disease that is prevalent under water stress conditions (Mayek-Pérez et al., 2001a; Mayek-Pérez et al.,

2001b). The third genotype, VAX 5, was developed at CIAT from an interspecific hybridization of common and tepary bean and selected for resistance to common bacterial blight (CBB) (*Xanthomonas campestris*) (Singh and Munoz, 1999). TLP 19 and VAX 5 were selected as parents for their adaptation to lowland-tropical conditions and good combining ability with B98311, adapted to temperate conditions. Additional traits such as commercial seed type, growth habit and disease resistance were considered in the selection of parents in order to hasten the utilization of any beneficial black genotypes resulting from this work in the Latin American/Caribbean region.

### Population Development

The original crosses made in 1998 were B98311/TLP 19 and B98311/VAX 5 which generated populations L88 and L91 respectively. In September 1999, single pods from each  $F_2$  plant were harvested in both populations.  $F_3$  seed was advanced to the  $F_4$  generation using single seed descent (SSD). Single pods were harvested from  $F_3$  plants and the SSD process was repeated. The last single plant selection was made in the  $F_3$  generation so that seed planted in the greenhouse was at the  $F_{3:4}$  generation. Seed from each  $F_{3:4}$  genotype was harvested in bulk. This  $F_{3:5}$  seed was planted in Saginaw, MI in 2000 to increase the amount of seed and  $F_{3:6}$  seed was shipped to Honduras for testing in 2001. A total of 81 RILs in L88 and 69 RILs in L91 population were produced for testing.

### Saginaw, MI 2000



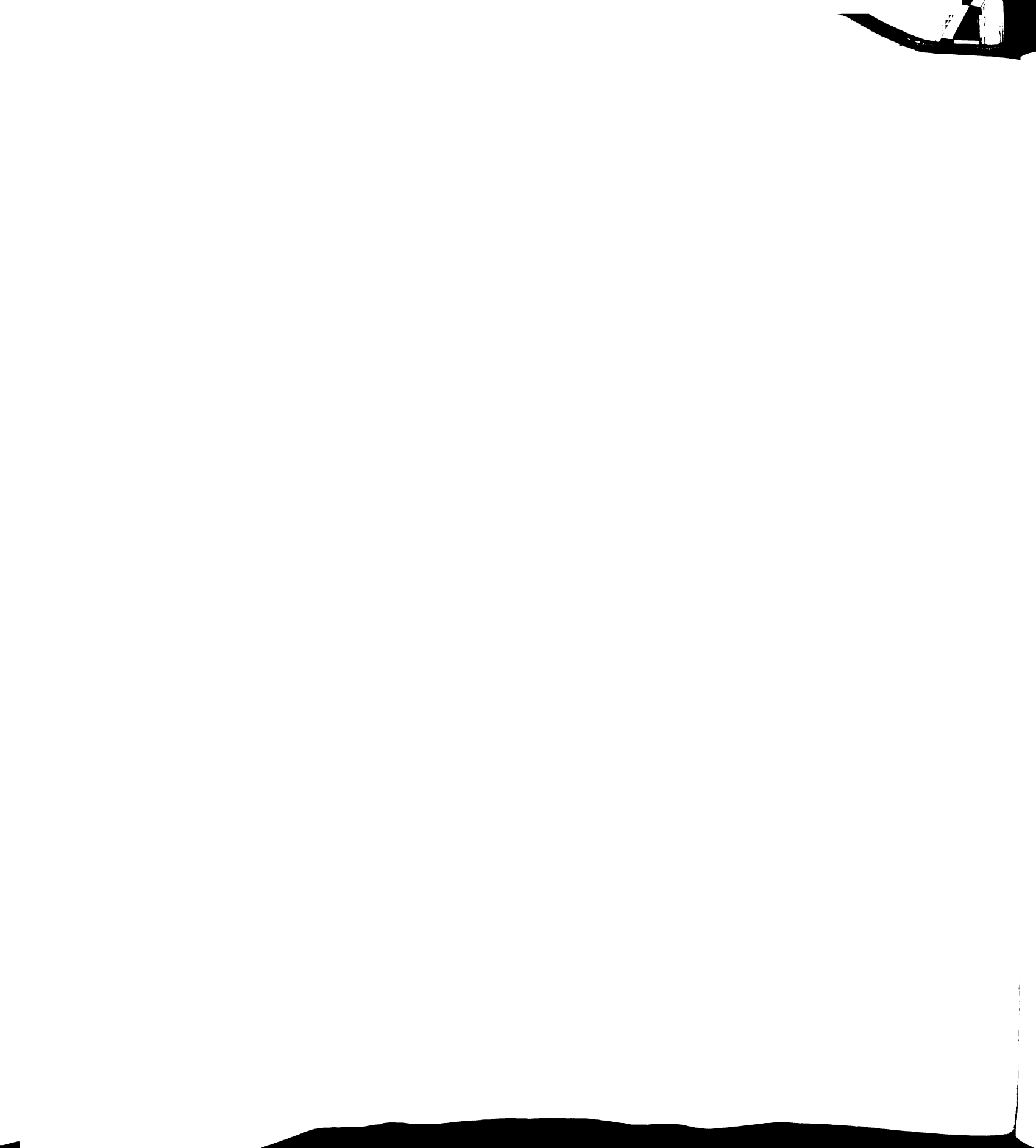
A randomized complete block design of 160 genotypes was planted with 3 replications on June 9<sup>th</sup>, 2000 at the Bean and Beet Farm in Saginaw Michigan (43°41' N, 84°08' W, 183m). The 150 (69 + 81) RILs and ten checks were space-planted in single rows of 20 seeds each. The ten checks included local Michigan cultivars Black Jack, Blackhawk, Jaguar, Phantom and T-39 along with drought resistant breeding lines B98311, N98122, T-3016 and V8025. At planting, 280 kg/ha of fertilizer 27:7:0 plus 4 % Mn and 1 % Zn were applied as a band next to the seed. The soil type at the Bean and Beet Farm in Saginaw, MI is a Misteguay (fine, mixed (calcerous), mesic Aeric Endoaquepts). Weeds were controlled by a pre-plant incorporation (ppi) of 5 L/ha Frontier (dimethenamid) and 10 L/ha Eptam (EPTC). Potato leaf-hoppers were controlled by a 2.5 L/ha application of Cygon (dimethoate) at 25 and 33 days after planting (dap). Benlate (benomyl) was applied at a rate of 3.6 kg/ha and Champ (copper hydroxide) at 5 L/ha on 35 and 46 dap to control fungal and bacterial diseases. Plant stand was recorded along with seed weight, percent moisture and 100 seed weight.

#### Zamorano, Honduras 2001

On January 23<sup>rd</sup>, 2001, 150 RILs, 3 parents and 7 checks were planted by hand in Zamorano, Honduras (14°00' N, 87°02' W, 800m) in collaboration with Programa de Investigaciones en Frijol (PIF). The seven checks included two PIF breeding lines (Tio Canela-75 and EAP 9510-77), two Mexican genotypes (Tacana and V8025) and three drought resistant genotypes (BAT 477, Rio Tibagi and SEA 5). This experiment was designed as a completely randomized design (CRD) with three replications per moisture treatment. Plots were 5.0m long and 0.70m wide. One-hundred seeds were planted in

each row and were thinned to 50 plants for uniform stands. Rows were hilled for furrow irrigation. Weeds were controlled by hand when needed. The type of soil was a sandy-loam, isohyperthermic Mollic Ustifluvent. At planting, one application of 130 kg/ha of diammonia phosphate 18:46:0 was applied. Urea 46:0:0 was applied 25 days after planting (dap) at the rate 65 kg/ha. Fertilizer 20:20:20 was applied at 300 L/ha before flowering at 37 dap. Two applications of Endosulfan (endosulfan) were applied at 18 and 32 dap at 1.5 L/ha to control white fly (*Bemisia Tabaci*). The fungicide Sapro (triforin) was applied 45 dap at 1.5 L/ha in order to control Macrophomina. Agrimicin which includes streptomycin and copper sulfate was applied 55 dap at 0.7 kg/ha to control CBB. A second insecticide, Basudín with the active ingredient, Diazinon, was applied at 28 dap at 1 L/ha to control corn rootworm beetles (*Diabrotica sp.*).

Moisture stress and non-stress treatments were applied through control of irrigation as January through April is the dry season in Honduras. The moisture stressed plots received 269 mm of rainfall and overhead irrigation and also 3 additional waterings by furrow irrigation. The non-stressed plots received 261 mm of rainfall and overhead irrigation along with 7 furrow irrigations. Tensiometers were installed to measure soil moisture. Readings above 60 cb signal that the soil is too dry and plants are being damaged by the water loss. Readings around 20 signify good moisture and aeration for optimal plant growth. Two weeks before flowering, they recorded 53 centibars (cb) in the stressed plots and 22 cb in the non-stressed plots. Two weeks after the first flowering, the stressed plots were experiencing 68 cb of soil suction while the non-stressed plots experienced 21 cb. Adequate moisture stress was recorded by soil moisture tests and differences in yield between treatments.



Only 30 plants were harvested per row to record yield. Agronomic field notes taken before harvest included days to flower, height, lodging, desirability score (DS), *Macrophomina phaseolina* incidence at 45 and 75 dap and plant stand. Desirability score is an overall rating from one to nine of plant architecture, number of pods, amount of disease, uniformity in maturity and uniformity of plants within the plot. Yield, biomass, percent moisture and 100 seed weight were recorded at harvest. Yield data was used to calculate GM, HI, DSI and DII for the experiment.

#### Montcalm, MI 2001

Using the geometric mean yield of the genotypes grown in Honduras as the selection criteria, the top and bottom 10 % of 150 RILs were selected for testing in Michigan. Although, RILs from population L88 tended to yield higher than L91 in Honduras, an equal number from each population was represented in the selections. From population L88, eleven resistant and five susceptible RILs were selected and from population L91, five resistant and ten susceptible RILs were selected (Table 3). Local cultivars, Phantom and T-39 and the three parents were included to complete a 36 entry, 6x6 Square Lattice experimental design, which was planted at the Montcalm Research Station (43°40' N, 85°20' W, 244m) on June 16<sup>th</sup>, 2001. The soil type is a McBride sandy loam (coarse-loamy, mixed, mesic Alfic Fragiorthods). Water stressed and non-stressed plots were irrigated by overhead sprayers. Irrigated plots received 38 mm more water than stressed plots. An early drought began seven days after planting where less than 5.1 mm of rain fell during the next 30 days. Herbicides, Treflan (trifluralin) at 2.5 L/ha and Dual (metolochlor) at 5 L/ha, were applied ppi to control weeds. At 27 dap, 1.25 L/ha Reflex





(fomesafen) was also applied. Weeds were pulled by hand when needed. An application of 280 kg/ha of 19:19:19 fertilizer was applied as a band at planting. An additional 34 kg/ha of N was applied 33 dap. Cygon (dimethoate) was applied to control potato leaf hoppers (*Empoasca fabae*) at 21 dap. Agronomic field notes taken before harvest included days to flower, height, lodging and DS. Seed weight, percent moisture and 100 seed weight was recorded at harvest.

### Root Protocol

The pouch method was used to collect root data (Yabba, 2001). Twenty to thirty seeds were germinated in the germination chamber. After four days, three seedlings from each genotype were transferred to pouches. A pouch consisted of a 25.4 cm x 35.6 cm clear plastic bag with 21.0 cm x 37.6 cm germination paper inside. The top of the germination paper is folded into a trough and a hole is cut so that the growing hypocotyls have a place to rest while the root adjusts. The pouch is stapled to a 24.8 cm x 35.6 cm piece of 14 ply cardboard. The pouch is then placed vertically into a slotted wooden box within a growth chamber. Growth chamber conditions included a 23/20°C day/night temperature and a 15 hr photoperiod. Each sample (pouch) received 360-400 ml of Hoagland's solution throughout the 14 day growth period.

At the end of this period the shoot was excised from the root. The root was removed from the pouch and put into a 0.1 g/L staining solution containing Methyl Violet. After a 24 hour period of staining, the root was transferred to a 30 cm x 20 cm plexiglass plate. Root laterals were separated using tweezers in order to minimize overlapping of roots. Root samples were scanned into a digital image using WinRhizo™ 4.10b (Régent

Instruments Inc., 2000). At 14 days after transplanting, the average root density was 0.03 mm root per mm<sup>2</sup> surface area. A resolution of 300 dpi and the automatic threshold for WinRhizo™ were used. Using the Batch Analysis, all samples in one replication were measured for the morphological traits and fractal dimension. Roots were stored in Whirl-pak bags (4 ounce) containing 50 ml water and staining solution.

Root characteristics such as total root length, root length according to diameter and fractal dimension were recorded. WinRhizo™ measures length according to pixel size and area covered. Root length according to root diameter was determined by using ten different root diameter ranges (A-J), each differing by 0.5 mm. The procedure to determine fractal dimension for root systems was summarized (Tatsumi et al., 1989);

A large square frame of a side 1 was placed over the object, then divided into  $(1/r)^2$  squares of side  $r$ . The number  $N(r)$  of the squares that intersected the object were counted, and  $\log N(r)$  was plotted against  $\log r$ . If, by measuring  $N(r)$  at small values of  $r$ , a straight line with negative slope,  $-D$ , is obtained, the interpretation is that the object is fractal and  $D$  is the fractal dimension ( $1 \leq D \leq 2$ ), since

$$\log N(r) = -D \log r + \log K$$

where  $K$  is a constant, whence

$$N(r) \propto r^{-D}$$

Note that at one extreme, for objects like straight lines  $D$  becomes 1, and at the other extreme, for plane-filling curves,  $D$  is 2.

### Marker Protocol

#### DNA Extraction

Leaf tissue from each F<sub>3,4</sub> RIL, check and parental genotype was harvested, lyophilized and ground. Lyophilized and ground tissue was allocated into 100 ml samples and DNA was extracted following the mini-prep procedure (Afanador et al., 1993). The

DNA concentration of each sample was quantified using a fluorometer (Hofer TKO100, Hofer Scientific, San Francisco, CA). This stock sample was diluted to a 10 ng/ml working solution for amplification by the polymerase chain reaction (PCR).

#### PCR protocol

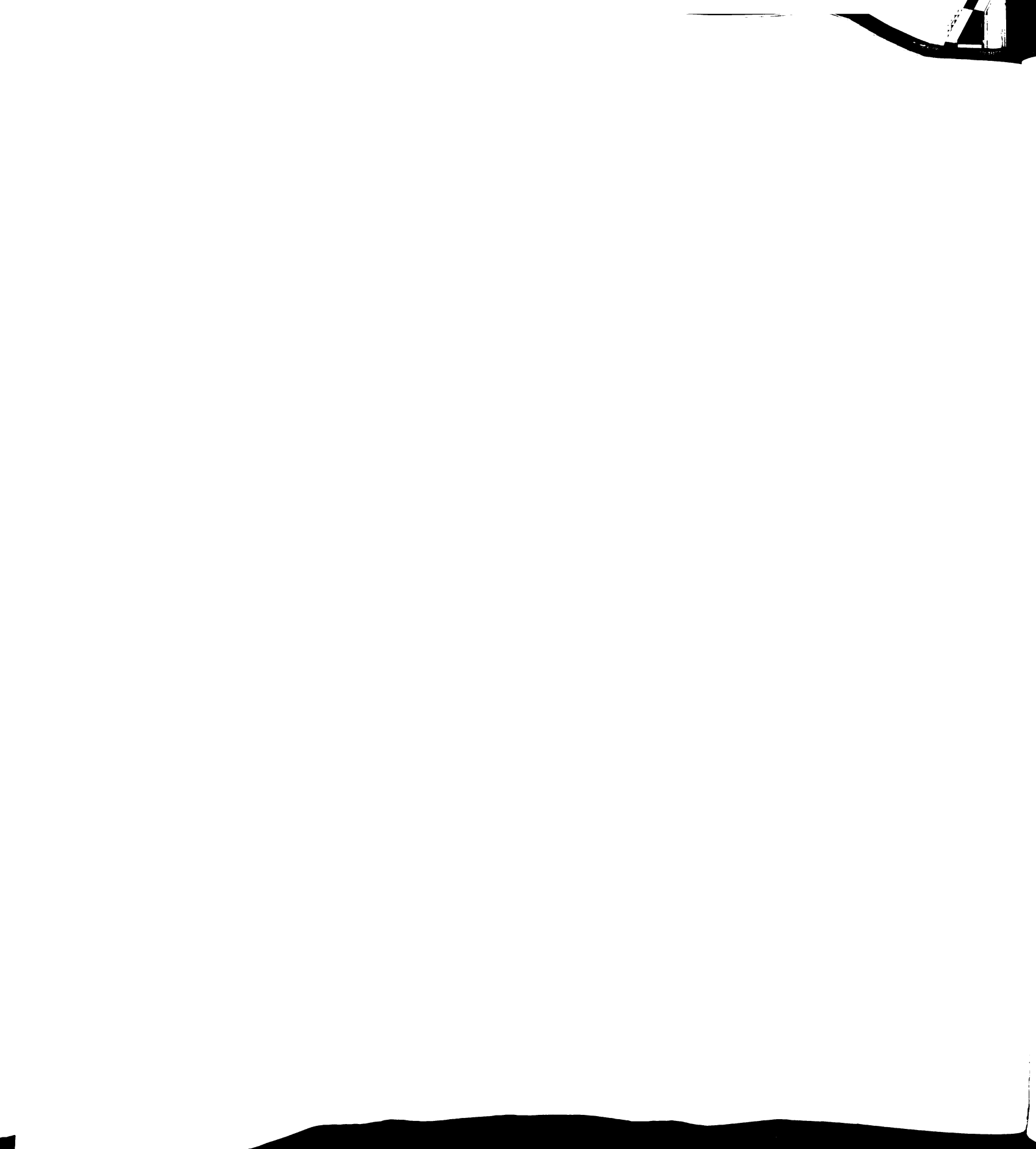
The modified PCR procedure (Haley et al., 1994) was used to amplify DNA. Primers reported to be associated with drought resistance (Schneider et al., 1997a) were used. The DNA was amplified using a Perkin Elmer Cetus DNA Thermal Cycler 480 (Perkin Elmer, Cetus, Norwalk, CT) with the following cycles: 1 min at 94°C, 1 min at 35°C, 2 min at 72°C for 3 cycles; 10 sec at 94°C, 20 sec at 40°C, 2 min at 72°C for 34 cycles; 5 min at 72°C; unlimited time at 4°C.

#### Electrophoresis

Approximately 20 µl of amplified DNA was separated by electrophoresis on a 1.4% agarose gel containing ethidium bromide 0.02 µg/ml, 40 mM Tris-acetate and 1mM EDTA. DNA was fluoresced by ultra-violet light and recorded by photograph.

#### **Statistical Analysis**

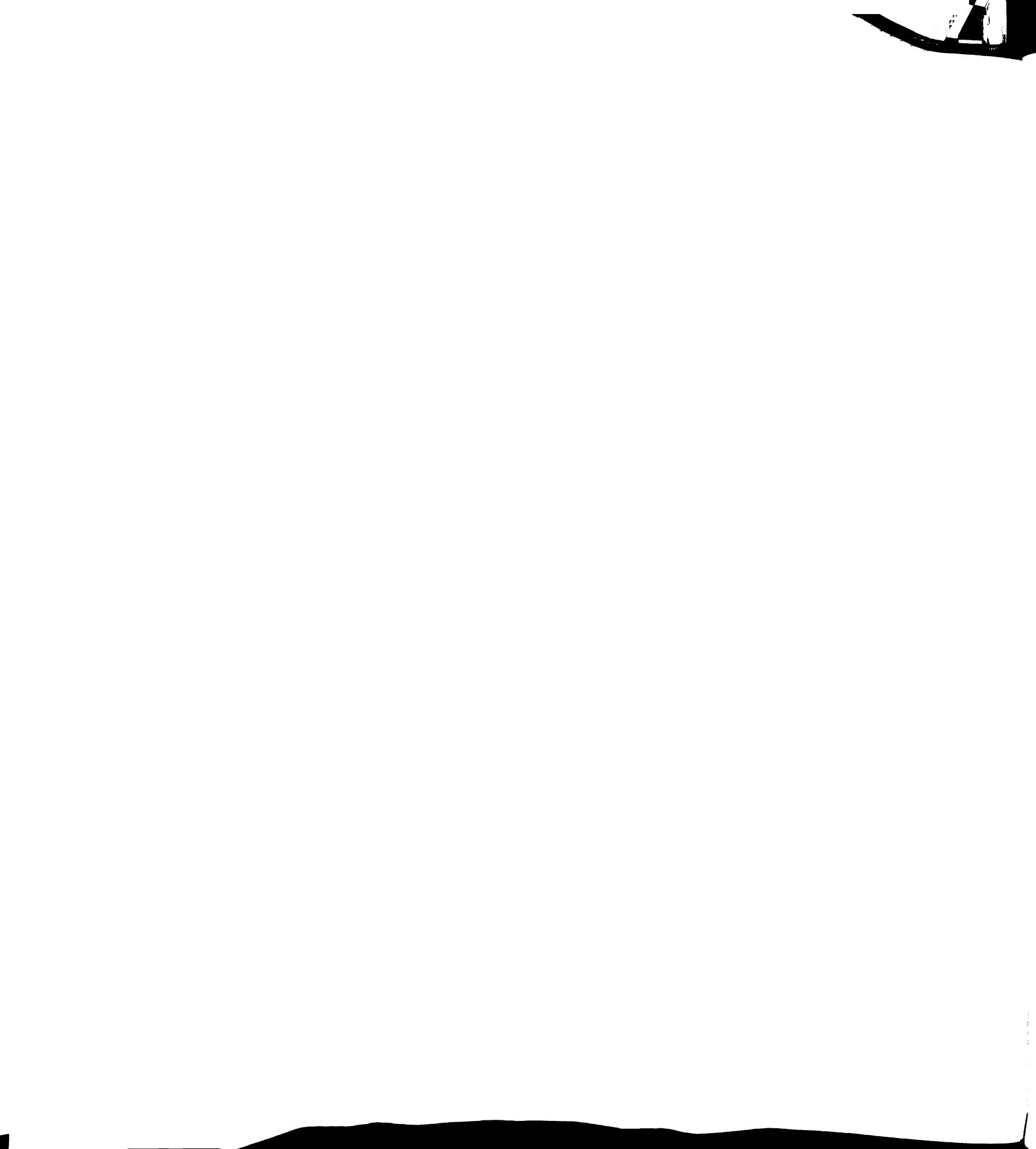
Analysis of variance (ANOVA) was calculated for each experiment. In the 2000 field experiment at Saginaw, data was analyzed as a randomized complete block design (RCBD) using PROC GLM with the number of harvested plants per plot as the covariant (SAS Institute Inc., 2000). In the Honduras 2001 experiment, the stress and non-stress treatments were analyzed as two CRDs. ANOVA was calculated for each treatment with



harvested plants per plot as the covariant. Each population was analyzed separately. Means, LSD values and CV values were calculated after being adjusted for the covariant. Yield means for individual RILs of the stress treatment were used with the corresponding yield means of the non-stress treatment to calculate GM and DSI. DII was calculated using the overall mean yields of each treatment.

In the Montcalm 2001 experiment, data from 36 genotypes were analyzed using a 6x6 square lattice design. ANOVA was calculated for each treatment. Mean yield, LSD and CV values were calculated for each treatment. Even though the DII was low, GM was calculated among genotypes. Regression analysis was conducted to compare yield trends between locations for the 31 selected RILs. The  $R^2$  values within corresponding figures were calculated by the regression function within Microsoft Excel. Correlations were made using PROC CORR (SAS Institute Inc., 2000) between yield, biomass, 100 seed weight, plant stand, disease incidence (DI) for *Macrophomina phaseolina* at 45 and 75 dap. Correlations were also made among yield, biomass, 100 seed weight, days to flowering, height, lodging, days to maturity, DS and PM.

Root measurements of RILs in population L88 were analyzed using PROC GLM for mean, LSD and CV values. Total root length, length according to diameter class and fractal dimension were correlated to Yd, Yp and GM using simple linear regression (PROC REG) and correlation (PROC CORR) methods of analysis (SAS Institute Inc., 2000). Both simple and multiple linear regression were used to associate molecular marker values to yield-based traits. The degree of association between traits was reported by the Pearson coefficient values ( $r$ ) and the Coefficient of Determination ( $R^2$ ). Multiple regression analysis was used to determine the best model of root and molecular marker



traits **that** explained the **highest** amount of variation for yield under **stress and non-stress** conditions.

## RESULTS

### Field Study

Three field experiments were conducted over two years and three locations to study the genetics of drought resistance in two black bean populations L88 and L91. Both populations showed marked differences in the first field test in Saginaw, MI in 2000. A seed increase was needed to meet the requirement of having sufficient seed for stress and non-stress treatments in Honduras. Space-planting allowed each plant to grow without competition. Therefore the yield results from Saginaw may be inflated, but evaluations of yield potential and comparisons between populations were performed.

Significant genotypic differences existed in both populations for yield and 100 seed weight (100 sw) grown in Saginaw (Table 1). Mean yield in L88 was 470 kg/ha higher ( $p < .05$ ) than the mean yield in L91, suggesting that L88 has a greater yield potential. Yield for individual RILs in L88 ranged from 2257 to 4926 kg/ha (Figure 1). The range of yield for L91 RILs represented lower yield potential and ranged from 1868 to 4323 kg/ha. Mean 100 sw for L88 was 23.0 g whereas 100 sw was 24.9 g in L91 (Table 1). Overall, the RILs in L88 produced a larger number of seeds whereas the RILs in L91 had larger seed size. This relationship is supported by differences in seed sizes between parents as VAX 5 is larger than TLP 19.

Yield potential ( $Y_p$ ) and the ability to yield under moisture stress ( $Y_d$ ) were tested in non-stress and stress treatments at Zamorano, Honduras in 2001. The DII for the Honduras experiment was 0.82 and the DSI of individual genotypes ranged from 0.62 to 1.20. Conditions within the tropical climate, such as high temperatures, short day length



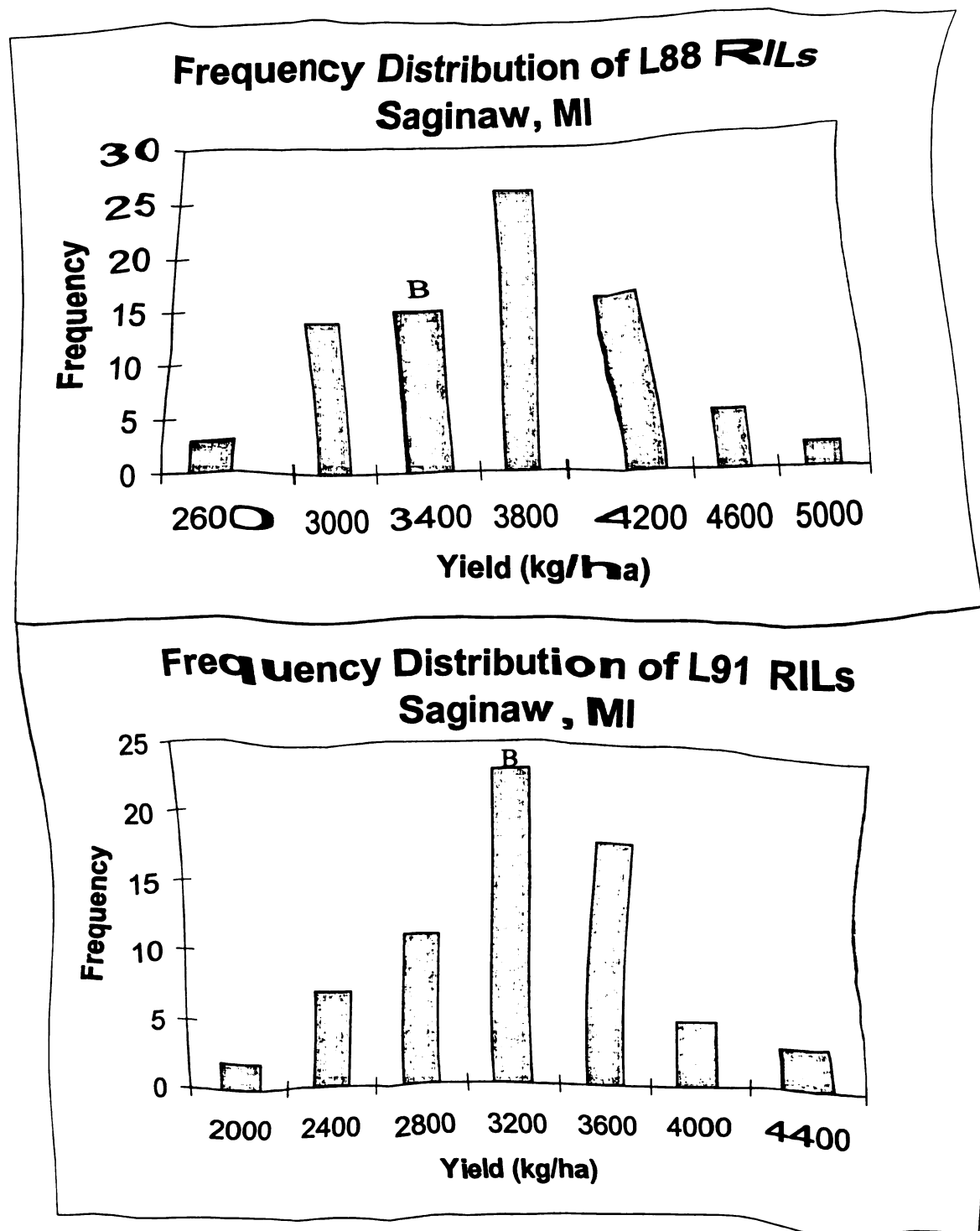


Figure 1. Frequency Distributions for yield using the adjusted means from each population in Saginaw, MI 2000. The drought resistant parent, B98311, is indicated by (B).

Table 1. Analysis of variance for the RILs at the F<sub>3.5</sub> generation in the L88 and L91 populations for yield and 100 seed weight at Saginaw, MI 2000.

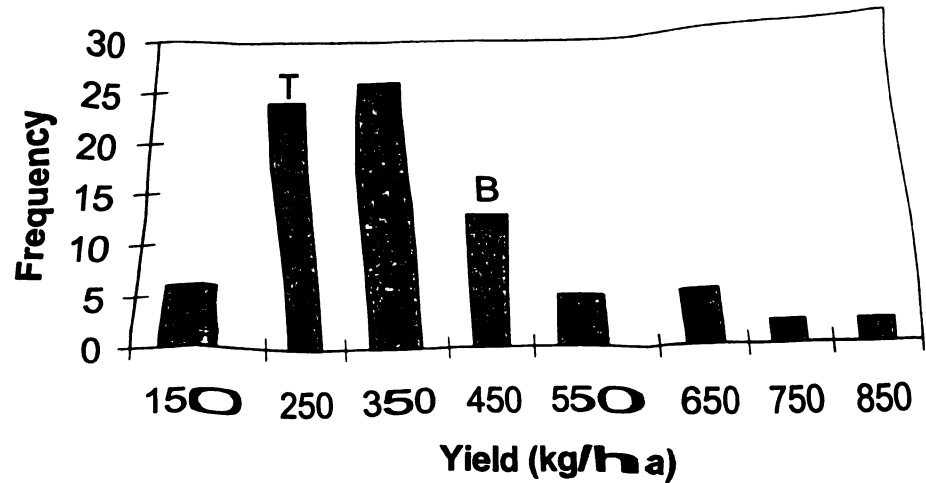
Source	L88			L91		
	DF	MS	F Test	DF	MS	F Test
			Yield (kg/ha)			Yield (kg/ha)
Grand Mean			3512			3042
LSD (0.05)			1056			977
CV			19			20
Replication	2	837809	1.94	2	1191759	3.26*
Genotype	80	898733	2.09****	68	759650	2.08***
Stand	1	9923410	23.03****	1	17782870	48.58****
Error	159	430847		135	366035	
			100 Seed weight (g)			100 Seed weight (g)
Grand Mean			23.0			24.9
LSD (0.05)			1.6			1.9
CV			4.3			4.8
Replication	2	19.9	20.8****	2	3.8	2.7
Genotype	80	9.4	9.8****	68	14.5	10.2****
Stand	1	0.6	0.6	1	0.1	0.1
Error	159	1.0		135	193.0	

\*P<.05; \*\*P<.01; \*\*\*P<.001; \*\*\*\*P<.0001

and low soil fertility, considerably decreased Y<sub>p</sub> compared to Michigan yet valid comparisons among most genetic traits could be conducted. Frequency distributions of mean yields showed the trends toward moisture stress and non-stress conditions (Figure 2). Distribution of yield was skewed with only 15 RILs yielding above 450 kg/ha. Mean yields for L88 RILs ranged from 77 to 842 kg/ha in the stress treatment (Figure 2) with an overall mean of 317 kg/ha. In the non-stress treatment, mean yields in the same population ranged from 1441 to 2922 kg/ha with an overall mean of 2060 kg/ha. The frequency distribution of RILs for Y<sub>p</sub> appears to resemble a normal Gaussian curve. Significant differences were recorded among genotypes for yield in the stress treatment, but not in the non-stress treatment (Table 2).

The frequency distribution in L91 followed a similar pattern to L88 (Figure 3). The histogram showing Y<sub>d</sub> in L91 was also skewed, yet only three RILs yielded above

### Frequency Distribution for $Y_d$ in L88, Zamorano



### Frequency Distribution for $Y_p$ in L88, Zamorano

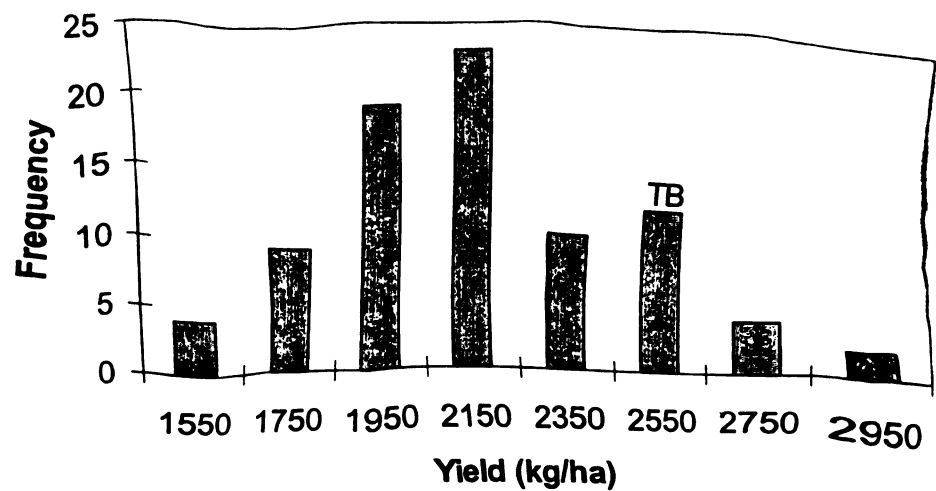


Figure 2. Frequency Distributions for yield under stress ( $Y_d$ ) and non-stress ( $Y_p$ ) using the adjusted means from each RIL from population L88 in Honduras. Mean yield of the parents, B98311 (B) and TLP 19 (T) are noted.

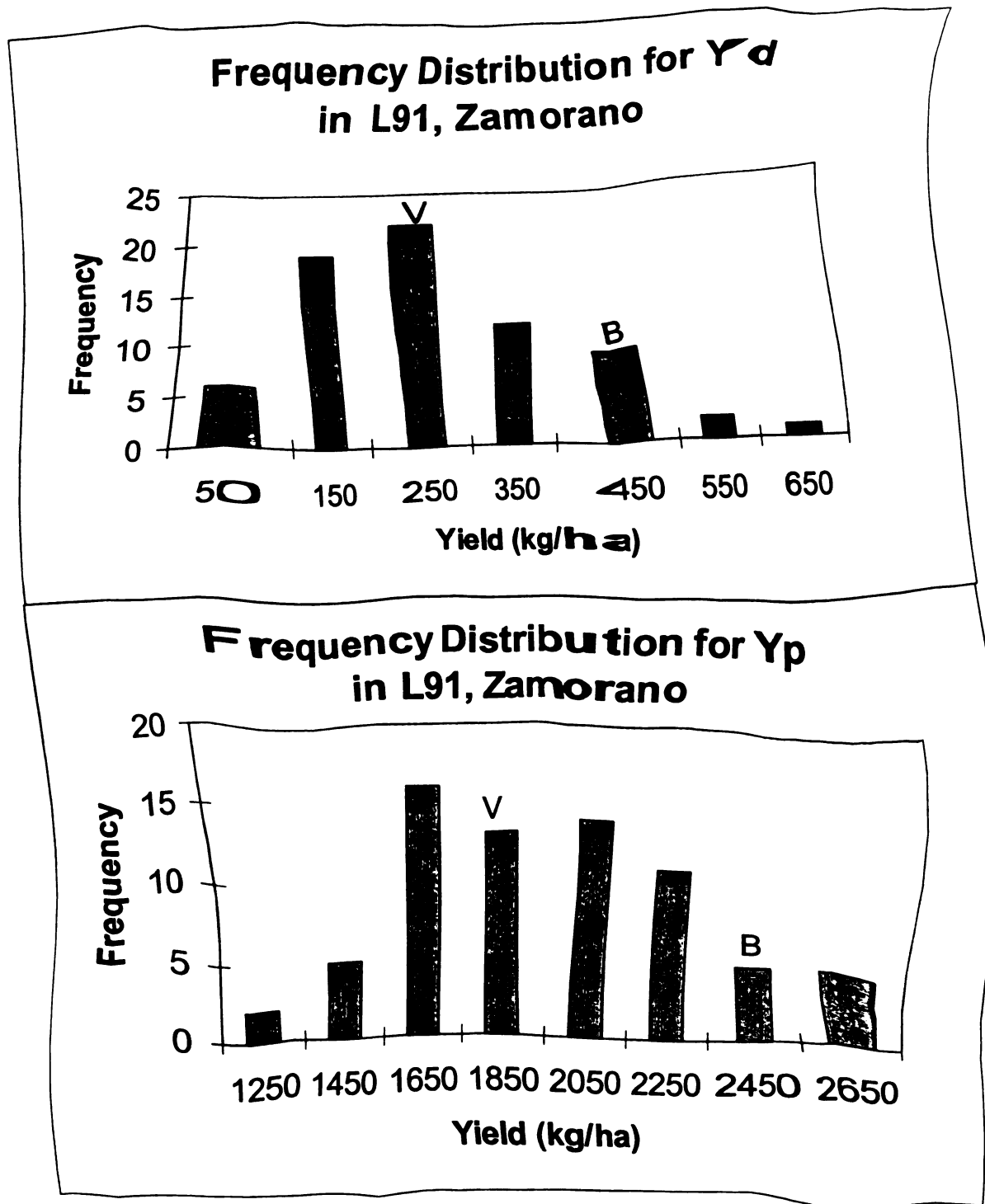


Figure 3. Frequency Distributions for yield under stress ( $Y_d$ ) and non-stress ( $Y_p$ ) using the adjusted means from each RIL from population L91 in Honduras. Mean yield of the parents, B98311 (B) and VAX 5 (V) are noted.

Table 2. Analysis of variance for 83 genotypes of population L88 in stress and non-stress treatments from Honduras 2001.

Source	DF	Stress		Non-stress	
		MS	F Test	MS	F Test
Yield (kg/ha)					
Grand Mean			317		2060
LSD (0.05)			357		916
CV			70		28
Replication	2	125133	2.55	1053485	3.27*
Genotype	82	72286	1.47*	308196	0.96
Stand	1	864683	17.60****	1088310	3.37
Error	163	49127		322544	
Biomass (kg/ha)					
Grand Mean			1676		3974
LSD (0.05)			1165		1582
CV			37		25
Replication	2	2075102	5.36**	4953171	5.17**
Genotype	82	539428	1.39*	826276	0.86
Stand	1	22548148	58.28****	1576618	1.64
Error	163	386921		958522	
100 Seed Weight (g)					
Grand Mean			17.9		18.9
LSD (0.05)			NA		2.7
CV			6.9		8.9
Replication	2	2.8	1.80	341.6	120.39****
Genotype	81	4.9	3.14****	6.9	2.43****
Stand	1	2.0	1.26	0.1	0.04
Error	125	1.6		2.8	

\*P<.05; \*\*P<.01; \*\*\*P<.001; \*\*\*\*P<.0001

450 kg/ha. Mean yields for L91 under stress ranged from 2 to 599 kg/ha with an overall mean of 211 kg/ha (Figure 3). RILs in the non-stress treatment ranged from 1130 to 2587 kg/ha and averaged 1863 kg/ha. Significant differences existed for yield among genotypes in the stress treatment but not among genotypes in the non-stress treatment (Table 3). As in Saginaw, mean yield in L88 was greater than in L91 for both stress ( $p<.05$ ) and non-stress conditions (not significant) in Honduras.

Data for 100 sw followed trends similar to the results in Saginaw (Table 2). In L88, mean values for 100 sw ranged from 13.8 to 21.6 g in the stress plots and 14.2 to 27.9 g in the non-stress plots with overall means of 17.9 and 18.9 g respectively. In L91,

Table 3. Analysis of variance for 71 genotypes of population L91 in stress and non-stress treatments from Honduras 2001.

Source	DF	Stress		Non-stress	
		MS	F Test	MS	F Test
Yield (kg/ha)					
Grand Mean			211		1863
LSD (0.05)			303		931
CV			89		31
Replication	2	104563	2.97	593066	1.78*
Genotype	70	49137	1.40*	360422	1.08
Stand	1	181353	5.15*	661116	1.99
Error	139	35199		332432	
Biomass (kg/ha)					
Grand Mean			1661		3709
LSD (0.05)			1267		1379
CV			43		23
Replication	2	2600809	5.17**	6465282	9.12***
Genotype	70	527540	1.05	894284	1.26
Stand	1	16358738	32.51****	3680182	5.19*
Error	139	503117		708965	
100 Seed Weight (g)					
Grand Mean			19.9		21.5
LSD (0.05)			NA		3.1
CV			6.9		9.0
Replication	2	21.2	11.33****	278.6	75.05****
Genotype	62	8.9	4.76****	12.7	3.42****
Stand	1	3.6	1.94	7.2	1.95
Error	73	1.9		3.7	

\*P<.05; \*\*P<.01; \*\*\*P<.001; \*\*\*\*P<.0001

seed weight values ranged from 16.3 to 27.8 g in the stress treatment and from 17.4 to 26.7 g in the non-stress treatment with overall means of 19.9 and 21.5 g respectively.

Population L91 had a greater CV value than L88 in every category except biomass under non-stress (Tables 2 and 3). RILs in L91 must have greater standard deviation from the mean or a lower mean value when compared to L88 RILs. The fact that the CV values were so high can be attributed to a large variance due to environmental conditions of stress and a lack of control of experimental error in the CRD.

Despite high CV values, significant genotypic differences were observed for yield, biomass and 100 sw (Tables 2 and 3). For both populations, 100 sw had significant

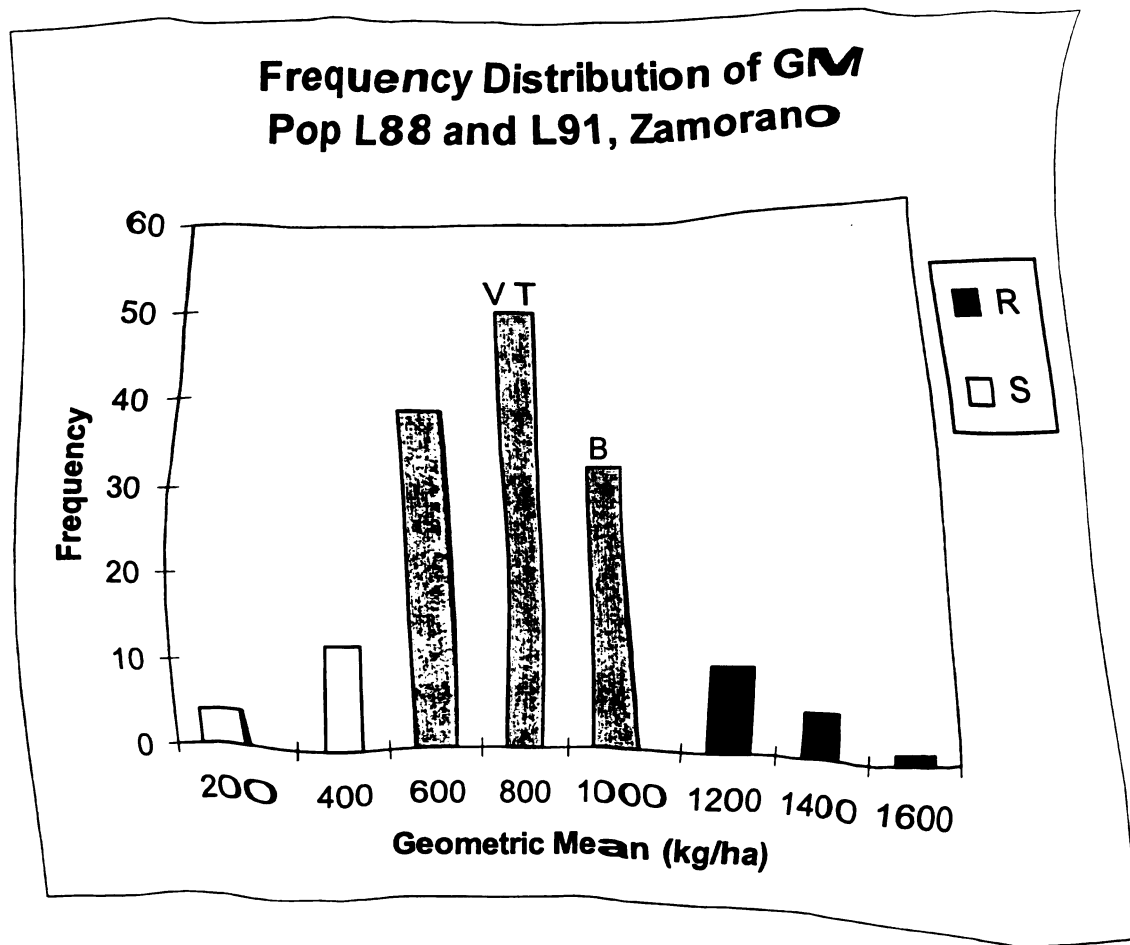


Figure 4. Frequency Distribution of 150 RILs showing selection of resistant (R) and susceptible (S) genotypes based on geometric mean. Parents VAX 5 (V), TLP 19 (T), and B98311 (B) are included.

genotypic differences in each treatment. In population L88, significant genotypic differences in yield and biomass were observed in the stress treatment, but not in the non-stress treatment. In population L91, only Yd showed significant genotypic differences whereas no significant genotypic differences were observed for Yp and biomass. Overall, the yield data from the stress treatment showed a separation of resistance and susceptible genotypes and yield potential of each genotype was expressed in the non-stress treatment.

To select genotypes with drought resistance, geometric mean (GM) between treatments was calculated. The frequency distribution of GM (Figure 4) appeared to follow a Gaussian curve. Resistant (R) and susceptible (S) RILs were selected based on the top and bottom 10 % of the curve. Other characteristics such as drought susceptibility (DSI) and harvest index (HI) for moisture stress and non-stress treatments were calculated (Table 6) but not used directly in the selection of resistant and susceptible individuals. Mean values for GM, DSI and HI were contrastingly different. The average GM value of the resistant RIL's was more than threefold greater than the susceptible RILs. A lower DSI value signifies a lower susceptibility to drought or a greater ability to tolerate moisture stress. The resistant RILs showed a greater tolerance to drought than the susceptible RILs. The resistant RILs had higher HI values and were more efficient than susceptible RILs in the stress and non-stress conditions.

Additional comparisons of the selected resistance and susceptible RILs from each population with checks is based and ranked on GM are shown in Tables 4 and 5. Parental values are also included to illustrate the transgressive segregation for different traits among the RILs. In every category, the parents are ranked in the middle while the resistant and susceptible RILs trended towards the extremes. The parents were not significantly different for yield or biomass in both treatments. The drought resistant parent, B98311, ranked higher than the susceptible parent in every category except biomass in L88. TLP 19 produced greater above-ground biomass than B98311 in both stress and non-stress treatments. VAX 5 out-yielded TLP 19 under stress, yet when comparing population mean, L88 out-yielded L91 in every category. In comparison to TLP 19, VAX 5 yielded much less under non-stress



Table 4. Yield (Yd or Yp), Biomass (BM), and 100 seed weight (100 sw) of the sixteen RILs selected as highest and lowest yielding based on Geometric Mean (GM) in population L88 grown under moisture stress and non-stress in Honduras 2001 †.

Genotype	GM kg/ha	Stress			Non-stress		
		Yd kg/ha	BM kg/ha	100 sw g	Yp kg/ha	BM kg/ha	100 sw g
<b>RILs</b>							
L88-63	1473 (1) ‡	842 (1)	2385 (15)	16.7	2576 (6)	4410 (26)	17.6
L88-74	1362 (2)	740 (3)	2610 (7)	16.9	2508 (12)	4725 (7)	17.9
L88-30	1328 (3)	779 (2)	1800 (60)	18.4	2263 (30)	4050 (52)	18.4
L88-69	1286 (4)	680 (4)	1890 (46)	17.6	2432 (18)	4500 (20)	18.2
L88-13	1285 (5)	565 (9)	2250 (20)	19.0	2922 (1)	5445 (1)	19.6
L88-66	1205 (6)	561 (10)	1575 (90)	18.3	2589 (4)	4590 (16)	17.4
L88-19	1126 (7)	579 (8)	1800 (59)	19.3	2188 (36)	4095 (46)	20.4
L88-3	1082 (8)	583 (7)	3015 (2)	19.4	2007 (70)	4050 (49)	18.7
L88-61	1050 (11)	507 (13)	1575 (91)	17.1	2173 (39)	3960 (62)	18.2
L88-31	1048 (12)	636 (5)	1935 (39)	18.9	1730 (117)	2970 (150)	18.8
L88-59	1033 (13)	455 (18)	2115 (28)	19.8	2344 (25)	4635 (12)	18.1
L88-37	577 (112)	224 (93)	990 (152)	21.3	1486 (151)	3600 (107)	21.8
L88-4	551 (122)	168 (119)	1440 (107)	19.7	1802 (105)	3600 (104)	18.2
L88-64	457 (139)	113 (141)	1215 (134)	18.3	1849 (97)	3825 (78)	18.7
L88-18	368 (146)	90 (144)	1755 (64)	-	1501 (148)	3420 (125)	21.6
L88-2	364 (147)	77 (150)	945 (155)	16.7	1729 (118)	4140 (49)	19.4
<b>Parents</b>							
B98311	951 (22)	375 (33)	1755 (71)	18.0	2411 (20)	4275 (36)	18.9
TLP 19	637 (95)	169 (118)	1890 (52)	18.4	2399 (22)	4545 (19)	19.6
<b>Checks</b>							
Tacana	667 (87)	213 (97)	1800 (62)	15.6	2097 (54)	4005 (61)	18.9
V8025	612 (102)	210 (99)	1395 (116)	16.7	1783 (108)	3240 (135)	17.3
Tio Canela	602 (105)	218 (95)	1845 (57)	17.8	1657 (129)	3870 (75)	20.2
EAP 9510-77	699 (78)	232 (89)	1710 (75)	19.5	2112 (50)	3465 (124)	20.8
BAT 477	784 (54)	400 (27)	2655 (6)	19.7	1536 (141)	2475 (160)	20.8
Rio Tibagi	886 (31)	372 (34)	3330 (1)	16.2	2108 (51)	3510 (120)	16.4
SEA 5	893 (30)	524 (11)	1260 (131)	20.9	1521 (145)	2835 (155)	25.4
Mean	699	269	1683	18.7	1957	3827	20.1
LSD (0.05)		333	1098	NA	933	1484	NA
CV		77	41	6.9	30	24	9.0

† Values of parents, checks and LSD values are included.

‡ Rankings (in parentheses), mean, LSD and CV values are derived from 160 genotypes.

Table 5. Yield (Yd or Yp), Biomass (BM), and 100 seed weight (100 sw) of the fifteen RILs selected as highest and lowest yielding based on Geometric Mean (GM) in population L91 grown under moisture stress and non-stress in Honduras 2001†.

Genotype	GM kg/ha	Stress			Non-stress		
		Yd kg/ha	BM kg/ha	100 sw g	Yp kg/ha	BM kg/ha	100 sw g
<b>RILs</b>							
L91-30	1073 (9)‡	599 (6)	1845 (55)	24.7	1922 (83)	3690 (98)	23.6
L91-25	1064 (10)	514 (12)	2520 (10)	19.6	2202 (34)	3825 (80)	24.2
L91-3	1023 (14)	435 (20)	1485 (105)	20.3	2406 (21)	4410 (27)	18.9
L91-59	1016 (15)	486 (15)	1890 (50)	19.9	2126 (46)	4455 (23)	20.9
L91-10	1004 (16)	448 (19)	1890 (47)	21.3	2250 (31)	4050 (54)	22.8
L91-37	402 (144)	77 (149)	1755 (70)	-	2092 (56)	3960 (67)	22.6
L91-22	392 (145)	79 (148)	1485 (106)	18.4	1938 (82)	3555 (111)	21.3
L91-41	282 (155)	47 (155)	810 (158)	17.8	1690 (120)	3690 (99)	21.2
L91-53	250 (156)	45 (156)	1845 (56)	-	1391 (157)	3060 (146)	21.0
L91-13	115 (158)	7 (158)	1755 (69)	-	1857 (96)	3780 (85)	20.9
L91-49	316 (151)	80 (147)	1035 (149)	-	1251 (158)	2655 (158)	22.7
L91-52	298 (154)	56 (154)	1125 (142)	18.3	1574 (139)	3600 (108)	19.9
L91-68	176 (157)	17 (157)	1710 (74)	-	1792 (106)	3735 (94)	20.5
L91-19	64 (159)	3 (159)	1935 (40)	-	1528 (143)	3645 (102)	26.6
L91-69	60 (160)	2 (160)	2745 (4)	-	1534 (142)	5175 (3)	26.7
<b>Parents</b>							
B98311	951 (8)	375 (9)	1755 (31)	18.0	2411 (6)	4275 (12)	18.9
VAX 5	663 (24)	249 (26)	1665 (39)	21.9	1765 (39)	3645 (40)	22.4
<b>Checks</b>							
Tacana	667 (87)	213 (97)	1800 (62)	15.6	2097 (54)	4005 (61)	18.9
V8025	612 (102)	210 (99)	1395 (116)	16.7	1783 (108)	3240 (135)	17.3
Tio Canela	602 (105)	218 (95)	1845 (57)	17.8	1657 (129)	3870 (75)	20.2
EAP 9510-77	699 (78)	232 (89)	1710 (75)	19.5	2112 (50)	3465 (124)	22.7
BAT 477	784 (54)	400 (27)	2655 (6)	19.7	1536 (141)	2475 (160)	20.8
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SEA 5	893 (30)	524 (11)	1260 (131)	20.9	1521 (145)	2835 (155)	25.4
Mean	699	269	1683	18.7	1957	3827	20.1
LSD (0.05)		333	1098	NA	933	1484	NA
CV		77	41	6.9	30	24	9.0

† Values of parents, checks and LSD values are included.

‡ Rankings (in parentheses), mean, LSD and CV values are derived from 160 genotypes.

Table 6. Resistant (16) and susceptible (15) RILs with geometric mean (GM), drought susceptibility index (DSI) and harvest index (HI) under stress and non-stress conditions, days to maturity (DTM) under the stress treatment and height under the stress and non-stress treatments in Honduras, 2001.

Resistant Genotype	GM	DSI	Stress HI	Non-stress HI	Stress DTM	Stress Height	Non-stress Height
	kg/ha				days	cm	cm
L88-63	1473	0.80	0.35	0.58	82	46	43
L88-74	1362	0.84	0.28	0.53	82	45	45
L88-30	1328	0.78	0.43	0.56	83	42	47
L88-69	1286	0.85	0.36	0.54	83	39	45
L88-13	1285	0.96	0.25	0.54	81	40	44
L88-66	1205	0.93	0.36	0.56	81	38	43
L88-19	1126	0.87	0.32	0.53	82	43	46
L88-3	1082	0.84	0.19	0.50	82	45	45
L91-30	1073	0.82	0.32	0.52	82	43	46
L91-25	1064	0.91	0.20	0.58	82	48	46
L88-61	1050	0.91	0.32	0.55	81	38	43
L88-31	1048	0.75	0.33	0.58	82	43	40
L88-59	1033	0.95	0.22	0.51	83	46	52
L91-3	1023	0.97	0.29	0.55	81	40	53
L91-59	1016	0.91	0.26	0.48	82	49	52
L91-10	1004	0.95	0.24	0.56	82	48	49
Mean	1153	0.88	0.30	0.54	81.9	43.3	46.1

Susceptible Genotype	GM	DSI	Stress HI	Non-stress HI	Stress DTM	Stress Height	Non-stress Height
	kg/ha				days	cm	cm
L88-37	577	1.01	0.23	0.41	82	36	47
L88-4	551	1.07	0.12	0.50	82	37	44
L88-64	457	1.11	0.09	0.48	83	30	44
L91-37	402	1.14	0.04	0.53	83	36	51
L91-22	392	1.14	0.05	0.55	82	37	46
L88-18	368	1.11	0.05	0.44	83	42	51
L88-2	364	1.13	0.08	0.42	82	34	45
L91-49	316	1.11	0.08	0.47	83	38	47
L91-52	298	1.14	0.05	0.44	83	39	49
L91-41	282	1.15	0.06	0.46	81	37	45
L91-53	250	1.15	0.02	0.45	82	36	40
L91-68	176	1.17	0.01	0.48	82	37	48
L91-13	115	1.18	0.00	0.49	83	39	47
L91-19	64	1.18	0.00	0.42	84	42	53
L91-69	60	1.18	0.00	0.30	84	41	51
Mean	311	1.13	0.06	0.46	82.6	37.4	47.2

Table 7. Analysis of variance for 36 genotypes grown under stress and non-stress treatments in Montcalm, MI 2001.

Source	DF	Stress		Non-stress	
		MS	F Test	MS	F Test
Yield (kg/ha)					
Grand Mean			2950		3006
LSD (0.05)			808		965
CV			16.7		19.5
Replication	2	2334.3		249.8	9.11***
Genotype	35	68.8	121.19***	105.3	3.84***
Block	15	62	3.57***	131.4	4.79***
Error	55	19.3	3.22***	27.4	
100 Seed Weight (g)					
Grand Mean			29.3		28.7
LSD (0.05)			2.1		2.2
CV			4.3		4.7
Replication	2	21.8	13.62***	8.4	4.64*
Genotype	35	21.1	13.18***	20.6	11.38***
Block	15	2.2	1.36	3.4	1.86
Error	55	1.6		1.8	

\*P<.05; \*\*P<.01; \*\*\*P<.001

conditions. VAX 5 might be better adapted to stress conditions yet lack the yield potential to remain competitive under non-stress conditions.

The 31 selected RILs, parents and local checks were evaluated for drought resistance in Montcalm, MI in 2001. Late rainfall during the season allowed for genotypes to negate the effects of the early drought stress in Montcalm. The DII for the Montcalm experiment was extremely low at 0.02. Treatment means were not significantly different and only varied by 56 kg/ha (Table 7). Mean yield under stress ranged from 1926 to 4015 kg/ha among the 36 genotypes. In the non-stress treatment, mean yield ranged from 1682 to 4340 kg/ha. Significant genotypic differences were present among stress and non-stress conditions for yield and 100 sw. Coefficients of variation for yield were moderately low, 16.7 and 19.5 %, for stress and non-stress treatments respectively and low LSD values allowed the separation of high and low yielding genotypes within both populations.

Table 8. Yield under stress ( $Y_d$ ) and non-stress ( $Y_p$ ) and 100 seed weight (100 sw) for sixteen genotypes ranked by Geometric Mean (GM) in population L88 grown in Montcalm, MI 2001 under stress and non-stress treatments †.

Genotype	GM kg/ha	Stress		Non-stress	
		$Y_d$ kg/ha	100 sw g	$Y_p$ kg/ha	100 sw g
<b>RILs</b>					
L88-69	3849 (2)‡	4015 (1)	28.3	3690 (5)	29.5
L88-63	3596 (3)	2995 (16)	25.5	4318 (2)	25.8
L88-30	3398 (9)	3432 (8)	26.9	3365 (12)	26.1
L88-61	3387 (10)	3432 (9)	27.2	3342 (13)	26.6
L88-59	3341 (11)	3241 (13)	28.9	3443 (10)	29.0
L88-74	3241 (15)	2838 (21)	27.2	3701 (4)	27.0
L88-66	3333 (12)	3488 (5)	26.3	3185 (14)	24.9
L88-19	3284 (13)	3174 (14)	28.9	3398 (11)	28.3
L88-31	2910 (19)	2894 (20)	27.3	2927 (22)	26.3
L88-37	3191 (17)	3376 (10)	31.3	3017 (19)	30.4
L88-64	2901 (20)	2759 (23)	28.6	3051 (18)	27.4
L88-2	2845 (23)	2703 (26)	29.3		
L88-13	2748 (26)	2748 (24)	28.9	2995 (20)	26.8
L88-3	2393 (30)	2322 (31)	30.4	2748 (24)	27.3
L88-4	2389 (31)	2355 (30)	29.6	2467 (29)	30.1
L88-18	2001 (34)	2075 (35)	27.8	2423 (30)	29.9
				1929 (33)	28.4
<b>Parents</b>					
B98311	3495 (6)	3903 (2)	28.6		
TLP 19	3527 (5)	3466 (7)	28.0	3129 (15)	27.5
				3589 (6)	26.4
<b>Checks</b>					
T 39	3533 (4)	3544 (4)	23.8		
Phantom	3494 (7)	3466 (6)	26.6	3522 (8)	24.8
				3522 (9)	26.3
Mean	2908	2950	29.3	3006	
LSD (0.05)		808	2.1	965	28.7
CV		16.7	4.3	19.5	2.2
					4.7

† Values of parents are included.

‡ Rankings (in parenthesis), mean, LSD and CV values are derived from 36 genotypes.

Table 9. Yield under stress (Yd) and non-stress (Yp) and 100 seed weight (100 sw) for fifteen genotypes ranked by Geometric Mean (GM) in population L91 grown in Montcalm, MI 2001 under stress and non-stress treatments†.

Genotype	GM kg/ha	Stress		Non-stress	
		Yd kg/ha	100 sw g	Yp kg/ha	100 sw g
<b>RILs</b>					
L91-10	4056 (1)‡	3791 (3)	31.7	4340 (1)	29.9
L91-30	3480 (8)	3051 (15)	33.8	3970 (3)	33.0
L91-49	3244 (14)	3376 (11)	32.9	3118 (16)	31.6
L91-3	3210 (16)	3353 (12)	29.9	3073 (17)	27.1
L91-25	3038 (18)	2613 (28)	32.8	3533 (7)	31.9
L91-22	2875 (22)	2781 (22)	29.1	2972 (21)	28.8
L91-52	2814 (24)	2905 (19)	31.1	2725 (26)	30.9
L91-41	2731 (27)	2714 (25)	29.3	2748 (25)	27.1
L91-53	2765 (25)	2938 (17)	26.5	2602 (28)	26.1
L91-68	2888 (21)	2927 (18)	27.5	2849 (23)	27.6
L91-13	2611 (28)	2501 (29)	33.2		
L91-59	2401 (29)	2636 (27)	30.6	2725 (27)	27.0
L91-37	2026 (33)	2288 (32)	30.7	2187 (32)	30.3
L91-19	1900 (35)	2131 (33)	35.6	1794 (34)	30.4
L91-69	1801 (36)	1929 (36)	34.4	1694 (35)	36.3
				1682 (36)	33.8
<b>Parents</b>					
B98311	3495 (6)	3903 (2)	28.6		
VAX 5	2244 (32)	2097 (34)	27.4	3129 (15)	27.5
				2400 (31)	26.9
<b>Checks</b>					
T 39	3533 (4)	3544 (4)	23.8		
Phantom	3494 (7)	3466 (6)	26.6	3522 (8)	24.8
				3522 (9)	26.3
Mean	2908	2950	29.3	3006	
LSD (0.05)		808	2.1	965	28.7
CV		16.7	4.3	19.5	2.2
					4.7

† Values of parents are included.

‡ Rankings (in parenthesis), mean, LSD and CV values are derived from 36 genotypes.

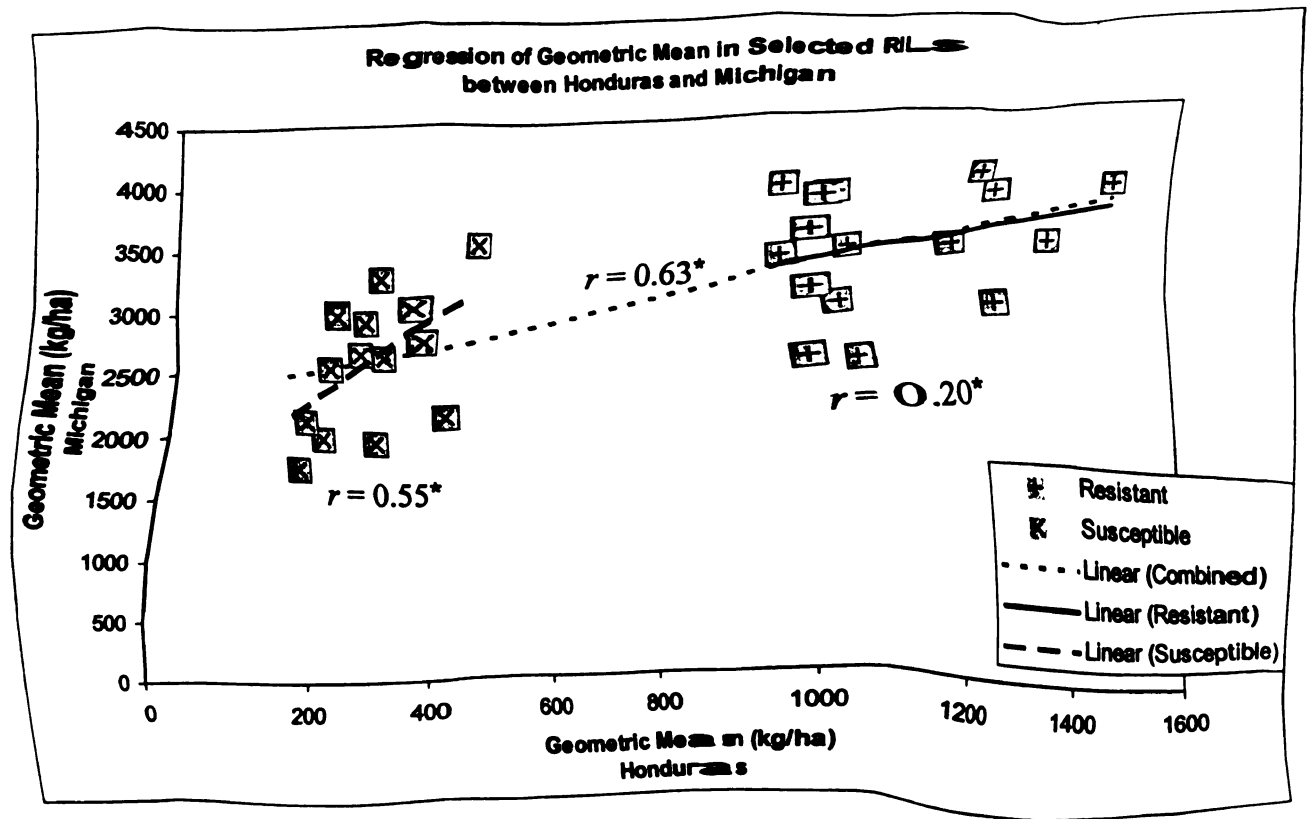


Figure 5. Regression analysis of Resistant and Susceptible RILs for Geometric Mean across the Honduran and Montcalm, MI locations.

Comparisons between populations, individual genotypes and checks were performed (Tables 8 and 9). RILs from L88 yielded 10% more than L91 RILs based on GM. Means for 100 sw in L91 were 10% larger than in L88. These results were consistent with previous results, but must be considered with the information that two-thirds of the resistant RILs were from L88 while two-thirds of the susceptible RILs came from L91.

Comparisons of the selected RILs between locations was performed by regression analysis. Yield data obtained in Montcalm was used to validate the results obtained in Honduras. Geometric mean was moderately correlated between locations,  $r = 0.63^*$  (Figure 5). This value is supported by the higher correlation of susceptible genotypes in

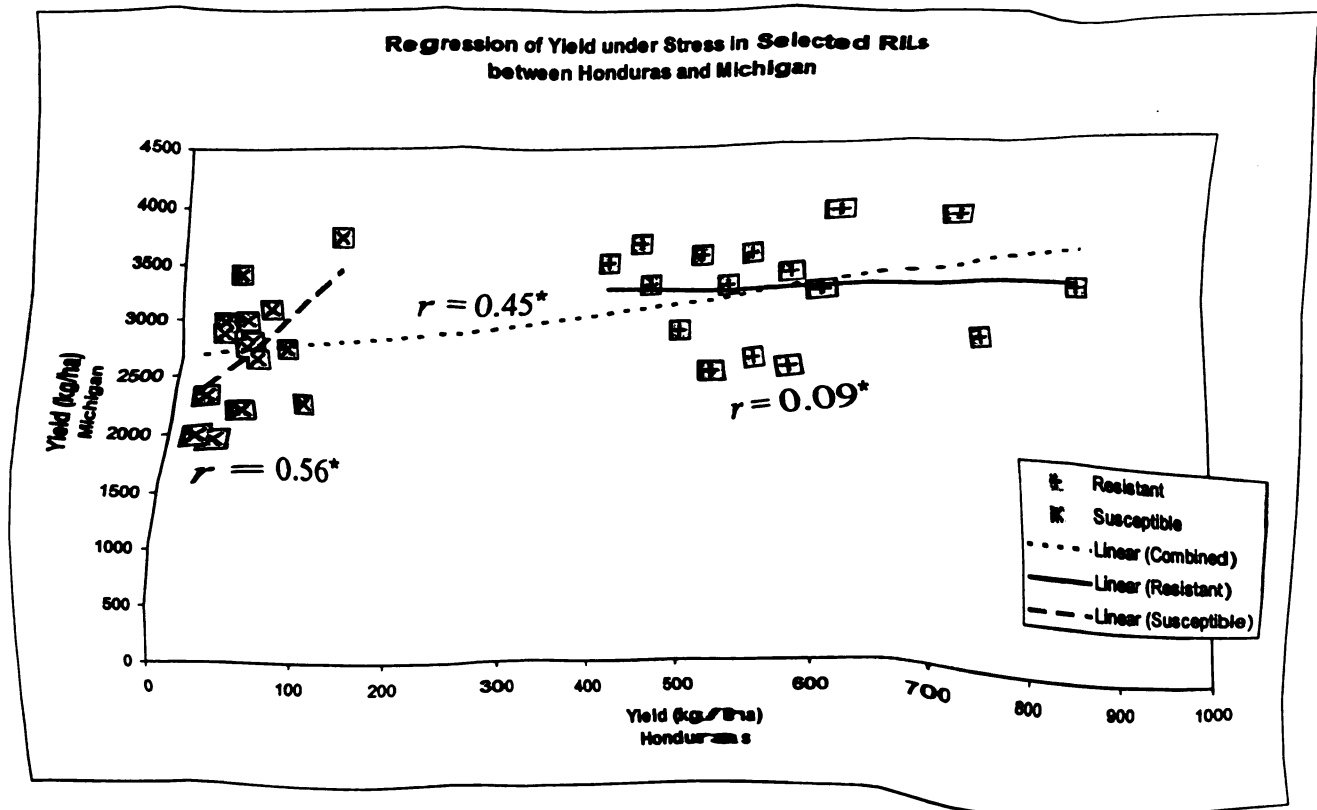


Figure 6. Regression of Selected RILs for yield under stress among the Honduran and Montcalm, MI locations.

GM ( $r = 0.55^*$ ) and Yd ( $r = 0.56^*$ ) regression analyses (Figure 6). Resistant genotypes were weakly correlated in GM ( $r = 0.20^*$ ) and Yd ( $r = 0.09^*$ ) regression analyses. All genotypes were moderately correlated in the non-stress treatments ( $r = 0.52^*$ ) (Figure 7). Resistant and susceptible genotypes were weakly correlated in the non-stress treatments ( $r = 0.16^*$ ;  $r = 0.07^*$ ) (Figure 7).

Agronomic traits may have contributed to yield in a positive or negative manner (Tables 10 and 11). Biomass had a significant impact on yield in stress and non-stress treatments. One hundred seed weight had a larger impact on yield and biomass in the stress treatment than in the non-stress treatment. In the stress treatment, plant stand was positively associated with yield and biomass, but was negatively associated with incidence



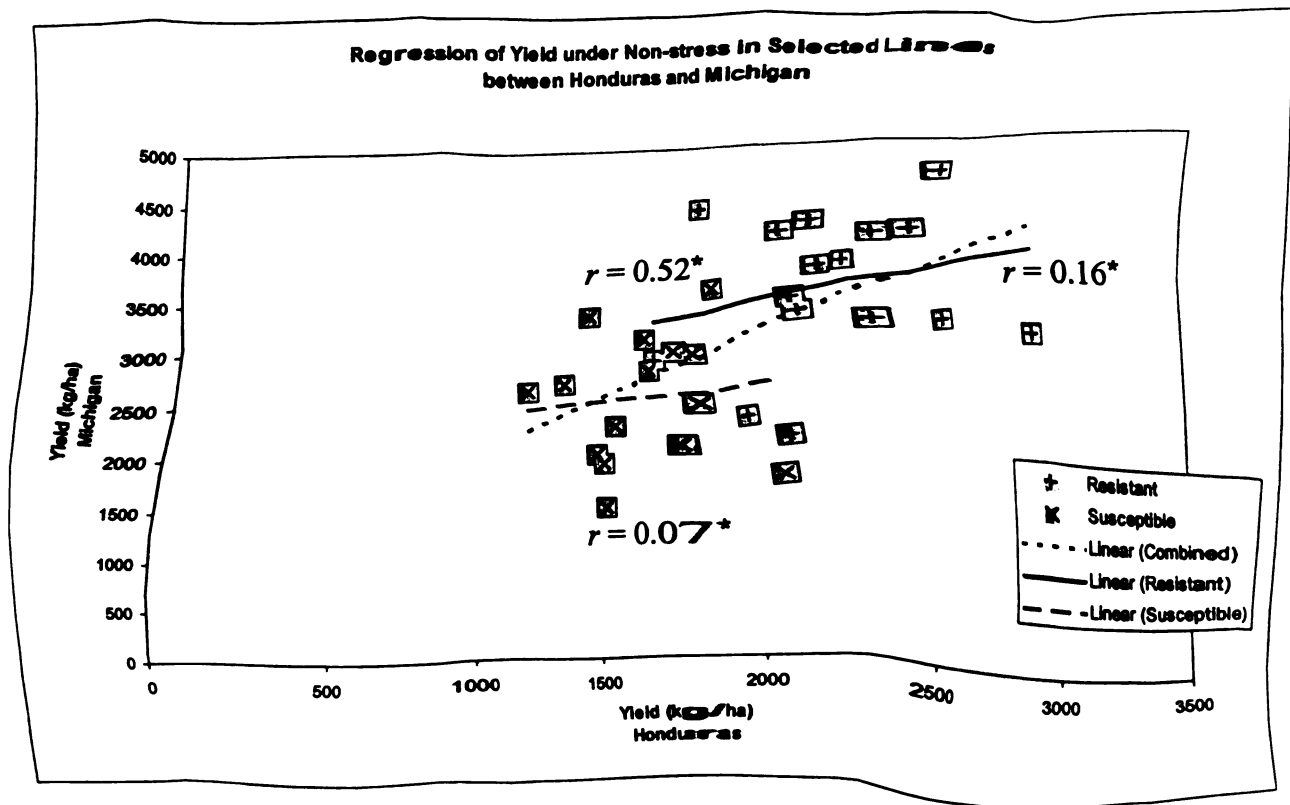


Figure 7. Regression of selected RILs for yield under non-stress conditions within the Honduran and Montcalm, MI locations.

of *Macrophomina phaseolina* at 45 and 75 dap. Disease incidence was negatively associated with yield, biomass, 100 sw and stand in both populations within the stress treatment. The negative association of DI to stand also affected yield and biomass.

Yield and biomass in L88 were closely associated and were associated with plant stand than in L91 (Table 10). Yet, 100 sw has a stronger association to yield and biomass in L91 rather than L88. Seed size in L91 is greater than L88, but did not show significant differences (Tables 4 and 5). Plant stand was affected more by DI in L88 ( $r = -0.41^{****}$ ) than in L91 ( $r = -0.33^{****}$ ). At 75 dap, Yd was more negatively affected by DI in L88 ( $r = -0.36^{****}$ ) than in L91 ( $r = -0.30^{****}$ ).

Table 10. Correlation between yield, biomass, 100 seed weight (100 sw), plant stand at harvest and disease incidence (DI) at 45 and 75 days after planting in the moisture stress and non-stress treatments for L88 and L91 RILs in Honduras, 2001.

Pop L88					
Stress	Yield	Biomass	100 sw	Stand	DI 45
Yield	-	-	-	-	-
Biomass	0.61****	-	-	-	-
100 sw	0.31****	0.35****	-	-	-
Stand	0.33****	0.46****	0.12	-	-
DI 45	-0.23**	-0.28***	-0.01	-0.27***	-
DI 75	-0.36****	-0.48****	-0.16*	-0.41****	0.58****
Non-stress					
Yield	Biomass	100 sw	Stand	DI 45	DI 75
Yield	-	-	-	-	-
Biomass	0.91****	-	-	-	-
100 sw	0.14*	0.20**	-	-	-
Stand	0.05	0.03	-0.09	-	-
DI 45	-0.16*	-0.18*	0.07	-0.23**	-
DI 75	-0.18**	-0.24****	-0.02	-0.12	0.81****

Pop L91					
Stress	Yield	Biomass	100 sw	Stand	DI 45
Yield	-	-	-	-	-
Biomass	0.53****	-	-	-	-
100 sw	0.37****	0.45****	-	-	-
Stand	0.21**	0.40****	0.11	-	-
DI 45	-0.25**	-0.32****	-0.07	-0.26**	-
DI 75	-0.30****	-0.48****	-0.05	-0.33****	0.53****
Non-stress					
Yield	Biomass	100 sw	Stand	DI 45	DI 75
Yield	-	-	-	-	-
Biomass	0.86****	-	-	-	-
100 sw	-0.07	0.11	-	-	-
Stand	0.05	0.10	-0.08	-	-
DI 45	-0.14	-0.15	0.05	-	-
DI 75	-0.20**	-0.24***	-0.09	-0.07	0.81****

\*P<.05; \*\*P<.01; \*\*\*P<.001; \*\*\*\*P<.0001

Yield under stress was potentially compromised in Honduras due to a severe infestation of ASB caused by *Macrophomina phaseolina*. The DI of ASB was characterized by 99 % of the stress plots having at least one dead plant compared to 50% in the non-stress treatment. DI values ranged from 0.05 to 0.54 across the 160 genotypes grown under stress (Figure 8). In population L88 and L91, a negative correlation was observed between plant stand and DI at 75 dap ( $r = -0.41****$  and  $r = -0.33****$ ). In the non-stress treatment, no correlations existed between plant stand and DI at 75 dap.

Table 11. Correlations between yield-based traits including 100 seed weight (100 sw) and harvest index (HI) and agronomic traits including desirability score (DS) in L88 (below diagonal) and L91 (above diagonal) in Honduras 2001.

Stress	Yield	Biomass	100 sw	HI	Flowering	Height
Yield	-	0.53****	0.37****	0.78****	-0.32***	0.53****
Biomass	0.61****	-	0.45****	ns	ns	0.53****
100 sw	0.31****	0.35****	-	ns	ns	0.37****
HI	0.77****	ns	0.14*	-	-0.39****	0.29****
Flowering	-0.16*	ns	ns	-0.29***	-	ns
Height	0.49****	0.62****	0.27****	0.18**	ns	-
Lodging	0.25***	0.26****	0.31****	0.17*	ns	0.20**
Maturity	-0.14*	ns	ns	-0.25**	0.29***	ns
DS	0.78****	0.46****	0.21**	0.72****	-0.22*	0.42****

Non-stress	Yield	Biomass	100 sw	HI	Flowering	Height
Yield	-	0.86****	ns	0.64****	-0.19*	0.29****
Biomass	0.91****	-	ns	0.19**	ns	0.39****
100 sw	0.14*	0.20**	-	-0.32****	0.31****	0.28****
HI	0.42****	ns	ns	-	-0.38****	ns
Flowering	ns	ns	0.29****	-0.22**	-	0.33****
Height	0.33****	0.46****	0.22***	-0.19**	0.32****	-
Lodging	0.30****	0.37****	ns	ns	ns	0.19**
Maturity	ns	0.18**	0.65****	-0.18**	0.49****	0.29****
DS	ns	ns	ns	0.29****	ns	-0.26****

\*P<.05; \*\*P<.01; \*\*\*P<.001; \*\*\*\*P<.0001

Stress	Lodging	Maturity	DS
Yield	0.36****	-0.25***	0.69****
Biomass	0.38****	ns	0.51****
100 sw	0.26**	ns	ns
HI	0.18*	-0.22**	0.56****
Flowering	ns	0.42****	-0.27**
Height	0.38****	ns	0.48****
Lodging	-	ns	0.29****
Maturity	-0.14*	-	-0.15*
DS	0.16*	-0.14*	-

Non-stress	Lodging	Maturity	DS
Yield	0.50****	-0.14*	0.20**
Biomass	0.47****	ns	ns
100 sw	-0.18*	0.61****	-0.26***
HI	0.29****	-0.30****	0.25****
Flowering	ns	0.47****	-0.32****
Height	ns	0.28****	-0.16*
Lodging	-	-0.30****	ns
Maturity	-0.17**	-	-0.37****
DS	-0.49****	ns	-

\*P<.05; \*\*P<.01; \*\*\*P<.001; \*\*\*\*P<.0001

Disease incidence data revealed a greater resistance to *ASB* in L91 than in L88, derived from the ASB resistant parent, TLP 19. Population L88 had a 6 % higher DI than L91 yet averaged 113 kg/ha more in yield (Table 12). Population and parental means were not significantly different ( $p < 0.05$ ). In the moisture stress and non-stress treatments, B98311 yielded more, yet had a two-fold higher DI in comparison to the two other parents. TLP 19 and VAX 5 had moderately low DI values at 0.15 and 0.20. Two RILs, L91-45 and L88-76, had the lowest DI in each population, whereas two other RILs, L91-30 and L88-69 that were selected as drought resistant based on GM had moderately low DI values.

Correlations between agronomic data and yield can assist breeders in designing plant phenotypes that thrive under moisture stress. Harvest index (HI) was highly significant and strongly associated with yield in both populations in each treatment. The moderate associations of height and lodging to yield and biomass suggest that tall plant that lodge positively influence yield and biomass. Desirability score was highly associated with yield and moderately associated with biomass in the stress treatment, but not in the non-stress treatment. In the non-stress treatment, 100 sw was highly associated with days to maturity. Days to maturity was negatively associated to yield in both populations and treatments except for L88 in the non-stress treatment. Height was positively associated to DS in the stress treatment and negatively associated to DS in the non-stress treatment. Individual agronomic traits did not associate strongly enough with yield under stress to support indirect selection, so direct selection based on yield is required in breeding for drought resistance in common bean.

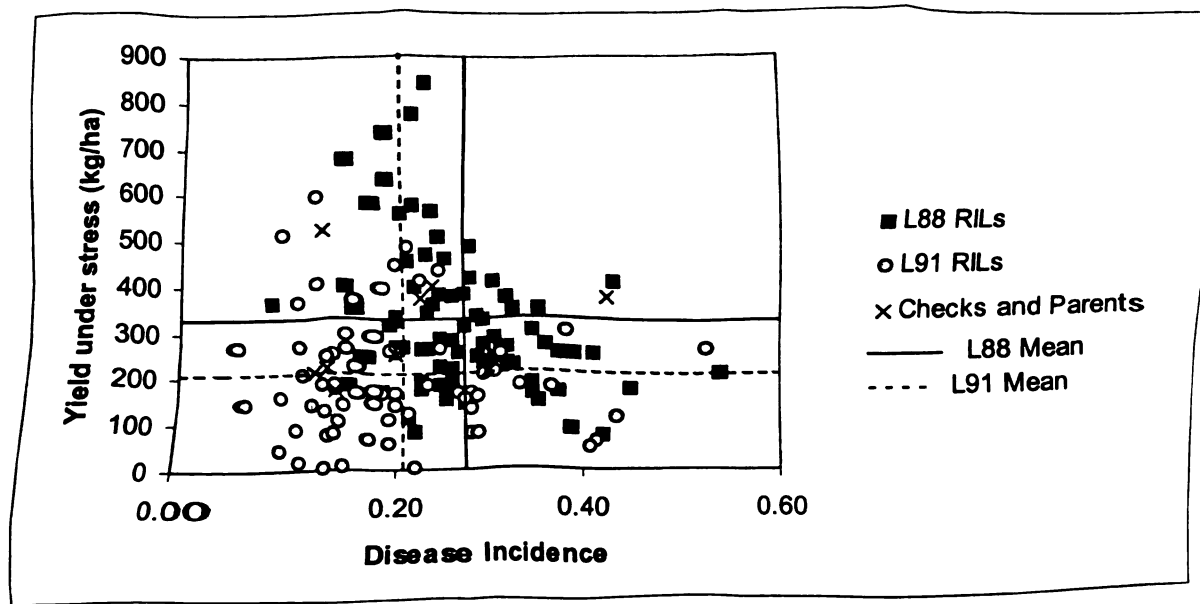


Figure 8. Field incidence of *Macrophomina phaseolina* compared to yield under stress in 160 genotypes grown in Honduras in 2001.

Table 12. Selected genotypes and means compared for their disease incidence (DI), plant stand at harvest, yield under stress (Yd), yield under non-stress (Yp), and geometric mean (GM) of moisture treatments grown in Honduras, 2001.

Genotype	DI	Stand %	Yield (kg/ha)		
			Yd	Yp	GM
L91-45	0.05 (1)†	97	266	2468	810
L88-76	0.09 (4)	90	363	1862	822
V8025	0.13 (13)	93	210	1783	612
L91-30	0.13 (15)	90	599	1922	1073
SEA 5	0.14 (20)	77	524	1521	893
Tío Canela 75	0.14 (22)	90	218	1657	602
TLP 19	0.15 (24)	100	169	2399	637
L88-69	0.16 (31)	90	680	2432	1286
VAX 5	0.20 (61)	90	249	1765	663
Rio Tibagi	0.23 (75)	90	372	2108	886
BAT 477	0.24 (88)	90	400	1536	784
EAP 9510-77	0.29 (120)	90	232	2112	699
Tacana	0.30 (125)	93	213	2097	667
B98311	0.42 (154)	90	375	2411	951
Mean, L88	0.27		320	2057	791
Mean, L91	0.21		207	1858	591

† Ranking based on DI for 160 genotypes.

## **Root Study**

Root traits were expected to differ due to associated differences in growth habit between the parents. TLP 19 has a type III growth habit while B98311 and VAX 5 exhibit a type II habit. At nine days after transplanting (dat), the root system of TLP 19 was significantly smaller than either B98311 or VAX 5. Due to root length differences between B98311 and TLP 19 and the lack of differences between B98311 and VAX 5, only the root characteristics of population L88 were studied.

The root characteristics measured in were total root length, length according to diameter class and fractal dimension. Significant genotypic differences were found for total root length and fractal dimension in L88 (Table 13). Within the ten root length diameter classes, the two classes for each extreme, A and B; I and J, showed significant genotypic differences while classes C through G did not. The ten different root diameter classes previously reported in common bean (Yabba, 2001) were grouped into fine (A-C), intermediate (D-G), and taproots (H-J). Fine roots described length for roots with 0-1.50 mm in diameter. Intermediate roots were classified as having diameters 1.51-3.50 mm. Taproot length is characterized as having a diameter greater than 3.51 mm. The extreme classes had low CV values whereas the intermediate classes had CV values that exceeded 100 %.

The correlations of root characteristics to yield data showed unexpected results. Beans having a high Yd were expected to have a deep taproot. The fine roots, which accounted for 99 % of the total root length, correlated to Yd and GM in Honduras whereas taproot length correlated to Yp (Table 14). The negative associations of the fine roots in class B suggest that as root length with a diameter of 0.50 mm to 1.00 mm decrease, yield

Table 13. Analysis of Variance for the 81 RILs in population L88 for Total root length, Fractal Dimension, and root length according to 10 different diameter widths (A-J).

Source	DF	Total Root Length		Fractal Dimension	
		MS	F Test	MS	F Test
genotype	77	295173	1.62**	0.0029	1.44*
block	3	3135560	17.20****	0.0746	36.91****
error	162	182248		0.002	

Source	DF	A†		B		C	
		MS	F Test	MS	F Test	MS	F Test
genotype	77	149161	1.79**	21685	1.43*	701	1.18
block	3	1980019	23.73****	270907	17.88****	28355	47.87****
error	162	83422		15153		592	

Source	DF	D		E		F		G	
		MS	F Test	MS	F Test	MS	F Test	MS	F Test
genotype	77	68	1.11	14	1.00	2	1.08	0.36	1.21
block	3	4977	81.29****	851	58.52****	135	66.45****	17.00	57.29****
error	162	61		15		2		0.30	

Source	DF	H		I		J	
		MS	F Test	MS	F Test	MS	F Test
genotype	77	0.12	0.99	0.05	1.48*	1.59	1.50*
block	3	3.75	30.50****	0.44	11.66****	5.98	5.64**
error	162	0.12		0.04		1.06	

\*P<.05; \*\*P<.01; \*\*\*P<.001; \*\*\*\*P<.0001

† Root diameter classes A, B, C, D, E, F, G, H, I, J are 0-0.5, 0.5-1.0, 1.0-1.5, 1.5-2.0, 2.0-2.5, 2.5-3.0, 3.0-3.5, 3.5-4.0, 4.0-4.5, and greater than 4.5 mm, respectively.

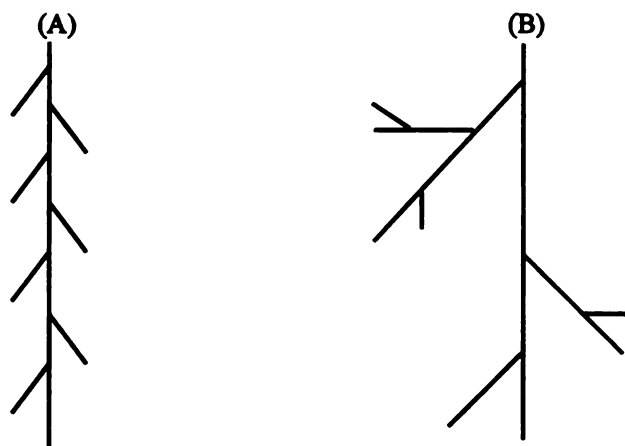


Figure 9. Representations of herringbone (A) and dichotomous (B) topologies.

Table 14. Correlation values between root characteristics and yields in the Saginaw 2000 and Honduras (Hon) 2001 experiments for the 81 RILs of population L88.

	<u>Total Root Length</u>	<u>Fractal Dimension</u>		
Hon Yd	ns	-0.13*		
Hon Yp	ns	ns		
Hon GM	ns	ns		
Saginaw	ns	ns		
<u>Fine roots</u>	<u>A†</u>	<u>B</u>	<u>C</u>	
Hon Yd	ns	-0.12†	ns	
Hon Yp	ns	ns	ns	
Hon GM	ns	-0.19†	ns	
Saginaw	ns	0.11†	ns	
<u>Intermediate Roots</u>	<u>D</u>	<u>E</u>	<u>F</u>	<u>G</u>
Hon Yd	ns	ns	ns	ns
Hon Yp	ns	ns	ns	ns
Hon GM	ns	ns	ns	ns
Saginaw	ns	ns	ns	ns
<u>Tap roots</u>	<u>H</u>	<u>I</u>	<u>J</u>	
Hon Yd	ns	ns	ns	
Hon Yp	ns	0.19**	ns	
Hon GM	ns	ns	ns	
Saginaw	ns	ns	ns	

† P<.10, \*P<.05; \*\*P<.01; ns - non-significant

‡ Root diameter classes A, B, C, D, E, F, G, H, I, J are 0-0.5, 0.5-1.0, 1.0-1.5, 1.5-2.0, 2.0-2.5, 2.5-3.0, 3.0-3.5, 3.5-4.0, 4.0-4.5, and greater than 4.5 mm, respectively.

will increase. The I class, which is representative of taproots, was positively associated to Yp in Honduras ( $r = 0.19^{**}$ ). Fractal dimension which measures root architecture was significantly correlated to Yd in Honduras ( $r = -0.13^*$ ). Significant differences were observed for the top and bottom five genotypes in total root length, fractal dimension and each root diameter class. The mean values of drought resistant and susceptible genotypes corresponded to the correlation values for each measurement. Drought resistant genotypes had less root length than drought susceptible genotypes in every root trait category except the I class (4.0-4.5 mm) (Table 15). Fractal dimension was also a lower value in drought resistant genotypes. The opposite effect was observed in the parents, in that, B98311 was greater than TLP 19 in every root length measurement and fractal dimension. Only in the



Table 15. Mean values of total root length, fractal dimension, fine roots (A-C) and taproots (H-J) of drought resistant and drought susceptible RILs and parents of population L88 obtained by the root pouch method.

genotype	Total mm	Fractal	A†	B	C	H	I	J
			----- mm -----			----- mm -----		
<b>Drought Resistant</b>								
L88-30	2106	1.52	1506	529	55	0.02	0.26	3.54
L88-63	2054	1.47	1645	366	32	0.28	0.10	2.95
L88-74	2019	1.47	1560	409	36	0.12	0.00	3.45
L88-13	1928	1.47	1464	413	38	0.15	0.13	3.51
L88-69	1532	1.46	1163	330	29	0.31	0.08	2.16
Mean	1928	1.48	1468	410	38	0.18	0.11	3.12
<b>Drought Susceptible</b>								
L88-64	2368	1.56	1648	614	79	0.25	0.04	4.17
L88-37	2119	1.53	1566	483	57	0.21	0.07	3.35
L88-18	2056	1.50	1562	441	42	0.26	0.08	2.41
L88-02	2007	1.49	1564	392	37	0.20	0.09	3.29
L88-04	1948	1.50	1391	504	41	0.33	0.16	3.09
Mean	2100	1.52	1546	487	51	0.25	0.09	3.26
<b>Parents and Ranges</b>								
B98311	2295	1.53	1660	547	62	0.32	0.19	3.56
TLP 19	1618	1.48	1174	391	40	0.16	0.06	2.52
Maximum	2660	1.61	1965	732	123	1.13	0.55	4.72
Minimum	1285	1.44	908	329	24	0.04	0.01	1.77
Mean‡	1959	1.50	1415	475	51	0.26	0.16	3.07
LSD (0.05)	688	0.07	466	198	39	0.57	0.31	1.66
CV	22	2.99	20	26	47	133.54	119.64	33.58

† Root diameter classes A, B, C, H, I, J are 0-0.5, 0.5-1.0, 1.0-1.5, 3.5-4.0, 4.0-4.5, and greater than 4.5 mm, respectively.

‡ 83 genotypes are included in the calculations of mean, LSD and CV values.

I class (4.0-4.5 mm) did B98311 and TLP 19 correspond to the correlation values observed among the RILs. A lower fractal dimension correlated to yield under drought stress, yet the drought resistant parent, B98311, had a greater fractal dimension and Yd than the susceptible parent, TLP 19. Significant differences between the parents were observed only in the A class (0-0.5 mm) category. Although the resistant and susceptible genotypes were not significantly different in the B and I root classes and fractal dimension, the correlation values indicate that these root characteristics are important in the yield performance of beans grown under drought stress in the lowland tropics.

## Marker Study

Molecular markers previously associated with drought resistance in pinto bean (Schneider et al., 1997a) were tested across both black bean populations. Only 40 of the 70 reported RAPD markers (Table A8) were tested for polymorphisms between T-3016 and Raven, the parents of B98311. The 40 RAPD markers selected for testing represented those that were significantly associated with GM in a previous study (Schneider et al., 1997a). Nine of the 40 RAPD markers tested were polymorphic between T-3016 and Raven suggesting that B98311 had the same marker phenotype as T-3016. Three of these markers were present on linkage group (LG) 4, while the other six were unlinked or on a LG with low association to performance under stress (Table A8). Of the nine reported LGs, only two (4 and 9) significantly associated with GM in a combined analysis across five locations (Schneider et al., 1997a). Markers on LG 9 were monomorphic between the parents, whereas markers on LG 4 were polymorphic (Table 16). RAPD primers F06<sub>970</sub>, I03<sub>1130</sub> and A16<sub>850</sub> from LG 4 were screened across both populations. The lack of clear amplification of A16<sub>850</sub> made the marker unscorable. F06<sub>970</sub> and I03<sub>1130</sub> were 100 % linked in population L88 while 3 recombinants existed in population L91. In L88, both primers were significantly associated with yield potential ( $R^2 = 0.05^*$ ) (Table 17). In multiple regression analysis, I03<sub>1130</sub> accounted for 5 % of the variation ( $R^2=0.05$ ;  $p<0.10$ ) in population L91.

Table 16. Presence/absence of RAPD markers in six bean genotypes

LG†	RAPD‡	T-3016	Sierra	B98311	Raven	VAX 5	TLP 19
9	AB18 <sub>850</sub>	+	+	+	+	+	+
	H19 <sub>890</sub>	-	+	-	-	-	-
4	V01 <sub>830</sub>	-	+	+	-	-	-
	F06 <sub>970</sub>	+	-	+	-	-	-
	A16 <sub>850</sub>	+	-	+	-	-	-
	I03 <sub>1130</sub>	+	-	+	-	-	-

† LG - Linkage Group

‡RAPD markers were previously associated to drought resistance in one pinto bean population (Schneider et al., 1997a).

Table 17. Coefficients of determination ( $R^2$ ) accounting for the variation in yield under drought (Yd), yield under non-stress (Yp) and geometric mean (GM) for two RAPD markers.

	L88		L91	
	F06 <sub>970</sub>	I03 <sub>1130</sub>	F06 <sub>970</sub>	I03 <sub>1130</sub>
Yd	0	0	0.03	0.03
Yp	0.05*	0.05*	0.05	0.05†
GM	0	0	0.03	0.03

† P<.10, \*P<.05

### Multiple Regression Analysis

For population L88, fractal dimension, root classes B and I and primer F06<sub>970</sub> were combined in a multiple regression analysis to identify which combination would explain the greatest variation for performance under drought. Root class I (4.0-4.5 mm) explained 12.7 % of the variation alone for Yp in Honduras (Table 19). A larger percentage, 16 %, of the variation was explained when additional measurements of fractal dimension, root class B (0.5-10. mm) and primer F06<sub>970</sub> were included. Statistically, the best model has the highest adjusted  $R^2$  value or the lowest C(p) value. Including primer F06<sub>970</sub> with the I class increased the  $R^2$  value by 2 %. This same effect occurred to a greater extent in the multiple regression analysis for Yd in Honduras. Alone, fractal dimension and F06<sub>970</sub> explained 3.5 and 3.1 % of the variation respectively, but together they explained 7.6 % of the variation (Table 18). This two-variable model also had the highest adjusted  $R^2$  value

and the lowest  $C(p)$  value. Using the four variables together in one model explained 8.8 % of the variation in  $Y_d$ . The combination of root characteristics and marker values explained a larger amount of the variation than any single variable.

Table 18. Coefficient of determination ( $R^2$ ) selection method for yield under stress in Honduras evaluating fractal dimension, root classes B (0.5-1.0 mm) and I (4.0-4.5 mm), and RAPD marker F06<sub>970</sub> (F06).

Number in Model	R-Square	Adjusted R-Square	C(p)	MSE	Variables in Model
1	0.035	0.0228	3.4114	3099.921	fractal
1	0.0314	0.0191	3.71	3111.433	F06
1	0.0297	0.0174	3.8552	3117.032	B
1	0.0001	-0.0125	6.3162	3211.906	I
2	0.0762	0.0525	1.9752	3005.5	fractal F06
2	0.0727	0.0489	2.2693	3016.98	B F06
2	0.0427	0.0182	4.7647	3114.414	fractal I
2	0.0386	0.0139	5.1141	3128.058	B I
2	0.035	0.0103	5.4067	3139.48	fractal B
2	0.0316	0.0068	5.6926	3150.645	I F06
3	0.0859	0.0503	3.1685	3012.624	fractal I F06
3	0.0851	0.0494	3.2365	3015.314	B I F06
3	0.0765	0.0405	3.9558	3043.764	fractal B F06
3	0.0431	0.0058	6.7394	3153.861	fractal B I
4	0.0879	0.0399	5	3045.512	fractal B I F06

Table 19. Coefficient of determination ( $R^2$ ) selection method for yield under non-stress in Honduras evaluating fractal dimension, root classes B (0.5-1.0 mm) and I (4.0-4.5 mm), and RAPD marker F06<sub>970</sub> (F06).

Number in Model	R-Square	Adjusted R-Square	C(p)	MSE	Variables in Model
1	0.1273	0.1163	2.0153	9974.918	I
1	0.0166	0.0042	12.0397	11240	F06
1	0.013	0.0005	12.3658	11282	fractal
1	0.012	-0.0005	12.4587	11293	B
2	0.1424	0.1204	2.6537	9928.709	I F06
2	0.1362	0.114	3.2161	10001	B I
2	0.1313	0.109	3.6562	10057	fractal I
2	0.0263	0.0013	13.1658	11273	fractal F06
2	0.0247	-0.0003	13.3061	11291	B F06
2	0.0131	-0.0122	14.3601	11425	fractal B
3	0.1562	0.1233	3.4011	9895.425	B I F06
3	0.1489	0.1157	4.0659	9981.525	fractal I F06
3	0.1394	0.1059	4.9188	10092	fractal B I
3	0.0263	-0.0116	15.1636	11419	fractal B F06
4	0.1606	0.1165	5	9972.991	fractal B I F06



## DISCUSSION

### Yield

The main objective of this study was to evaluate the performance of two RIL populations under terminal drought stress and non-stress conditions in the lowland tropics of Central America. The lowland tropics of Honduras provide appropriate conditions to evaluate terminal drought. By identifying genotypes resistant to terminal drought, breeding programs located in Honduras and other Central American countries will be able to use these genotypes to improve local varieties for performance under drought. RILs selected as drought resistant out-performed all local checks, drought resistant checks and the drought resistant parent based on GM (Tables 4, 5 and 6). Likewise, drought susceptible RILs performed well below all checks and parents. The stability of the drought resistant RILs was evaluated across locations with different intensities of drought stress. An experiment that included the resistant and susceptible genotypes selected in Honduras was conducted in Montcalm county, Michigan under moisture stress and non-stress conditions. Despite the absence of a significant drought stress in Montcalm in 2001, GM values across treatments were moderately correlated between experiments in Honduras and Montcalm (Figure 5). Drought resistant genotypes selected in Honduras were among the highest yielding genotypes while drought susceptible genotypes were among the lowest yielding in Montcalm (Tables 8 and 9).

The Honduran field experiment in the lowland tropics experienced a severe terminal drought stress,  $DII = 0.82$ . This stress was more severe than previous experiments conducted with beans under rain-fed conditions in the Mexican highlands

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(DII = 0.49; Schneider et al., 1997b) and under rain shelter controlled, drought treatments in Michigan (DII = 0.63; Ramirez-Vallejo and Kelly, 1998). Lowland tropical areas can experience decreasing soil moisture and increasing temperatures, both of which contributed to the substantial reduction in Yd in Honduras.

Resistant and susceptible RILs were identified among the populations based on yield in relation to the parents. B98311 was selected as the drought resistant parent because it was the highest yielding genotype in drought experiments conducted in Michigan in 1998 (Kolkman and Kelly, 1999). TLP 19 and VAX 5 were selected for adaptation to lowland tropics without prior knowledge of their response to drought stress. TLP 19 and VAX 5 yielded 50 and 30 % less than B98311 under stress. The corresponding population means of each parent did not follow the same relationship. Even though TLP 19 had a lower yield mean under stress than VAX 5, the corresponding population L88 mean was greater than the population mean of L91 where VAX 5 was the parent (Tables 4 and 5). Population L91 had a lower mean yield than its drought susceptible parent, VAX 5. Many of the RILs in L91 in the drought stress treatment had high biomass but low seed yield. This relationship was displayed by RIL L91-69, which under stress, ranked last in yield, but first in biomass (Table 15). Under stress, this RIL remained longer in a vegetative stage and began to flower late into the period of stress producing a low yield as a result of low HI (Table 6). Many RILs flowered late in population L91 in Honduras and exhibited low HI. The biomass means between populations differed by 15 kg/ha whereas the yield means differed by 106 kg/ha. Harvest index under stress for populations L88 and L91 was low, 0.19 and 0.13, respectively, yet

the RILs in population L88 were more efficient in partitioning nutrients to the seed than those in population L91.

Biomass has been suggested as an indirect selection criterion for Yd since it is highly correlated with biomass (Acosta-Gallegos, 1988). This correlation is logical in that high yielding genotypes need to fix greater biomass to partition to the seed. Yet, selection for biomass can indirectly increase days to maturity. In populations L88 and L91, biomass was moderately correlated with Yd ( $r = 0.61^{****}$  and  $r = 0.53^{****}$ ) and highly correlated with Yp ( $r = 0.91^{****}$  and  $r = 0.86^{****}$ ), respectively (Table 10). These values are greater than the correlation between Yd and Yp in each population. Therefore, under severe stress, biomass would be a better indirect measurement of Yd than Yp. For temperate climates less affected by drought like Michigan, selection for biomass would be counter-productive since days to maturity would increase. In Michigan, HI would be a better selection criteria for Yd. HI and biomass should always be used together when selecting for performance under stress, so that plants with high biomass and low reproductive efficiency are not selected.

Growth habit in common bean has also been associated with Yd. Indeterminate genotypes are more suitable than determinate genotypes for production in semi-arid areas (Samper, 1984; Acosta-Gallegos and Adams, 1991). Indeterminate genotypes exhibit early vigorous establishment and accumulate a greater biomass with the ability to transfer assimilates to the seed (Samper, 1984; Acosta-Gallegos and Adams, 1991). All three parents in this study were indeterminate, yet they exhibited different types of indeterminacy. B98311 and VAX 5 have a type II, short vine, erect growth habit while TLP 19 has a type III, prostrate vine. Comparisons between type II and type III

indeterminacy for drought resistance were not made in this study. Population L88 segregated for growth habit whereas population L91 exhibited only type II growth habit. The range of growth habits present in L88 might have given individual genotypes an opportunistic edge over the type II individuals in population L91. Therefore, a strict adherence to type II growth habit may not be beneficial in selecting for drought resistance in Central America.

The field experiment in Montcalm, Michigan was conducted to validate the Honduran results. The resistant and susceptible RILs selected in Honduras experienced minimum drought stress in Michigan and consequently the effect of stress could not be evaluated at a second location. Despite the lack of drought in Michigan, relationships of yield between Honduras and Montcalm were compared using regression analysis. The strongest correlation for Yd was shown within the susceptible genotypes. The negative affects of late flowering and inability to partition nutrients to the seed were also detrimental to yield in Michigan. Resistant genotypes showed a higher correlation between locations than susceptible genotypes for Yp (Figure 7). The moderate correlations of Yd ( $r = 0.45^*$ ), Yp ( $r = 0.52^*$ ), and GM ( $r = 0.63^*$ ) supported the adaptability of selected RILs to temperate conditions and the consistent performance between locations. Selection under drought conditions in Honduras was successful in identifying high and low yielding RILs that expressed similar potential in the Michigan environment.

In Honduras, a high CV was recorded due to high experimental error resulting from genetic susceptibility of RILs to diseases such as ASB in the stress treatment and the higher environmental variation. The high CV in our experiment was considerably higher

than previously published studies of bean grown under drought stress (Schneider et al., 1997b). The experimental design used in Honduras was a CRD which accounts for less of the total error than a RCBD or lattice designs that better control environmental variation. Plant stand was reduced and consequently, yield due to ASB infestation under stress conditions only. The mean yield reduction due to stress was 85 and 89 % for populations L88 and L91, respectively. In breeding for resistance to terminal drought stress, ASB resistance among genotypes must be considered.

Although variation was high within the drought stress treatment, the 150 RILs in the non-stress treatment can be directly compared between locations. The top yielding genotypes in Honduras were poor performers in non-replicated trials in Saginaw, Michigan in 2000. The lack of consistent performance between these locations can relate to adaptation of the genotypes, environmental differences and different planting populations. All plots were adjusted by plant stand using covariate analysis, so that valid comparisons could be made. One genotype, L88-69, had consistent high yield in all locations and treatments.

Bean genotypes that yield well under stress do not always yield well under non-stress. A strong relationship between  $Y_d$  and  $Y_p$  was not reported in previous experiments (Ramirez-Vallejo, 1992). Different mechanisms within the plant contribute to high  $Y_d$  and  $Y_p$ . A breeding strategy for drought resistance should combine in a genotype both high  $Y_d$  and high  $Y_p$  (Schneider et al., 1997b). This strategy would be more effective in population L88. B98311 yielded above average under drought (375 kg/ha) in Honduras while TLP 19 (169 kg/ha) yielded below the mean. TLP 19 was a poor yielding genotype under stress in Honduras, but was equivalent to B98311 in  $Y_p$  in Honduras. TLP 19 out-

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yielded B98311 in Montcalm, Michigan by 13 %. This parental combination resulted in several progeny L88-63 and L88-69 with superior yield in each treatment in Honduras and Michigan (Tables 4 and 5).

The parent of population L91, VAX 5, was derived from tepary bean which is known to be drought tolerant (Thomas et al., 1983). Genes from tepary bean present in VAX 5 could have aided in tolerance to stress. VAX 5 produced a moderate Yd, yet poor combining ability for performance with B98311 was observed that could have resulted from the tepary ancestry.

Both populations belong to common bean race, Mesoamerica in the Middle American gene pool. Germplasm from race Mesoamerica is recognized as a source of yield genes for stressed or non-stressed environments in Central America (White et al., 1994a). The two other races of common bean in the Middle American gene pool are Durango and Jalisco. These races have been exclusively screened for additional drought resistant genes (Singh, 1995; Terán and Singh, 2002). Durango race cultivars have shown a higher yield under drought stress than Jalisco race cultivars (Terán and Singh, 2002). Moderate success in breeding for drought resistance has been achieved in the Durango race (Acosta-Gallegos and Adams, 1991; Schneider et al., 1997b), which could result from its limited adaptation. Mesoamerican genotypes have a broader adaptation than Durango genotypes. Both races could endure drought periods by different mechanisms. Interracial crosses of Durango and Mesoamerican genotypes may provide a strategy to improve drought resistance by combining different adaptation and yield performance traits from both races.

Mesoamerican genotypes deriving their drought resistance from Durango varieties could have an advantage in complementation of drought resistance from both races. B98311 from race Mesoamerica was derived from a drought resistant Durango genotype, T-3016 which is a non-commercial, Durango race genotype previously identified as the most drought resistant genotype based on GM from a cross of Sierra/AC1028 (Schneider et al., 1997b). Drought resistance was transferred from the Durango genotype to the Mesoamerican genotype as the other Mesoamerican parent, Raven, demonstrated no drought resistance. The most drought resistant RILs in both populations, L88-63 and L91-30, exceeded the yield of previously recognized drought resistant genotypes BAT 477, V8025 and Rio Tibagi by 40 and 17 %, respectively. Mesoamerican drought resistant genotypes BAT477, V8025 and Rio Tibagi have shown moderate Yd and high Yp (White et al., 1994a). The stability of performance under stress for the drought resistant RILs will be determined across additional locations and years. A Mesoamerican genotype with complimentary drought resistance genes from a Durango genotype might have a greater impact on drought-prone areas than either a Mesoamerican or Durango genotype.

The goal in breeding for drought resistance in common bean was to combine mechanisms of drought tolerance and avoidance into a broadly-adapted genotype that produced high yields under stress and non-stress conditions. The resistant mechanisms of avoidance and tolerance are so integrated that separation is not always possible. Improving drought resistance will require combining plant traits known to be beneficial in performance under stress. In traditional breeding, selection based on GM across treatments accounts for all traits contributing to yield without distinguishing between drought resistance mechanisms. If avoidance traits could be combined with tolerance

traits, drought resistance could be improved in common bean. Traits such as growth habit, root architecture, osmotic adjustment, or indirect selection using linked molecular markers could be useful in population development for drought resistance in common bean.

### **Root Study**

The hypothesis that deep-penetrating roots contribute to drought resistance in common bean was tested. Separate field experiments have supported this hypothesis (Sponchiado et al., 1989; White and Castillo, 1989; White and Castillo, 1992). Since only a small numbers of genotypes have been compared and BAT 477 was mainly used in associating drought resistance to rooting depth, new studies were deemed necessary.

The pouch method was used to ascertain whether seedling root growth correlated to field performance. Genetic differences in root systems can be measured in early stages of development of common bean (less than 20 dap) . The pouch method was designed to study root vascular systems (McMichael et al., 1985) and was modified to study drought resistance in bean (Yabba, 2001). It was also used to study plant response to phosphorus availability (Liao et al., 2001). Genotypes responding in a phosphorus-efficient manner allocated roots to shallow soil horizons during phosphorus stress. Inefficient genotypes would continue to grow deeper. These differences in root length for phosphorus accumulation substantiate an investigation of root length correlations to drought stress.

One disadvantage of the pouch study is that it yields a two-dimensional root. Although not representative of natural conditions where roots can grow in three-dimensional directions, it makes digital scanning easier. Digital root images from the pouches were used to measure root length and root architecture. Computer analysis using



WinRhizo™ increases root measurement efficiency so that more tedious measurements can be performed in a shorter time and with less error.

Root length and root architecture were measured in population L88 in which the parents contrasted in root length (Table 15). Root architecture among RILs did correlate with yield performance under stress, whereas total root length did not (Table 14). Using fractal dimension to describe root architecture, drought resistant genotypes exhibited a root structure equipped for deep soil-water extraction while drought susceptible genotypes did not possess the same root architecture. This relationship was not observed among the parents. The drought resistant parent, B98311, exhibited a highly branched root system unlike the deep taproot structure considered to be important in terminal drought conditions (Sponchiado et al., 1989). Additional drought resistant genes unrelated to root structure must be present in B98311 to account for its yield performance under stress.

### **Root Length**

The measurement of root length for the whole root system and for specific diameter classes provides useful information into root components. Ten different root classes based on root diameter and used in previous root studies (Yabba, 2001) were measured to provide a better understanding of root architecture in different bean genotypes. Root components were primarily classified into four classes: adventitious, basal, tap and lateral roots (Stoffella et al., 1979). The acquisition of root data through WinRhizo™ in the present study did not measure root length based on these root components, but on different components that have the same diameter. Even though

correlations can not be made to individual root components, the diameter of root segments will govern its importance and use in water accumulation.

Results were ascertained based on the assumption that root diameters will continue to expand such that the large diameters will always be larger than the small diameters. Therefore, root classes of small diameters (0-1.5 mm) represent fine roots and large diameters (>3.5mm) represent taproots. Taproots positively correlated to  $Y_p$  and fine roots negatively correlated to  $Y_d$  (Table 14). The correlation with taproots were opposite than expected as taproots were expected to associate with  $Y_d$ . This data showed that short fine root length supports yield performance under stress conditions and long taproots contribute to yield potential. Roots that are longer at a greater diameter have more potential to transport increased volumes of water from the soil. Potentially these roots would continue to increase in growth so that the root genotype is representative of a deep and large-width taproot. Large taproots are able to keep the shoot well supplied with water. The I class (4.0-4.5 mm), representing the taproots, was significantly correlated with  $Y_p$ ,  $r = 0.19^{**}$  (Table 14). Since the parents showed the same relationship as the drought resistant and susceptible RILs in the I class, this taproot measurement is suggested as a selection criterion for  $Y_p$ .

Fine roots were negatively correlated to yield performance under stress. Greater fine root length would be detrimental to performance under stress. Although fine roots, measured at the 0.5-1.0 mm diameter, negatively correlated to  $Y_d$  in Honduras, they positively correlated to yield in Saginaw, Michigan. Since the plots in Saginaw were space-planted, individual plants grew without competition nor water stress. More fine

roots allow a more extensive exploration of the soil which would logically account for more yield at this location.

This conclusion was supported by a previous study in which root characteristics were studied in drought resistant and susceptible bean genotypes (Yabba, 2001). Eight genotypes were compared in the pouch method under non-stress and water stressed treatments induced by abscisic acid. Drought resistant genotypes had less fine root length than drought susceptible genotypes suggesting that fine roots did not impart resistance to drought (Yabba, 2001). Total root length of these eight genotypes averaged at 1899 mm (Yabba, 2001). The RILs of population L88 had a slightly larger total root length of 1959 mm. The larger root length can be attributed to the root length of B98311. This breeding line was derived from Michigan germplasm whereas the eight other genotypes were mainly derived from Latin America (Yabba, 2001). The range of total root length was also greater in population L88 than among the eight genotypes. In population L88 mean values ranged from 1230 to 2694 compared to 1530 to 2350 mm from the eight tested genotypes (Yabba, 2001). Greater variation was recorded in population of L88, which encourages the use of populations segregating for root characteristics. However, the differences among individual genotypes for total root length were not significant enough to be used as an indirect selection criterion.

### **Root Architecture**

Fractal dimension was first described to mathematically explain complex objects of nature (Mandelbrot, 1977). Recently in common bean, fractal dimension has been used to describe root architecture which aids in drought avoidance (Lynch and van Beem, 1993;

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Nielsen et al., 1997). Fractal dimension is promising as a selection criterion because it describes root branching patterns independently of root size (Fitter et al., 1988). It is also easy to measure and is a single number amenable to mass screening (Lynch and van Beem, 1993).

In a breeding program, screening methods cannot be time consuming nor laborious as large numbers of individuals need to be evaluated. The pouch method and WinRhizo™ computer analysis make data collection for fractal dimension relatively easy while the digital root image provides a permanent record. The WinRhizo™ program has simplified a difficult mathematical calculation. Complete root systems must be measured and need to be in a two-dimensional format. This orientation is not representative of the root system growth *in situ*. In order to fully exploit the genetic variation of root architecture, root measurement technology must consider three-dimensional models (Lynch and van Beem, 1993).

Measurement of fractal dimension for roots grown in a narrow space or excavated and flattened prior to analysis may be problematic (Nielsen et al., 1997). Three-dimensional root structures can be measured in trench excavations by marking the root intersections on the exposed planes of soil. This method is not suitable for screening large numbers of individuals. Currently, screening for root characteristics in a breeding program is only possible with two-dimensional representations of roots.

Fractal dimension is always reported in values between one and two. Roots exhibiting a deep penetrating taproot or herringbone structure (Figure 9) should have a low fractal dimension, less than 1.50 whereas, highly branching roots exhibiting a dicotymous structure should have a high fractal dimension greater than 1.50. This derivation was

supported in a study (Lynch and van Beem, 1993), where root systems from short, bush type I bean plants had a larger fractal dimension than climbing type IV plants with a long taproot and fewer root meristems. The range of fractal dimension in the 81 RILs of population L88 ranged from 1.41 to 1.65 with a mean of 1.50. This range is similar to the reported fractal dimension of other leguminous species, peanut (1.56), garden pea (*Pisum sativum* L.) (1.57) (Tatsumi et al., 1989), and common bean (1.33 to 1.59) (Lynch and van Beem, 1993). The top five drought resistant RILs had a mean fractal dimension of 1.48 whereas mean fractal dimension of the corresponding five susceptible RILs was 1.52. The resistant RILs were not significantly different than the susceptible RILs for fractal dimension.

The negative correlations ( $r = 0.13^*$ ) of fractal dimension to Yd indicates that as fractal dimension gets smaller, Yd increases. Fractal dimension did not adequately separate drought resistant genotypes from susceptible genotypes. Since only field data under stress was available from one location, results are tentative.

Root characteristics associated with drought resistance allow breeders to combine root traits with tolerance traits and to select genotypes with specific root characteristics for drought resistance. Detailed root measurements can help explain the plant's response to drought stress. Avoidance and tolerance traits could be separately identified and combined to improve drought resistance in common bean.

Since the fractal dimensions of B98311 and TLP 19 do not correlate with drought resistant and drought susceptible RILs, fractal dimension as a technique for selecting parents is not suggested. Out of the three root characteristics that correlated to yield performance under stress only the I class (4.0-4.5 mm) reflected the parental values.

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Therefore, selection for the larger taproot (I class) is suggested as an indirect screening technique for selecting parents of future drought resistant populations.

### **Molecular markers**

RAPD markers previously associated with drought resistance (Schneider et al., 1997a) were tested for their usefulness in this study. Two linkage groups (4 and 9) which explained more than 10 % in yield variation under drought stress in pinto bean populations were analyzed in the two black bean populations L88 and L91 (Table A8). Only one of these LGs was polymorphic between three parents.

RAPD markers associated with drought resistance in common bean were previously used in MAS (Schneider et al., 1997a). Nine LGs were present in the one population. Although two LGs (4 and 9) explained over 10 % in the combined analysis across seven locations, only LG 9 with two flanking markers was used in MAS. A 3 % gain in Yd was obtained in one pinto population using the four markers as selection criteria. The markers were inefficient in previous studies because Yd was a moderately heritable trait in one pinto population being tested (Schneider et al., 1997a).

The most resistant genotype, T-3016 from one pinto population, was a parent of B98311. Most markers linked to drought resistance in T-3016 were not passed on to B98311, except for three of four markers on LG 4 (Table A8). Linkage group 4 explained 12 % of the variation in Yd and GM across seven locations (Schneider et al., 1997a). Only two markers from LG 4 were tested in populations L88 and L91 since the other two markers were either not scoreable (A16<sub>850</sub>) or monomorphic between parents (V01<sub>830</sub>). The two markers, F06<sub>970</sub> and I03<sub>1130</sub>, explained 5 % of the variation in Yp in populations



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L88 and were tightly linked with no cross-overs. Every RIL that showed a band presence for F06<sub>970</sub> also showed a presence in I03<sub>1130</sub> and vice versa, whereas they were mapped 8 cM apart in a previous study (Schneider et al., 1997a).

The linkage relationship of F06<sub>970</sub> and I03<sub>1130</sub> in population L88 and the extent of variation explained suggest that a QTL was transferred to the L88 population from T-3016 through the resistant parent, B98311. Further research is needed to validate the continued usefulness of these markers in MAS for drought resistance and locate the markers on the bean core map (Freyre et al., 1998).

Regression analysis was conducted to determine the best variables to use for indirect selection for Yd. Root characteristics and molecular markers associated with yield potential, explained 9 % of the variation of Yd and 16 % of the variation in Yp. The reliability of screening techniques is necessary for plant breeders to consistently select for the desired trait. Since drought resistance is a quantitative trait, indirect selection using plant attributes or molecular markers will vary according to the environmental conditions. Selection criterion must be highly associated to drought resistance across multiple locations to substantiate its potential in breeding for drought resistance in common bean.

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## CONCLUSIONS

Terminal drought stress negatively affected bean productivity in the lowland tropics. Transgressive segregation for yield was shown by 15 % of the RILs that outperformed the three parents and seven checks. Drought resistant RILs yielded 700 kg/ha under stress and 2500 kg/ha under non-stress conditions in Honduras. In Montcalm, Michigan, drought resistant genotypes yielded up to 4000 kg/ha in minimal drought stress conditions preventing confirmation of drought resistance. Broad adaptation and performance of genotypes under stress across different environments could not be evaluated. Drought resistance results are based on one location and will be confirmed. However, the drought resistant genotypes show great potential for increasing yields of common bean in Honduras and Michigan. Breeding programs can incorporate yield and drought resistance traits from the black bean RILs into locally adapted material with commercially acceptable seed types. Areas of the Latin American/Caribbean region where common bean is planted into dry season will benefit the most. Further testing in different locations will identify the zone of adaptation for the drought resistant RILs.

Root length, root architecture and molecular markers were correlated to yield in common bean. Under drought stress conditions, root length at the 0.5-1.0 mm diameter and fractal dimension correlated to yield. Fine roots correlating to yield under stress suggesting that highly branched root systems are not favored in drought conditions in Honduras. Low fractal dimension values suggest that root architecture designed for deep soil exploration are present in drought resistant RILs. Taproot length had the highest correlation to yield potential. In conjunction with molecular markers associated to yield potential, the root measurements explained 9 % of the variation of yield under stress and

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16 % of the variation of yield potential. Since the combination of root traits and molecular markers explained greater variation than either trait alone, bean breeders should consider combining different approaches to improve drought resistance in common bean.

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## APPENDIX A

### DATA TABLES FROM DROUGHT EXPERIMENTS IN MICHIGAN AND HONDURAS

Table A1. Yield, rank and 100 seed weight for the 150 RILs in Saginaw, MI 2000.

Line	entry	Yield kg/ha	Rank	100 sw g	Line	entry	Yield kg/ha	Rank	100 sw g
L88-1	1	3847	21	26.5	L91-1	82	3106	32	24.5
L88-2	2	3048	62	24.3	L91-2	83	3272	24	26.3
L88-3	3	3295	52	23.5	L91-3	84	2006	67	23.4
L88-4	4	3923	16	22.8	L91-4	85	2867	46	28.3
L88-5	5	3171	59	22.4	L91-5	86	3541	10	26.4
L88-6	6	2981	66	23.0	L91-6	87	3186	27	24.5
L88-7	7	3662	35	25.6	L91-7	88	3285	23	28.0
L88-8	8	3529	41	22.2	L91-8	89	3444	14	25.2
L88-9	9	3249	56	21.5	L91-9	90	2926	45	24.5
L88-10	10	4583	3	21.7	L91-10	91	3303	21	24.1
L88-11	11	2491	80	20.0	L91-11	92	2046	66	20.7
L88-12	12	3281	54	23.6	L91-12	93	3142	29	26.7
L88-13	13	3418	49	24.0	L91-13	94	2930	44	25.6
L88-14	14	4540	4	24.8	L91-14	95	2208	65	25.4
L88-15	15	3588	39	23.4	L91-15	96	3337	19	26.8
L88-16	16	3840	22	23.3	L91-16	97	3594	9	29.6
L88-17	17	3483	44	23.7	L91-17	98	3699	7	24.8
L88-18	18	2644	78	22.5	L91-18	99	3139	30	24.7
L88-19	19	3644	37	22.6	L91-19	100	3865	5	26.2
L88-20	20	3951	15	21.5	L91-20	101	2813	49	26.4
L88-21	21	4926	1	25.4	L91-21	102	2393	61	23.2
L88-22	22	3459	45	23.3	L91-22	103	3074	33	23.7
L88-23	23	4071	13	24.1	L91-23	104	3163	28	22.5
L88-24	24	3649	36	23.4	L91-24	105	2422	60	24.3
L88-25	25	3012	63	20.7	L91-25	106	2538	58	28.8
L88-26	26	2863	71	21.3	L91-26	107	4042	3	26.6
L88-27	27	2257	81	19.0	L91-27	108	2628	56	22.5
L88-28	28	3663	34	21.9	L91-28	109	2219	64	21.3
L88-29	29	4159	9	25.4	L91-29	110	2951	43	22.5
L88-30	30	3485	43	22.1	L91-30	111	3488	13	28.8
L88-31	31	2694	76	21.5	L91-31	112	3505	11	23.7
L88-32	32	2671	77	23.8	L91-32	113	3022	39	28.7
L88-33	33	2928	70	24.0	L91-33	114	3977	4	27.1
L88-34	34	3139	60	25.2	L91-34	115	2757	52	21.3
L88-35	35	4621	2	22.6	L91-35	116	2670	54	25.8
L88-36	36	3788	25	24.1	L91-36	117	2991	42	26.8
L88-37	37	4115	12	25.1	L91-37	118	2866	47	26.2
L88-38	38	3887	20	20.6	L91-38	119	2630	55	23.4
L88-39	39	3327	51	20.3	L91-39	120	3839	6	25.5
L88-40	40	2566	79	19.7	L91-40	121	3233	25	25.4
L88-41	41	3721	30	24.9	L91-41	122	3405	17	24.2
L88-42	42	3458	46	23.3	L91-42	123	1922	68	21.2
L88-43	43	4140	10	24.3	L91-43	124	3133	31	25.8
L88-44	44	3002	64	18.9	L91-44	125	2242	63	23.8
L88-45	45	2713	74	22.0	L91-45	126	3050	35	22.1

Table A1. Continued.

Line	entry	Yield kg/ha	Rank	100 sw g	Line	entry	Yield kg/ha	Rank	100 sw g
L88-46	46	4161	8	24.8	L91-46	127	3012	40	25.1
L88-47	47	2735	73	23.8	L91-47	128	3001	41	24.4
L88-48	48	4367	6	24.0	L91-48	129	3291	22	24.4
L88-49	49	3785	26	21.5	L91-49	130	4109	2	26.4
L88-50	50	3546	40	22.0	L91-50	131	3492	12	23.9
L88-51	51	2949	68	24.3	L91-51	132	3643	8	25.6
L88-52	52	3223	58	23.6	L91-52	133	3429	16	22.9
L88-53	53	3907	17	26.0	L91-53	134	2564	57	22.0
L88-54	54	3779	27	25.8	L91-54	135	2261	62	19.3
L88-55	55	3450	47	22.4	L91-55	136	2757	51	24.1
L88-56	56	3517	42	20.4	L91-56	137	2814	48	25.4
L88-57	57	3241	57	22.9	L91-57	138	3022	38	28.9
L88-58	58	2856	72	21.8	L91-58	139	3217	26	25.0
L88-59	59	3968	14	23.1	L91-59	140	3054	34	25.7
L88-60	60	3896	19	22.9	L91-60	141	3322	20	24.7
L88-61	61	2995	65	21.8	L91-61	142	3026	37	25.0
L88-62	62	3441	48	23.0	L91-62	143	3040	36	21.3
L88-63	63	3813	23	21.8	L91-63	144	4323	1	28.9
L88-64	64	3066	61	22.3	L91-64	145	2459	59	24.4
L88-65	65	3293	53	25.8	L91-65	146	2779	50	21.6
L88-66	66	3793	24	21.7	L91-66	147	2728	53	21.9
L88-67	67	2963	67	19.6	L91-67	148	3440	15	28.9
L88-68	68	4277	7	23.2	L91-68	149	1868	69	22.4
L88-69	69	3697	33	24.8	L91-69	150	3396	18	26.0
L88-70	70	2949	69	21.7					
L88-71	71	3603	38	23.4					
L88-72	72	3738	28	20.7					
L88-73	73	2706	75	21.3					
L88-74	74	3250	55	21.8					
L88-75	75	4453	5	25.2					
L88-76	76	3389	50	25.3					
L88-77	77	3720	31	23.3					
L88-78	78	3724	29	25.5					
L88-79	79	3902	18	23.8					
L88-80	80	3713	32	22.3					
L88-81	81	4129	11	26.8					

Table A2. Field data for 160 genotypes from drought treatment in Honduras 2001.

Line	Flort	Hght	Lodg	Matr	DS	PM	Mac 45d	Mac 75d	Stnd	Yield	Pct moist	100 sw	Bio mass	HI
	d	cm	d		d					g/m <sup>2</sup>		g	g/m <sup>2</sup>	
L88-1	42	43	2	84	3	73	6	13	28	39.3	-	17.6	510.3	0.08
L88-2	42	34	2	82	2	71	5	21	30	21.2	-	16.9	198.5	0.12
L88-3	43	45	2	82	4	73	2	9	30	128.5	12.3	19.5	633.1	0.17
L88-4	42	37	2	82	2	72	2	19	25	23.8	-	19.6	302.4	0.07
L88-5	41	40	3	81	4	70	2	11	27	79.8	-	15.8	396.9	0.20
L88-6	41	41	1	82	5	71	6	12	28	79.1	10.2	18.5	396.9	0.23
L88-7	42	37	3	82	3	74	4	12	30	60.2	-	19.3	472.5	0.14
L88-8	42	38	2	82	3	73	1	19	26	46.2	-	16.5	321.3	0.13
L88-9	45	35	2	82	2	75	7	27	24	31.1	-	17.4	179.6	0.15
L88-10	42	34	2	82	4	72	10	14	29	71.2	-	17.4	302.4	0.24
L88-11	40	38	1	80	4	70	7	16	26	63.7	11.5	17.8	264.6	0.22
L88-12	43	45	2	84	2	76	3	11	30	22.3	-	16.8	368.5	0.07
L88-13	42	40	2	81	3	72	0	12	29	121.6	11.3	19.0	472.5	0.25
L88-14	42	43	2	82	3	74	4	9	31	43.9	-	18.4	406.3	0.14
L88-15	42	38	2	82	3	71	4	15	26	53.0	-	18.7	378.0	0.15
L88-16	42	36	3	82	5	71	7	8	30	89.4	-	18.3	387.4	0.22
L88-17	41	39	2	81	4	70	1	13	29	99.4	-	19.1	302.4	0.33
L88-18	43	42	1	83	2	75	4	19	25	7.4	-	-	368.5	0.04
L88-19	41	43	2	82	4	74	5	11	27	117.3	-	19.2	378.0	0.31
L88-20	41	39	2	80	3	70	1	16	27	67.2	-	14.8	387.4	0.21
L88-21	44	37	2	82	3	73	1	8	28	37.5	-	18.9	585.9	0.07
L88-22	41	46	2	81	3	71	1	15	27	52.9	-	16.0	321.3	0.17
L88-23	42	32	1	81	3	72	3	11	28	36.1	-	15.2	207.9	0.17
L88-24	42	43	2	82	2	74	1	19	27	48.9	-	19.0	368.5	0.13
L88-25	42	44	2	81	4	72	6	14	30	85.2	11.5	18.6	434.7	0.16
L88-26	43	36	2	83	2	75	1	13	29	55.5	12.1	15.4	321.3	0.14
L88-27	42	41	2	82	4	73	1	12	30	80.4	-	15.7	340.2	0.27
L88-28	42	41	2	83	3	75	5	15	27	46.4	-	17.6	264.6	0.18
L88-29	42	35	2	83	3	73	6	18	26	48.9	-	17.6	226.8	0.19
L88-30	42	42	1	83	5	72	0	11	26	156.2	11.6	18.3	378.0	0.36
L88-31	41	43	2	82	5	72	2	10	29	135.4	11.6	18.9	406.3	0.33
L88-32	42	41	1	82	3	72	5	16	30	60.2	-	20.1	340.2	0.17
L88-33	42	40	2	82	3	71	11	16	22	58.1	-	17.2	311.8	0.18
L88-34	41	37	2	83	2	75	3	14	22	44.4	11.7	19.2	359.1	0.10
L88-35	41	38	1	81	3	72	1	10	30	61.6	-	18.1	283.5	0.22
L88-36	42	35	2	82	4	73	4	12	30	43.6	-	18.2	274.0	0.15
L88-37	43	36	2	82	2	72	4	16	24	34.4	-	21.0	207.9	0.14
L88-38	42	40	2	81	3	72	7	20	29	54.3	-	17.9	236.3	0.23
L88-39	42	37	2	82	3	72	3	13	29	47.3	-	15.8	255.2	0.17
L88-40	42	36	1	83	3	71	6	15	24	53.3	-	17.2	311.8	0.14
L88-41	43	44	2	84	2	74	3	12	28	46.0	-	19.9	368.5	0.12
L88-42	42	44	2	82	3	75	1	10	29	68.4	-	17.8	500.8	0.13
L88-43	43	31	2	82	2	73	5	11	26	33.5	-	19.6	302.4	0.08
L88-44	42	43	1	83	3	72	1	10	30	74.2	11.6	17.5	567.0	0.15
L88-45	41	38	2	82	3	71	6	17	30	39.5	-	16.3	283.5	0.15

Table A2. Continued.

Line	Flor	Hght	Lodg	Matr	DS	PM	Mac 45d	Mac 75d	Stnd	Yield	Pct moist	100 sw	Bio mass	HI
	d	cm		d		d				g/m <sup>2</sup>		g	g/m <sup>2</sup>	
L88-46	41	36	1	82	3	72	3	22	27	33.2	-	17.2	217.4	0.14
L88-47	42	39	2	82	2	73	3	9	30	56.4	-	17.9	472.5	0.11
L88-48	42	39	2	82	3	72	3	13	28	32.4	-	17.8	292.9	0.12
L88-49	42	41	2	83	3	74	6	12	30	76.7	-	18.5	491.4	0.16
L88-50	40	42	2	80	4	70	1	12	30	104.0	-	17.4	368.5	0.28
L88-51	41	37	2	80	5	70	2	14	30	92.2	-	19.0	321.3	0.28
L88-52	43	39	2	83	2	74	2	18	29	32.2	-	18.2	349.6	0.09
L88-53	41	36	2	82	3	70	2	8	30	79.2	11.8	19.9	340.2	0.23
L88-54	44	43	2	83	3	73	3	14	30	56.5	-	19.1	415.8	0.16
L88-55	41	35	2	82	3	71	4	13	30	42.8	-	17.7	283.5	0.14
L88-56	40	36	2	80	4	71	1	21	26	76.9	-	18.6	236.3	0.32
L88-57	42	34	1	82	3	72	5	14	30	35.4	-	16.3	255.2	0.14
L88-58	40	36	2	82	5	73	3	14	30	107.4	-	18.2	311.8	0.33
L88-59	42	46	2	83	4	74	1	11	30	100.6	11.6	19.9	444.1	0.23
L88-60	41	36	2	81	3	72	0	11	28	55.0	11.3	17.2	396.9	0.12
L88-61	41	38	2	81	6	72	4	12	30	111.6	-	17.1	330.7	0.33
L88-62	41	40	2	82	2	71	2	17	30	44.2	-	17.2	311.8	0.15
L88-63	41	46	2	82	5	72	3	12	30	181.8	19.9	16.7	500.8	0.34
L88-64	40	30	1	83	2	74	6	11	27	18.4	-	-	255.1	0.07
L88-65	42	40	2	82	3	73	5	16	26	46.7	-	19.7	255.1	0.20
L88-66	39	38	2	81	5	71	2	10	30	122.8	-	18.3	330.7	0.38
L88-67	42	39	2	82	2	73	2	15	26	41.9	-	14.5	207.9	0.21
L88-68	41	39	2	82	3	71	8	15	30	90.9	-	20.9	302.4	0.29
L88-69	42	39	2	83	4	73	2	8	27	138.5	12.7	17.5	396.9	0.28
L88-70	40	40	2	82	4	72	3	10	30	73.5	-	18.0	453.6	0.17
L88-71	41	41	2	83	3	74	2	16	30	53.3	-	15.9	255.2	0.20
L88-72	42	41	2	81	4	71	5	14	30	70.9	-	17.9	425.2	0.16
L88-73	42	33	2	80	3	72	0	11	25	44.9	11.2	18.3	311.8	0.15
L88-74	41	45	2	82	4	69	0	10	30	160.3	12.7	16.9	548.1	0.28
L88-75	42	45	2	82	4	73	1	13	30	84.8	-	19.7	472.5	0.19
L88-76	42	40	3	82	4	73	1	4	30	81.3	-	20.0	415.8	0.20
L88-77	42	34	2	84	3	74	14	17	24	50.0	-	16.6	217.4	0.20
L88-78	41	41	2	83	3	72	0	12	30	64.7	-	19.0	463.0	0.13
L88-79	43	40	2	82	4	75	6	13	30	62.9	-	19.0	349.6	0.17
L88-80	41	35	2	82	3	69	6	18	25	63.6	-	20.0	217.4	0.25
L88-81	42	40	2	81	3	70	1	10	29	56.9	-	16.2	349.6	0.17
L91-1	41	40	2	80	3	69	3	10	29	36.0	-	18.0	207.9	0.17
L91-2	44	43	2	82	3	74	2	7	30	21.1	-	19.5	368.5	0.06
L91-3	41	40	2	81	3	71	1	12	28	91.1	11.2	20.3	311.8	0.23
L91-4	41	42	3	82	2	72	4	18	29	38.7	-	22.7	330.7	0.09
L91-5	41	36	1	82	1	74	9	26	19	25.2	-	20.3	141.8	0.15
L91-6	42	46	2	82	3	73	2	7	30	43.4	-	19.1	321.3	0.15
L91-7	40	43	3	82	3	72	0	6	30	81.5	-	22.9	453.6	0.18
L91-8	43	43	2	83	2	73	4	10	27	17.3	-	19.9	283.5	0.07
L91-9	42	41	2	83	2	74	2	13	30	39.8	-	24.0	255.1	0.14

Table A2. Continued.

Line	Flor	Hght	Lodg	Matr	DS	PM	Mac 45d	Mac 75d	Stnd	Yield	Pct moist	100 sw	Bio mass	HI
	d	cm		d		d				g/m <sup>2</sup>		g	g/m <sup>2</sup>	
L91-10	42	48	2	82	3	73	2	10	30	98.1	12.8	21.3	396.9	0.23
L91-11	43	43	2	82	2	72	1	17	28	36.2	-	17.2	217.4	0.15
L91-12	42	37	2	83	2	73	2	9	24	22.7	-	17.9	160.7	0.13
L91-13	43	39	2	83	2	74	1	7	33	13.9	-	-	368.5	0.04
L91-14	48	43	2	83	3	72	0	7	28	21.9	-	20.2	415.8	0.06
L91-15	45	44	3	82	3	73	4	9	29	62.5	-	20.9	538.6	0.12
L91-16	44	42	2	83	3	75	1	7	30	57.3	-	21.4	425.2	0.14
L91-17	42	44	2	83	4	73	1	6	29	32.9	-	19.0	396.9	0.08
L91-18	41	43	1	79	3	70	4	6	30	60.6	-	17.9	283.5	0.21
L91-19	47	42	2	84	2	75	3	6	30	5.6	-	-	406.3	0.01
L91-20	42	47	2	83	3	75	1	8	33	50.4	-	23.0	510.3	0.10
L91-21	42	43	2	82	3	73	2	8	27	50.0	11.6	19.1	349.6	0.11
L91-22	41	37	1	82	2	73	1	14	27	13.4	-	18.1	311.8	0.04
L91-23	45	51	2	85	2	74	1	7	30	41.9	-	21.4	510.3	0.08
L91-24	43	39	2	83	3	72	3	12	27	35.4	-	20.6	406.3	0.09
L91-25	41	48	2	82	4	70	0	5	30	113.0	10.6	19.7	529.2	0.23
L91-26	41	41	3	82	3	71	3	8	28	76.0	-	21.0	415.8	0.17
L91-27	41	39	2	81	3	71	0	12	22	36.6	-	18.6	189.0	0.19
L91-28	44	48	2	83	3	75	1	2	30	35.9	11.3	20.3	340.2	0.20
L91-29	40	46	3	82	4	71	0	7	27	79.1	-	19.6	349.6	0.23
L91-30	42	43	2	82	4	73	1	7	30	130.7	-	24.7	387.4	0.33
L91-31	43	42	2	82	3	73	3	15	28	42.1	-	18.9	302.4	0.12
L91-32	42	39	2	82	2	72	1	21	29	14.2	-	-	245.7	0.06
L91-33	41	50	2	81	3	71	2	8	30	82.3	10.5	20.0	453.6	0.18
L91-34	42	44	1	81	3	72	3	14	30	40.3	-	17.8	264.6	0.18
L91-35	41	37	2	82	2	72	1	6	30	48.5	10.5	17.2	292.9	0.14
L91-36	43	34	2	83	3	73	5	9	26	27.5	-	20.6	245.7	0.10
L91-37	44	36	2	83	2	75	1	7	27	10.9	-	-	368.5	0.05
L91-38	42	38	2	82	4	73	4	16	30	57.8	-	18.6	264.6	0.22
L91-39	43	41	2	82	2	74	0	9	28	27.9	-	19.8	264.6	0.09
L91-40	42	42	2	83	3	72	3	12	30	60.0	12.1	19.2	359.1	0.15
L91-41	42	37	2	81	3	71	5	20	29	12.8	-	17.7	170.1	0.08
L91-42	42	44	2	82	3	72	1	4	30	37.9	-	17.5	491.4	0.08
L91-43	42	40	2	83	3	72	1	10	30	58.3	-	19.7	406.3	0.14
L91-44	48	46	2	83	3	75	1	8	30	34.9	-	17.3	434.7	0.09
L91-45	41	47	2	81	3	71	0	2	30	60.9	10.8	17.3	453.6	0.14
L91-46	44	45	2	81	3	72	1	8	30	66.9	-	24.5	538.6	0.14
L91-47	40	36	1	80	2	70	2	7	30	43.4	-	16.3	274.0	0.16
L91-48	43	46	2	82	3	71	0	11	28	86.1	12.9	21.2	500.8	0.13
L91-49	45	38	2	83	3	73	2	14	27	12.5	-	-	217.4	0.06
L91-50	40	39	2	83	3	72	2	15	29	44.1	-	18.5	226.8	0.20
L91-51	41	46	3	81	3	72	1	7	30	56.6	-	24.4	302.4	0.19
L91-52	47	45	2	83	2	75	1	10	27	8.6	-	18.2	236.3	0.04
L91-53	43	41	2	82	3	74	1	4	30	14.5	-	-	387.4	0.03
L91-54	40	33	2	82	4	72	1	9	30	19.3	-	27.8	217.4	0.09
L91-55	43	45	2	83	2	73	1	10	28	52.9	10.2	23.5	349.6	0.12



Table A2. Continued.

Line	Flor	Hght	Lodg	Matr	DS	PM	Mac 45d	Mac 75d	Stnd	Yield	Pct moist	100 sw	Bio mass	HI
	d	cm		d		d				g/m <sup>2</sup>		g	g/m <sup>2</sup>	
L91-56	42	48	2	82	3	71	1	19	30	68.0	-	20.8	396.9	0.17
L91-57	42	39	2	84	2	75	0	14	25	18.0	-	20.5	349.6	0.06
L91-58	42	41	3	80	3	71	2	11	30	30.8	-	20.6	340.2	0.10
L91-59	41	49	2	82	3	72	2	11	30	107.0	12.2	19.7	396.9	0.21
L91-60	42	43	2	83	3	72	2	14	29	35.6	-	19.6	283.5	0.13
L91-61	42	38	2	82	2	74	0	14	30	37.9	-	20.2	321.3	0.13
L91-62	42	45	2	82	3	74	2	9	24	69.7	-	21.8	425.2	0.12
L91-63	42	37	1	83	2	72	7	22	30	29.3	-	20.4	283.5	0.09
L91-64	42	44	2	81	3	71	5	8	29	49.2	-	17.6	378.0	0.12
L91-65	45	39	2	83	3	75	2	5	30	23.2	-	22.8	349.6	0.07
L91-66	43	43	2	82	3	74	0	7	30	31.9	-	19.0	330.7	0.10
L91-67	43	41	2	83	3	73	2	10	28	29.6	-	18.7	396.9	0.07
L91-68	44	37	2	82	2	74	0	5	30	8.7	-	-	359.1	0.02
L91-69	47	41	2	84	2	75	2	11	30	4.5	-	-	576.4	0.01
Tacana	42	36	2	84	2	73	8	15	25	34.1	-	15.4	378.0	0.10
V8025	42	30	3	81	3	72	2	6	30	49.1	-	16.8	292.9	0.18
Tio Canela	40	33	2	80	3	71	1	7	30	49.9	-	17.6	387.4	0.14
B98311	41	47	2	80	3	69	3	21	26	71.4	11.8	17.8	368.5	0.19
VAX 5	41	39	2	81	3	72	2	10	27	48.0	-	21.8	349.6	0.15
TLP 19	45	35	2	84	3	76	1	7	30	40.6	-	18.5	396.9	0.10
BAT 477	41	33	3	80	2	71	0	12	26	76.7	-	19.7	557.5	0.10
Rio Tibagi	41	43	2	82	3	72	2	11	26	69.8	-	16.1	699.3	0.11
EAP	41	29	2	82	3	72	4	15	26	41.2	-	19.4	359.1	0.11
9510-77														
SEA 5	35	37	2	72	4	65	1	7	27	104.8	-	20.8	264.6	0.40

† Flor - days to flowering, Hght - Height, Lodg - Lodging (1-5), Matr - days to maturity, DS - Desirability Score (1-9), PM - Physiological maturity, Mac 45d - Macrophomina incidence at 45 days, Mac 75d - Macrophomina incidence at 75 days, Stnd - Stand, Pct moist - percent moisture, 100 sw - 100 seed weight, HI - Harvest Index

Table A3. Field data for 160 genotypes from non-stress treatment in Honduras 2001.

Line	Flort	Hght	Lodg	Matr	DS	PM	Mac 45d	Mac 75d	Stnd	Yield	Pct moist	100 sw	Bio mass	HI
	d	cm	d	d						g/m <sup>2</sup>		g	g/m <sup>2</sup>	
L88-1	43	45	3	84	3	75	1	1	30	304.1	7.5	20.1	604.8	0.50
L88-2	41	45	3	82	3	73	2	2	29	360.5	8.2	19.4	869.4	0.44
L88-3	42	45	4	82	3	75	1	0	30	423.0	7.8	18.7	850.5	0.49
L88-4	42	44	4	81	3	72	1	2	30	379.9	8.5	18.2	756.0	0.50
L88-5	41	44	4	83	3	72	2	2	30	453.4	8.0	18.7	822.1	0.55
L88-6	42	46	4	86	4	78	4	4	25	487.4	8.7	20.3	907.2	0.53
L88-7	42	41	3	82	4	76	0	3	30	342.4	7.6	20.0	699.3	0.49
L88-8	42	44	4	83	4	74	1	2	30	461.3	7.2	20.2	859.9	0.53
L88-9	44	44	3	85	4	77	0	2	30	382.4	8.1	18.7	812.7	0.47
L88-10	42	41	3	80	4	73	0	1	30	385.2	7.2	17.8	756.0	0.51
L88-11	41	42	3	79	5	70	1	1	30	437.2	8.3	16.8	746.5	0.59
L88-12	44	48	4	85	3	79	1	1	30	528.7	7.7	19.8	973.3	0.54
L88-13	42	44	4	82	5	75	0	1	30	615.2	7.4	19.6	1143.4	0.54
L88-14	43	51	3	85	3	78	0	2	30	373.2	10.1	20.3	812.7	0.45
L88-15	41	42	5	79	2	71	0	1	30	395.5	8.0	18.4	680.4	0.62
L88-16	41	44	4	81	4	71	3	2	30	351.8	8.8	20.0	670.9	0.52
L88-17	43	44	3	83	4	75	0	1	30	414.3	8.3	17.1	841.0	0.49
L88-18	44	51	3	85	2	78	0	3	30	316.8	7.1	21.6	718.2	0.43
L88-19	43	46	3	84	4	77	0	0	30	461.0	8.1	20.4	859.9	0.53
L88-20	42	45	3	83	4	72	1	2	30	431.0	8.2	17.4	812.7	0.53
L88-21	44	46	4	85	3	78	0	1	30	348.0	8.4	21.7	954.4	0.37
L88-22	42	48	3	81	4	72	0	0	30	413.8	8.0	17.1	859.9	0.48
L88-23	41	48	3	79	4	74	1	1	30	369.9	7.5	18.1	718.2	0.51
L88-24	41	48	3	83	3	77	0	2	30	447.2	7.2	19.4	850.5	0.53
L88-25	42	45	3	81	4	72	2	2	30	428.7	7.9	17.8	793.8	0.55
L88-26	42	46	3	82	5	76	0	0	30	454.5	8.3	17.6	888.3	0.51
L88-27	41	40	3	82	5	74	0	1	30	411.4	8.3	16.5	784.3	0.52
L88-28	41	50	4	83	3	76	1	0	30	496.0	7.8	16.9	963.9	0.51
L88-29	42	48	3	81	5	73	1	1	30	446.7	8.0	17.4	850.5	0.52
L88-30	42	47	3	83	6	75	0	1	30	476.7	7.5	18.4	850.5	0.56
L88-31	42	40	3	81	5	75	0	0	30	364.7	7.6	18.8	623.7	0.58
L88-32	42	41	3	82	4	74	1	2	30	452.4	8.6	19.9	841.0	0.54
L88-33	41	44	3	82	5	74	4	6	30	389.0	7.9	19.1	803.2	0.50
L88-34	42	45	3	82	4	77	1	0	30	466.4	8.4	21.7	916.6	0.51
L88-35	41	43	3	80	4	74	1	1	30	417.2	7.3	18.1	765.4	0.55
L88-36	42	44	3	85	5	74	4	3	30	351.2	7.9	19.0	756.0	0.47
L88-37	44	47	3	84	3	76	1	2	30	313.6	7.1	21.8	756.0	0.42
L88-38	41	45	3	81	3	73	2	3	30	317.4	8.8	18.6	576.4	0.56
L88-39	42	44	3	81	4	75	1	1	30	499.4	8.7	16.1	926.1	0.53
L88-40	42	46	3	82	4	74	1	1	30	395.2	7.6	16.4	812.7	0.48
L88-41	43	50	3	82	4	75	0	2	30	413.8	7.6	21.0	774.9	0.53
L88-42	42	45	4	83	4	76	3	3	28	415.1	8.1	19.7	803.2	0.50
L88-43	44	48	3	82	4	74	0	0	30	525.5	7.8	19.9	982.8	0.53
L88-44	42	47	2	83	6	76	0	1	30	510.4	9.0	16.2	935.5	0.54
L88-45	40	44	3	81	5	71	3	5	30	353.1	7.7	19.5	708.7	0.50

Table A3. Continued.

Line	Flor	Hght	Lodg	Matr	DS	PM	Mac 45d	Mac 75d	Stnd	Yield	Pct moist	100 sw	Bio mass	HI
	d	cm	d	d						g/m <sup>2</sup>		g	g/m <sup>2</sup>	
L88-46	42	46	3	82	4	73	1	2	30	429.3	8.5	18.7	916.6	0.47
L88-47	42	44	4	82	3	75	0	0	30	492.3	8.3	19.9	907.2	0.54
L88-48	42	46	3	81	4	72	4	3	30	424.2	7.3	20.3	841.0	0.51
L88-49	43	45	3	83	4	78	0	0	30	385.8	7.1	20.5	727.6	0.53
L88-50	41	43	3	81	6	72	1	4	30	442.8	7.6	19.8	737.1	0.60
L88-51	40	39	3	81	5	72	2	5	30	441.5	7.4	20.1	784.3	0.57
L88-52	43	49	4	85	3	76	0	0	30	402.3	7.8	19.7	878.8	0.46
L88-53	43	40	4	84	2	74	5	6	30	370.9	7.1	22.6	737.1	0.49
L88-54	43	50	4	87	3	77	0	0	30	603.8	8.9	21.2	1124.5	0.54
L88-55	41	51	3	80	4	72	1	1	30	522.3	8.0	20.4	1001.7	0.52
L88-56	41	43	4	81	4	76	1	1	30	481.9	8.1	17.3	869.4	0.56
L88-57	42	48	4	83	4	75	3	4	30	541.4	8.4	18.3	982.8	0.55
L88-58	41	42	2	81	5	73	1	4	27	336.4	9.4	17.6	623.7	0.55
L88-59	42	52	4	84	3	76	1	0	30	493.8	7.4	18.1	973.3	0.51
L88-60	42	46	4	83	3	77	0	3	30	545.3	8.0	21.2	1001.7	0.54
L88-61	42	43	3	82	5	74	3	3	30	457.9	7.9	18.2	831.6	0.54
L88-62	42	46	4	81	3	72	0	0	30	401.1	6.4	20.2	869.4	0.46
L88-63	41	43	3	81	6	74	0	2	30	542.5	8.0	17.6	926.1	0.59
L88-64	41	44	3	82	4	74	1	1	30	389.9	8.2	18.7	803.2	0.48
L88-65	42	43	4	82	4	77	5	5	30	383.6	8.5	18.3	784.3	0.49
L88-66	40	43	3	82	5	72	1	5	30	545.2	8.4	17.4	963.9	0.57
L88-67	42	44	2	80	4	74	2	3	30	399.3	10.1	15.7	765.4	0.52
L88-68	42	45	4	80	4	72	0	0	30	440.2	7.9	17.9	897.7	0.49
L88-69	41	45	3	80	5	75	1	1	30	512.2	7.9	18.2	945.0	0.55
L88-70	41	44	3	83	4	75	0	1	30	446.0	8.3	19.1	850.5	0.52
L88-71	42	51	3	84	4	76	2	1	30	438.3	8.3	17.1	869.4	0.51
L88-72	42	39	4	82	4	71	1	2	30	402.5	7.9	16.3	784.3	0.51
L88-73	41	42	4	83	3	74	0	2	30	411.7	8.2	18.9	841.0	0.49
L88-74	42	45	4	82	3	73	0	0	30	528.3	8.0	17.9	992.2	0.54
L88-75	42	46	3	82	4	75	2	4	30	394.7	8.2	18.5	822.1	0.48
L88-76	42	43	3	80	4	74	2	2	30	392.6	7.7	20.3	718.2	0.55
L88-77	42	42	3	80	5	73	0	0	30	383.7	7.7	18.4	661.5	0.57
L88-78	42	43	4	83	4	74	0	1	30	532.9	8.7	21.7	973.3	0.54
L88-79	42	47	4	82	3	74	3	3	30	425.8	8.1	18.8	831.6	0.51
L88-80	41	44	3	81	3	71	0	0	30	412.8	7.5	19.1	803.2	0.51
L88-81	42	45	3	81	5	73	1	1	30	364.8	7.3	17.0	680.4	0.53
L91-1	41	44	4	79	4	71	0	0	30	480.0	7.6	18.5	841.0	0.56
L91-2	46	53	3	85	3	76	2	2	30	321.3	8.7	23.1	670.9	0.48
L91-3	42	53	3	80	5	72	0	0	30	506.8	8.5	18.9	926.1	0.55
L91-4	42	45	3	83	3	74	1	1	30	371.8	9.0	22.5	774.9	0.47
L91-5	42	42	2	85	3	77	1	2	30	349.7	8.3	21.3	652.0	0.53
L91-6	41	46	3	81	4	73	2	1	30	459.2	8.5	20.9	831.6	0.55
L91-7	41	47	3	82	4	72	0	0	30	535.3	7.1	24.5	973.3	0.55
L91-8	43	45	3	83	3	77	1	4	30	339.3	9.0	24.0	623.7	0.54
L91-9	43	53	3	83	3	76	0	1	30	442.9	8.0	23.2	831.6	0.53

Table A3. Continued.

Line	Flor	Hght	Lodg	Matr	DS	PM	Mac 45d	Mac 75d	Stnd	Yield	Pct moist	100 sw g	Bio mass g/m <sup>2</sup>	HI
	d	cm	d	d						g/m <sup>2</sup>				
L91-10	42	49	3	86	3	75	1	1	30	474.1	9.3	22.8	850.5	0.55
L91-11	41	47	3	79	5	72	0	0	30	402.6	8.2	18.2	737.1	0.54
L91-12	42	48	3	82	3	76	1	1	30	419.0	7.3	22.5	793.8	0.54
L91-13	43	47	3	81	4	74	2	1	30	391.6	7.4	20.9	793.8	0.49
L91-14	43	45	3	84	4	74	0	2	30	256.3	7.9	21.0	689.8	0.38
L91-15	44	46	3	85	3	74	1	1	30	343.6	7.6	22.7	774.9	0.44
L91-16	44	43	3	84	3	76	3	3	30	336.9	7.2	23.9	708.7	0.47
L91-17	43	46	4	83	3	75	0	1	30	414.8	8.0	20.2	841.0	0.49
L91-18	41	44	3	82	5	73	2	3	25	421.5	8.6	19.8	784.3	0.54
L91-19	47	53	2	86	3	76	4	3	30	322.4	11.1	26.6	765.4	0.41
L91-20	42	44	3	82	4	76	1	1	30	352.4	19.6	24.2	737.1	0.48
L91-21	43	48	3	84	3	78	0	1	30	436.6	7.6	20.5	888.3	0.49
L91-22	42	46	3	85	3	75	1	2	30	408.5	10.0	21.3	746.5	0.52
L91-23	45	51	3	85	4	77	2	1	30	490.0	8.1	22.4	1020.6	0.48
L91-24	43	45	2	85	4	75	0	1	30	305.0	8.8	19.9	642.6	0.48
L91-25	42	46	4	84	3	72	3	2	30	464.0	7.9	24.2	803.2	0.58
L91-26	41	45	4	80	5	73	0	2	30	472.7	7.9	21.2	831.6	0.56
L91-27	43	45	3	80	4	72	0	0	30	413.9	8.7	19.9	746.5	0.55
L91-28	43	47	3	86	3	79	0	1	30	307.5	7.9	19.7	633.1	0.48
L91-29	41	43	3	84	4	71	0	0	30	371.6	8.0	19.1	670.9	0.55
L91-30	42	46	2	83	3	74	1	1	29	399.1	8.8	23.6	774.9	0.51
L91-31	43	48	3	81	3	74	1	2	30	326.8	10.1	20.8	623.7	0.51
L91-32	42	50	3	85	3	76	1	0	30	429.1	7.8	22.3	897.7	0.49
L91-33	42	47	3	83	4	72	2	1	30	544.9	8.3	23.1	992.2	0.55
L91-34	41	46	2	81	5	73	2	2	30	419.8	8.5	18.1	737.1	0.57
L91-35	41	39	3	85	3	73	0	0	30	377.5	7.8	20.7	727.6	0.52
L91-36	42	44	3	83	4	74	1	1	30	315.8	8.8	24.2	727.6	0.43
L91-37	44	51	4	86	3	78	0	0	30	440.9	9.3	22.6	831.6	0.53
L91-38	43	45	2	82	5	75	1	3	30	316.3	7.2	19.7	633.1	0.50
L91-39	41	49	3	83	2	75	0	0	30	369.8	8.6	21.5	746.5	0.50
L91-40	43	46	3	83	3	74	1	1	30	336.1	9.2	20.0	670.9	0.50
L91-41	42	45	3	84	4	72	1	1	30	356.5	8.6	21.2	774.9	0.46
L91-42	42	42	3	83	4	74	1	2	30	350.9	9.6	18.6	670.9	0.52
L91-43	43	44	3	83	4	73	1	1	30	522.7	7.7	21.4	963.9	0.54
L91-44	43	45	3	84	3	76	0	0	30	402.8	7.7	21.2	803.2	0.50
L91-45	42	48	3	83	5	73	1	1	30	519.7	9.3	19.3	926.1	0.56
L91-46	42	50	3	86	3	74	0	1	30	427.4	10.0	24.9	859.9	0.49
L91-47	41	47	2	82	4	72	0	1	30	353.6	8.2	18.9	670.9	0.51
L91-48	42	48	3	85	3	74	0	1	30	297.9	8.6	21.9	670.9	0.42
L91-49	43	47	3	84	3	76	0	0	30	264.2	8.4	22.7	557.5	0.47
L91-50	41	42	3	81	4	74	1	2	30	338.7	7.6	20.4	633.1	0.54
L91-51	42	45	3	84	3	74	2	2	30	429.6	8.5	23.0	793.8	0.53
L91-52	45	51	4	82	2	76	1	1	30	332.1	7.7	19.9	756.0	0.43
L91-53	44	49	2	86	3	78	4	3	30	293.5	7.8	21.0	642.6	0.46
L91-54	42	37	3	82	3	73	2	3	30	296.8	9.5	18.2	557.5	0.52
L91-55	43	47	3	84	3	75	2	1	30	384.4	7.5	22.6	784.3	0.49

Table A3. Continued.

Line	Flor	Hght	Lodg	Matr	DS	PM	Mac 45d	Mac 75d	Stnd	Yield	Pct moist	100 sw	Bio mass	HI
	d	cm		d		d				g/m <sup>2</sup>		g	g/m <sup>2</sup>	
L91-56	42	48	3	81	3	73	0	0	30	537.6	8.2	23.1	935.5	0.57
L91-57	42	47	3	82	4	74	1	0	30	449.4	8.0	24.5	907.2	0.49
L91-58	44	46	3	87	3	77	0	0	30	341.2	11.3	23.6	822.1	0.40
L91-59	43	52	3	84	3	76	0	0	30	448.1	11.2	20.9	935.5	0.46
L91-60	41	45	3	82	3	74	0	0	30	340.0	8.2	18.8	803.2	0.42
L91-61	42	45	3	80	4	73	0	1	30	397.3	8.3	18.9	756.0	0.53
L91-62	44	50	3	83	4	76	0	0	30	358.2	8.7	21.4	737.1	0.48
L91-63	40	44	3	84	5	74	1	0	30	318.6	8.6	22.5	680.4	0.48
L91-64	42	43	4	83	3	73	1	0	30	448.6	7.2	17.4	869.4	0.52
L91-65	44	45	3	86	3	76	0	0	30	238.8	8.4	22.5	576.4	0.41
L91-66	44	43	3	84	3	76	0	1	30	402.8	8.1	21.1	793.8	0.50
L91-67	43	48	3	84	3	74	4	4	30	460.0	9.5	20.5	935.5	0.49
L91-68	44	48	3	83	4	78	0	0	30	377.9	8.1	20.5	784.3	0.48
L91-69	44	51	3	87	3	79	0	1	30	323.6	12.1	26.7	1086.7	0.31
Tacana	43	45	3	83	4	74	0	0	30	441.8	9.1	18.9	841.0	0.51
V8025	42	48	4	80	3	73	0	2	30	376.0	7.6	17.3	680.4	0.56
Tio Canela	41	42	4	82	3	75	0	1	30	349.4	8.6	20.2	812.7	0.43
B98311	41	46	3	83	5	73	2	1	30	507.9	6.9	18.9	897.7	0.56
VAX 5	41	46	3	85	4	75	0	3	30	372.3	9.2	22.4	765.4	0.47
TLP 19	45	44	3	87	4	80	3	3	28	493.1	7.5	19.6	954.4	0.51
BAT 477	41	39	3	85	3	74	1	2	22	276.1	9.8	19.7	519.7	0.40
Rio Tibagi	42	49	3	84	5	75	0	0	22	397.8	7.8	16.7	737.1	0.54
EAP 9510-77	40	33	3	80	4	75	0	3	27	426.8	10.8	22.8	727.6	0.57
SEA 5	34	39	2	82	5	69	7	5	28	308.7	11.0	25.5	595.3	0.53

† Flor - days to flowering, Hght - Height, Lodg - Lodging (1-5), Matr - days to maturity, DS - Desirability Score (1-9), PM - Physiological maturity, Mac 45d - Macrophomina incidence at 45 days, Mac 75d - Macrophomina incidence at 75 days, Stnd - Stand, Pct moist - percent moisture, 100 sw - 100 seed weight, HI - Harvest Index

Table A4. Yield under stress (Yd), yield under non-stress (Yp), and geometric mean (GM) of 160 genotypes adjusted for plant stand by covariate analysis for the Honduras experiment 2001.

Line	Entry	Yd	Yp	GM	Line	Entry	Yd	Yp	GM
		kg/ha	kg/ha	kg/ha			kg/ha	kg/ha	kg/ha
L88-1	1	193	1441	527	L91-1	82	167	2279	617
L88-2	2	77	1729	364	L91-2	83	77	1523	342
L88-3	3	583	2007	1082	L91-3	84	435	2406	1023
L88-4	4	168	1802	551	L91-4	85	175	1763	556
L88-5	5	400	2152	928	L91-5	86	264	1658	661
L88-6	6	382	2468	971	L91-6	87	183	2179	631
L88-7	7	263	1623	653	L91-7	88	364	2542	962
L88-8	8	250	2189	740	L91-8	89	108	1609	416
L88-9	9	213	1814	621	L91-9	90	166	2102	590
L88-10	10	335	1827	782	L91-10	91	448	2250	1004
L88-11	11	343	2075	844	L91-11	92	178	1910	583
L88-12	12	82	2510	454	L91-12	93	168	1988	578
L88-13	13	565	2922	1285	L91-13	94	-7	1857	115
L88-14	14	170	1770	549	L91-14	95	105	1213	357
L88-15	15	287	1876	734	L91-15	96	289	1629	686
L88-16	16	402	1668	818	L91-16	97	249	1597	631
L88-17	17	459	1966	950	L91-17	98	142	1968	529
L88-18	18	90	1501	368	L91-18	99	270	2144	760
L88-19	19	579	2188	1126	L91-19	100	3	1528	64
L88-20	20	345	2045	840	L91-20	101	167	1671	528
L88-21	21	184	1650	551	L91-21	102	263	2072	739
L88-22	22	272	1963	731	L91-22	103	79	1938	392
L88-23	23	177	1754	558	L91-23	104	175	2326	639
L88-24	24	248	2122	726	L91-24	105	184	1445	516
L88-25	25	382	2034	881	L91-25	106	514	2202	1064
L88-26	26	255	2157	742	L91-26	107	368	2244	908
L88-27	27	359	1952	837	L91-27	108	269	1964	726
L88-28	28	241	2355	754	L91-28	109	147	1457	463
L88-29	29	268	2120	754	L91-29	110	402	1762	842
L88-30	30	779	2263	1328	L91-30	111	599	1922	1073
L88-31	31	636	1730	1048	L91-31	112	211	1549	572
L88-32	32	263	2147	751	L91-32	113	64	2036	360
L88-33	33	371	1845	827	L91-33	114	368	2587	976
L88-34	34	311	2214	829	L91-34	115	168	1992	579
L88-35	35	270	1979	730	L91-35	116	207	1791	609
L88-36	36	183	1665	553	L91-36	117	171	1497	506
L88-37	37	224	1486	577	L91-37	118	77	2092	402
L88-38	38	250	1504	613	L91-38	119	251	1499	614
L88-39	39	221	2371	724	L91-39	120	143	1754	501
L88-40	40	324	1875	779	L91-40	121	262	1593	646
L88-41	41	225	1963	664	L91-41	122	47	1690	282
L88-42	42	317	2037	803	L91-42	123	157	1664	510
L88-43	43	195	2495	697	L91-43	124	253	2482	793
L88-44	44	334	2423	900	L91-44	125	142	1911	522
L88-45	45	164	1674	524	L91-45	126	266	2468	810

Table A4. Continued.

Line	Entry	Yd	Yp	GM	Line	Entry	Yd	Yp	GM
		kg/ha	kg/ha	kg/ha			kg/ha	kg/ha	kg/ha
L88-46	46	174	2037	595	L91-46	127	295	2028	773
L88-47	47	245	2337	756	L91-47	128	183	1677	553
L88-48	48	155	2013	559	L91-48	129	411	1411	761
L88-49	49	341	1830	790	L91-49	130	80	1251	316
L88-50	50	471	2101	995	L91-50	131	206	1606	575
L88-51	51	415	2095	933	L91-51	132	246	2039	708
L88-52	52	144	1909	525	L91-52	133	56	1574	298
L88-53	53	353	1759	788	L91-53	134	45	1391	250
L88-54	54	245	2868	838	L91-54	135	68	1406	309
L88-55	55	180	2480	668	L91-55	136	258	1823	685
L88-56	56	406	2287	964	L91-56	137	300	2553	875
L88-57	57	145	2571	610	L91-57	138	131	2133	528
L88-58	58	487	1681	905	L91-58	139	123	1617	445
L88-59	59	455	2344	1033	L91-59	140	486	2126	1016
L88-60	60	263	2590	825	L91-60	141	156	1612	501
L88-61	61	507	2173	1050	L91-61	142	157	1885	543
L88-62	62	187	1903	596	L91-62	143	397	1698	821
L88-63	63	842	2576	1473	L91-63	144	115	1510	417
L88-64	64	113	1849	457	L91-64	145	225	2129	692
L88-65	65	258	1819	685	L91-65	146	86	1130	312
L88-66	66	561	2589	1205	L91-66	147	128	1911	494
L88-67	67	235	1894	667	L91-67	148	142	2183	556
L88-68	68	409	2089	924	L91-68	149	17	1792	176
L88-69	69	680	2432	1286	L91-69	150	-2	1534	60
L88-70	70	326	2117	831	Tacana	151	213	2097	667
L88-71	71	230	2080	692	V8025	152	210	1783	612
L88-72	72	314	1910	774	Tio Canela	153	218	1657	602
L88-73	73	264	1953	718	B98311	154	375	2411	951
L88-74	74	740	2508	1362	VAX 5	155	249	1765	663
L88-75	75	380	1872	843	TLP 19	156	169	2399	637
L88-76	76	363	1862	822	BAT 477	157	400	1536	784
L88-77	77	298	1820	737	Rio Tibagi	158	372	2108	886
L88-78	78	284	2530	848	EAP	159	232	2112	699
					9510-77				
L88-79	79	280	2021	753	SEA 5	160	524	1521	893
L88-80	80	348	1959	825					
L88-81	81	267	1730	679					

Table A5. Field data of 36 genotypes under drought stress at Montcalm, MI 2001.

Line	Entry	Yield cwt/acre	100 sw† g	flor d	matr d	lodg	hght cm	DS
L88-63	1	26.7	25.5	45.2	97.7	3.0	39.7	3.6
L88-74	2	25.3	27.2	44.0	102.7	3.7	36.4	3.7
L88-13	3	24.5	28.9	44.8	98.0	3.0	34.7	4.7
L88-30	4	30.6	26.9	46.2	106.7	3.0	41.4	4.0
L88-69	5	35.8	28.3	42.9	104.7	3.0	38.3	4.1
L88-66	6	31.1	26.3	41.6	97.0	3.0	35.1	3.7
L88-3	7	20.7	30.4	46.8	107.0	3.0	38.9	3.6
L88-19	8	28.3	28.9	45.1	104.7	3.7	38.8	3.9
L91-25	9	23.3	32.8	44.6	107.7	2.7	41.3	4.5
L91-30	10	27.2	33.8	45.7	100.0	2.7	44.2	4.0
L88-61	11	30.6	27.2	44.0	102.0	2.7	39.3	4.0
L88-59	12	28.9	28.9	45.5	105.7	3.7	40.3	3.7
L88-31	13	25.8	27.3	43.8	94.7	2.7	42.2	4.6
L91-59	14	23.5	30.6	46.0	104.7	3.0	40.2	4.6
L91-10	15	33.8	31.7	44.6	104.7	3.0	42.6	3.9
L91-3	16	29.9	29.9	45.1	98.3	2.7	42.8	4.8
I81066	17	31.6	23.8	45.5	101.7	4.0	30.7	2.6
B98311	18	34.8	28.6	44.1	107.0	2.7	43.3	5.0
TLP 19	19	30.9	28.0	44.7	107.0	3.3	35.4	3.0
VAX 5	20	18.7	27.4	45.3	95.7	2.3	42.5	4.8
B95204	21	30.9	26.6	46.9	108.0	2.7	46.5	4.7
L88-37	22	30.1	31.3	45.8	106.7	3.3	35.6	2.7
L88-4	23	21.0	29.6	44.8	108.0	4.0	34.6	2.2
L88-2	24	24.1	29.3	44.9	105.0	4.0	32.1	3.7
L88-64	25	24.6	28.6	45.1	104.0	3.7	37.9	2.9
L91-22	26	24.8	29.1	44.5	99.7	4.0	35.3	2.5
L91-13	27	22.3	33.2	45.2	108.7	3.7	36.8	2.7
L91-37	28	20.4	30.7	46.9	106.0	3.3	36.6	2.9
L91-41	29	24.2	29.3	44.9	105.7	3.0	42.8	3.1
L91-53	30	26.2	26.5	44.7	103.7	3.0	38.7	3.6
L91-68	31	26.1	27.5	45.8	97.7	2.7	46.6	4.7
L91-49	32	30.1	32.9	46.1	103.0	3.7	38.6	3.0
L91-52	33	25.9	31.1	45.1	95.7	3.0	38.7	4.8
L88-18	34	18.5	27.8	46.7	108.7	3.7	46.0	1.7
L91-19	35	19.0	35.6	48.7	112.7	3.7	41.3	1.7
L91-69	36	17.2	34.4	47.0	107.7	3.0	41.6	2.0

† 100 sw - 100 seed weight, flor - days to flowering, matr - days to maturity, lodg - lodging (1-5), hght - height and DS - desirability score (1-9)



Table A6. Field data of 36 genotypes under non-stress at Montcalm, MI 2001.

Line	Entry	Yield cwt/acre	100 sw† g	flor d	matr d	lodg	hght cm	DS
L88-63	1	38.5	25.8	45.0	103.5	3.3	40.1	3.4
L88-74	2	33.0	27.0	44.3	108.8	3.4	39.7	2.9
L88-13	3	24.5	27.3	44.4	97.9	3.5	39.8	3.0
L88-30	4	30.0	26.1	44.6	107.6	3.8	44.1	3.6
L88-69	5	32.9	29.5	43.7	107.0	3.2	43.6	2.9
L88-66	6	28.4	24.9	43.0	101.3	3.0	37.3	3.3
L88-3	7	22.0	30.1	47.0	110.9	3.2	43.1	2.1
L88-19	8	30.3	28.3	44.0	109.6	3.3	37.9	3.2
L91-25	9	31.5	31.9	43.7	107.5	3.2	41.2	3.4
L91-30	10	35.4	33.0	44.6	105.0	3.3	44.2	3.0
L88-61	11	29.8	26.6	43.1	104.6	3.1	42.4	3.6
L88-59	12	30.7	29.0	45.0	107.7	3.8	37.3	2.9
L88-31	13	26.1	26.3	44.3	96.2	3.1	42.9	2.5
L91-59	14	19.5	30.3	47.0	109.4	3.2	45.4	3.4
L91-10	15	38.7	29.9	45.7	109.3	3.0	42.4	4.2
L91-3	16	27.4	27.1	45.7	101.3	2.4	46.0	4.6
I81066	17	31.4	24.8	45.4	109.8	4.0	36.6	2.1
B98311	18	27.9	27.5	44.0	107.8	2.9	44.6	3.1
TLP 19	19	32.0	26.4	45.3	107.5	3.2	42.4	3.2
VAX 5	20	21.4	26.9	45.1	97.0	2.7	45.5	3.3
B95204	21	31.4	26.3	46.3	107.4	2.3	44.2	5.0
L88-37	22	26.9	30.4	45.6	105.9	3.4	38.8	2.9
L88-4	23	21.6	29.9	46.0	111.3	4.1	39.0	2.2
L88-2	24	26.7	26.8	43.4	108.6	3.7	36.4	2.7
L88-64	25	27.2	27.4	44.0	110.5	3.6	37.7	2.1
L91-22	26	26.5	28.8	44.4	109.0	3.6	39.6	2.0
L91-13	27	24.3	31.3	45.6	108.8	3.4	38.5	2.4
L91-37	28	16.0	30.4	46.7	112.8	3.8	41.9	2.4
L91-41	29	24.5	27.1	45.7	109.6	3.1	42.0	2.9
L91-53	30	23.2	26.1	45.7	107.0	3.4	39.1	2.8
L91-68	31	25.4	27.6	46.3	105.4	2.3	49.2	4.1
L91-49	32	27.8	31.6	46.3	107.0	3.7	39.3	3.5
L91-52	33	24.3	30.9	46.0	106.6	3.8	38.0	2.0
L88-18	34	17.2	28.4	47.0	117.5	4.5	46.4	1.6
L91-19	35	15.1	36.3	48.3	113.9	4.2	42.4	1.4
L91-69	36	15.0	33.8	46.3	111.5	3.9	43.4	2.0

† 100 sw - 100 seed weight, flor - days to flowering, matr - days to maturity, lodg - lodging (1-5), hght - height and DS - desirability score (1-9)

Table A7. Mean values of Fractal Dimension, total root length, fine roots (A-C) and taproots (H-J) for the 81 RILs of population L88.

Line	Fractal Dimension	Total Root Length	A† mm	B mm	C mm	H mm	I mm	J mm
L88-1	1.53	2455.57	1854.07	521.04	58.39	0.19	0.13	3.61
L88-2	1.49	2007.44	1563.84	392.08	37.31	0.20	0.09	3.29
L88-3	1.52	2212.16	1633.82	496.22	60.73	0.43	0.11	2.76
L88-4	1.50	1947.68	1391.05	503.58	41.34	0.33	0.16	3.09
L88-5	1.48	1899.39	1471.23	380.29	36.55	0.15	0.04	2.69
L88-6	1.51	2185.40	1653.08	472.95	46.02	0.12	0.09	2.30
L88-7	1.42	1271.89	914.79	331.65	20.60	0.13	0.08	1.95
L88-8	1.52	1876.98	1338.21	458.98	55.97	0.15	0.22	2.64
L88-9	1.55	1970.53	1340.70	503.64	83.74	0.92	0.15	2.30
L88-10	1.51	2396.09	1887.57	444.67	48.55	0.07	0.17	2.87
L88-11	1.45	1711.56	1357.87	315.11	28.45	0.10	0.13	2.15
L88-12	1.46	1972.86	1579.40	358.10	26.26	0.09	0.16	2.67
L88-13	1.47	1927.67	1463.76	413.17	38.34	0.15	0.13	3.51
L88-14	1.51	1998.46	1417.98	516.58	51.30	0.05	0.18	3.13
L88-15	1.49	1767.68	1223.30	494.38	39.55	0.33	0.12	3.18
L88-16	1.49	1919.91	1425.55	430.37	49.10	0.19	0.08	3.06
L88-17	1.48	1703.16	1311.29	339.99	38.76	0.20	0.20	2.67
L88-18	1.50	2056.45	1561.53	441.47	42.38	0.26	0.08	2.41
L88-19	1.51	1834.76	1284.52	485.45	52.37	0.14	0.17	2.50
L88-20	1.52	2065.64	1480.86	510.47	59.37	0.52	0.13	2.86
L88-21	1.49	1940.02	1394.31	491.68	43.81	0.10	0.06	2.58
L88-22	1.50	2073.12	1517.26	500.21	42.85	0.13	0.05	4.51
L88-23	1.54	2398.38	1710.68	615.65	56.83	0.22	0.23	3.28
L88-24	1.46	1716.39	1241.52	431.61	33.62	0.04	0.11	3.21
L88-25	1.47	1316.23	922.90	353.02	32.44	0.16	0.18	2.19
L88-26	1.48	1719.07	1182.08	483.59	43.07	0.13	0.08	2.66
L88-27	1.52	1638.26	1160.32	397.33	57.34	0.59	0.00	2.56
L88-28	1.53	2271.56	1621.88	572.69	62.31	0.08	0.22	2.68
L88-29	1.51	2200.98	1558.71	578.17	50.80	0.17	0.24	3.24
L88-30	1.52	2105.69	1506.48	529.22	54.99	0.02	0.26	3.54
L88-31	1.50	2139.44	1599.37	473.33	48.35	0.14	0.31	3.66
L88-32	1.57	2373.22	1659.38	592.89	83.36	0.54	0.32	2.87
L88-33	1.50	2001.49	1476.10	478.29	36.86	0.30	0.30	3.27
L88-34	1.47	1366.42	997.94	336.13	26.24	0.09	0.16	1.54
L88-35	1.52	2384.38	1730.78	575.34	58.57	0.17	0.14	3.82
L88-36	1.46	1538.65	1041.41	449.87	36.44	0.32	0.34	2.72
L88-37	1.53	2119.42	1565.76	482.71	57.11	0.21	0.07	3.35
L88-38	1.50	1965.69	1420.34	500.34	35.23	0.27	0.16	2.45
L88-39	1.49	1786.83	1283.44	449.15	43.56	0.17	0.05	2.45
L88-40	1.60	2423.41	1539.43	705.47	113.00	0.81	0.56	3.30
L88-41	1.55	2109.80	1500.17	518.63	70.38	0.41	0.08	3.13
L88-42	1.54	2213.90	1570.30	550.31	72.55	0.07	0.17	3.21
L88-43	1.49	1554.04	1095.50	402.06	42.32	0.10	0.21	3.39
L88-44	1.46	1450.88	1034.19	380.83	28.64	0.15	0.18	1.91
L88-45	1.51	1732.21	1252.49	420.01	46.44	0.32	0.22	2.18

Table A7. Continued.

Line	Fractal Dimension	Total Root Length	A† mm	B mm	C mm	H mm	I mm	J mm
L88-46	1.50	1995.22	1452.22	485.73	46.35	0.28	0.26	2.49
L88-47	1.51	2506.79	1949.99	496.44	44.63	0.16	0.12	4.89
L88-48	1.55	2245.70	1585.05	576.18	64.98	0.13	0.05	4.59
L88-49	1.45	1252.36	882.84	339.38	23.70	0.30	0.17	1.65
L88-50	1.45	1372.76	974.05	368.06	24.75	0.07	0.03	1.81
L88-51	1.50	2364.49	1849.15	456.56	42.81	0.26	0.13	2.95
L88-52	1.50	1803.74	1322.38	427.06	42.26	0.18	0.10	3.16
L88-53	1.54	2171.95	1507.55	578.22	65.40	0.23	0.03	3.59
L88-54	1.59	2375.49	1421.89	720.48	138.11	2.13	1.20	4.83
L88-55	1.52	1939.71	1371.82	497.38	53.28	0.07	0.12	3.83
L88-56	1.51	1835.58	1337.55	425.94	56.86	0.10	0.23	3.42
L88-57	1.50	1804.26	1282.39	453.78	53.46	0.16	0.09	3.15
L88-58	1.45	1770.94	1311.95	421.33	29.73	0.24	0.07	2.88
L88-59	1.60	2369.07	1495.41	693.40	119.46	0.86	0.34	3.85
L88-60	1.50	1987.14	1407.94	519.93	46.82	0.10	0.01	3.93
L88-61	1.49	2128.10	1641.02	427.71	44.60	0.35	0.10	3.29
L88-62	1.44	1421.27	1030.18	361.12	23.28	0.07	0.26	2.66
L88-63	1.47	2053.61	1644.52	365.70	32.35	0.28	0.10	2.95
L88-64	1.56	2367.52	1648.38	614.17	78.64	0.25	0.04	4.17
L88-65	1.45	1666.80	1206.45	421.87	29.64	0.34	0.04	2.61
L88-66	1.45	1355.56	960.30	352.16	35.33	0.04	0.09	2.19
L88-67	1.60	2519.12	1715.64	660.12	96.69	0.62	0.28	3.93
L88-68	1.54	2694.40	2080.43	533.31	60.04	0.16	0.00	4.22
L88-69	1.46	1531.77	1163.28	330.08	29.23	0.31	0.08	2.16
L88-70	1.43	1456.09	997.63	419.92	30.43	0.21	0.32	2.11
L88-71	1.45	1566.58	1152.80	377.83	29.55	0.07	0.24	1.95
L88-72	1.44	1229.69	859.99	332.48	28.53	0.30	0.09	2.26
L88-73	1.53	2385.51	1790.29	509.82	61.67	0.08	0.08	3.91
L88-74	1.47	2019.02	1560.40	409.47	36.38	0.12	0.00	3.45
L88-75	1.65	3122.16	2050.44	862.90	137.70	0.88	0.20	4.59
L88-76	1.49	1570.25	1096.46	423.28	39.30	0.05	0.13	3.58
L88-77	1.53	1984.24	1421.91	478.41	65.57	0.06	0.25	3.56
L88-78	1.51	1887.86	1392.12	426.98	55.85	0.18	0.05	3.99
L88-79	1.61	2403.66	1556.08	676.27	108.40	0.87	0.25	4.71
L88-80	1.51	1832.25	1319.99	453.87	45.41	0.19	0.05	2.54
L88-81	1.48	1971.53	1451.65	468.34	40.65	0.17	0.22	3.22

† Root diameter classes A, B, C, H, I, J are 0-0.5, 0.5-1.0, 1.0-1.5, 3.5-4.0, 4.0-4.5, and greater than 4.5 mm, respectively.

Table A8. Presence/absence of RAPDs associated with drought resistance in T-3016 (T), Sierra (S), B98311 (B), Raven (R), N98122 (N), Huron (H), VAX 5 (V) and TLP 19 (P).

LG†	RAPD	T	S	B	R	N	H	V	P	unlinked	T	S	B	R	N	H	V	P
9	H19.960	-	+	-	-	-	-	-	-	A09.860								
	AB18.650	+	+	+	+	+	+	+	+	A16.1220								
	V01.830	-	+	-	-	-	-	-	-	A18.800	-	+	+	+	-	-		
4	F06.970	+	-	+	-	-	-	-	-	A18.1400								
	A16.850	+	-	+	-	-	-	-	-	F01.520	+	-	+	-	-	-	-	-
	I03.1130	+	-	+	-	-	-	-	-	F10.1000								
	A08.510	-	+	-	-	+	+			G04.330	-	+	+	+	-	-		
	F05.440	-	+	-	-	-	-			G08.1240								
3	I18.1400	-	+	-	-	-	-			G08.720								
	H03.1060									G09.1070	+	+	+	+	+	+		
	I03.870	-	+	-	-	-	-	-	-	H01								
	R16.1180	+	-	+	+	+	+			H12.523	-	+	-	+	+	+		
	H02.760									H18.520								
	I14.770	+	-	+	+	+	+			H18.710	+	+	+	-	-	-		
										L07.900								
7	O02.1010	+	+	-	-	+	-			M05								
	Z03.1010									N03								
	G06.400	-	+	-	-			-	-	Q06.970								
	Z01.780	-	+	-	-	-	-			T01								
2	G11.500	+	-	+	+	+	+			T16								
	AB18.600	+	-	+	+	+	+	+	+	U03								
	L12.420									U03								
	I19.840									U10.1600	-	-	+	-	-	-		
	G05.620	+	-	+	-	+	+			W20.300								
	P03.700									W20.1300	-	+	-	-	-	-		
5	A09.600	+	+	+	+	+	+	+	+	X01.850	+	-	+	+	+	-		
	G10.550	-	+	+	+	+	+	+	+	X03.850	+	-	+	+	+	-		
	T18.550	+	-	+	+													
6	L08.1090																	
	N09.860	+	-	+	+	+				SL-1								
8	A09.500									A04.560	+	+	+	+			+	+
	R10.1000	-	+	-	+	-	+			X11.680	+	-	+	+	+	+	-	-
	Q06.900									X18.980	+	-	+	+	+	+	+	+
	AC03.570									A08.780	+	+	+	-	+	+		
1	I03.830	-	+	-	-			-	-	Z08.750								
	I10.500																	
	I10.950																	
	A07.740	+	+	+	+	+	+											
	AB14.450																	
	R11.540																	
	G02.1010																	
	Z04.580	+	-	+	+	-	-											
	H08.490	+	-	+	+	+	+											

† LG - Linkage groups identified in a previous study (Schneider et al., 1997a).

## APPENDIX B

### INTROGRESSION OF ROOT ROT RESISTANCE FROM MIDDLE AMERICAN LANDRACE BEAN TO CULTIVATED ANDEAN BEAN GENOTYPES

## INTRODUCTION

Large-seeded dry beans (55-65 grams per 100 seeds) are highly susceptible to root rot, *Fusarium solani* pv. *phaseoli* (Fsp). Irrigation increases production and provides suitable conditions for Fsp to proliferate. Relatively, no resistant sources can be found among large-seeded kidney and cranberry beans in the Andean gene pool. Resistance to root rot in small-seeded genotypes in the Middle American gene pool behaves as a quantitative trait (Schneider et al., 2001). Due to intrinsic genetic differences, the transference of quantitative traits across gene pools of common bean is a difficult task (Kelly, 1988).

A major barrier for gene exchange across gene pools is the phenomenon of dwarf lethality. Andean germplasm is characterized as possessing  $dl_1dl_1Dl_2Dl_2$  genes, whereas Middle American germplasm possess  $Dl_1Dl_1dl_2dl_2$  genes for dwarf lethality. The  $Dl_1$  and  $Dl_2$  genes are differentially expressed in bean roots and shoots, respectively (Shii et al., 1981). When both loci are dominant, lethal and sub-lethal phenotypes occur (Shii et al., 1980). The symptoms of dwarf lethality are stunted growth, chlorosis, crippled leaf formation and plant death (Shii et al., 1980). The restricted root growth experienced by dwarf lethal  $F_1$  hybrids between two gene pools has been overcome by hormonal treatment (Beaver, 1992).

The inbred backcross method can be used to introgress the quantitative traits from the wild source to elite cultivars of bean (Bliss, 1993). After the initial cross is made,  $F_1$  plants are crossed back to the recurrent parent. The favorable genes of the recurrent parent are recovered more quickly when the recurrent parent is used as the female. When a suitable number of backcrosses have been made, the lines are advanced to near

homozygosity by single seed descent without selection. The desired quantitative trait can be evaluated among the progeny lines in the F<sub>4</sub> or later generations in common bean.

Advanced backcross QTL analysis is a method that combines QTL analysis with the inbred backcross method (Tanksley and Nelson, 1996). Mapping of QTLs is delayed until the BC<sub>2</sub> or BC<sub>3</sub> generation. RAPD markers conferring root rot resistance have already been discovered in bean populations (Schneider et al., 2001). These markers could be used to check for QTL presence in the inbred backcross populations developed in the project. An additional backcross to the recurrent parent, inter-mating between sister lines or crossing to other genotypes that acquired root rot resistance from a different source could be facilitated through MAS to develop a superior variety with improved levels of root rot resistance.

In this project, a source of root rot resistance was identified in the Mexican landrace, Negro San Luis (NSL). It is a small-seeded black bean from the Middle American gene pool. Its lack of adaptation in temperate latitudes make it difficult to obtain viable offspring when crossed to Andean genotypes. Parental crosses were made between the Middle American source of resistance, NSL, and the elite Michigan Andean lines, Redhawk (dark red kidney) and C97407 (cranberry). This project was initiated in order to introgress root rot resistance genes into the large-seeded bean class.

## MATERIALS AND METHODS

### **Backcross #1**

NSL was crossed to Redhawk and C97407. Since crosses between gene pools is known to produce dwarf lethals a modified protocol (Beaver, 1992) was implemented on October 4<sup>th</sup>, 1999. Three F<sub>1</sub> seeds from each cross were planted, one seed per pot. F<sub>1</sub> seed was planted 2 cm deep in a shallow 4 cm soil. Significantly less root growth develops in F<sub>1</sub> since it produces deleterious effects for the plant (Shii et al., 1981). The stem grew unusually long. Soil and Hormex, 1000 ppm Indol 3-Butyric Acid was added to encourage adventitious root growth in the F<sub>1</sub> hybrids.

A shorter day length was required to induce flowering. Two large trash cans were placed on top of each other to create the dark period. Three pots were placed inside the trash cans and the brims were sealed with duct tape to prevent any light from entering. A photoperiod of 14 hours of darkness was kept in order to induce flowering. Recurrent parents were planted at different time intervals to ensure that viable crosses would be made. All crosses were made without emasculation. All F<sub>1</sub> progeny from a cross between gene pools are expected to be dwarf lethals showing semi-lethality (Figure B1).

### **Backcross #2**

In January 2000, 24 BC<sub>1</sub>F<sub>1</sub> individuals were planted at three seeds per pot. Parental material and eleven genotypes consisting of commercial varieties and breeding lines was planted along with the BC<sub>1</sub>F<sub>1</sub> seeds. BC<sub>1</sub>F<sub>1</sub> plants that resembled recurrent parent phenotypes were preferentially crossed to the recurrent parent. Morphological information was recorded to ensure a cross-fertilization was made (Table B1).



### **DNA Preparation**

Leaf tissue from NSL, Redhawk and C97407 was collected and ground into a powder using liquid Nitrogen. DNA samples were extracted according to the mini-prep method (Afanador et al., 1993). The DNA concentration of each sample was quantified using a fluorometer (Hoefer TKO100, Hoefer Scientific, San Francisco, CA). This stock sample was diluted to a 10 ng/ml working solution for amplification by PCR.

### **PCR Protocol**

The fragment size of the RAPD markers associated to root rot resistance (Schneider et al., 2001) varied from 2000 base pairs to 800 base pairs. Gibco enzyme was used for RAPD fragments greater than 1200 base pairs while the Stoffel enzyme was used for fragments less than 1200 bp. Each RAPD primer was ran across NSL, Redhawk and C97407. PCR reactions were performed only for primers that were present in the lab. Samples were separated by electrophoresis on a 1.4% agarose gel.

## RESULTS AND DISCUSSION

### Backcross Study

Flowering occurred in seven days after the short day length treatment began. Within 14 days, all  $F_1$ s were flowering. A total of 22 successful cross-fertilizations were made in the  $BC_1$  generation. From the successful crosses, 50% of the  $BC_1F_1$ s were expected to be dwarf lethals (Figure B1) and the actual percentage was 42%.

A concern of backcrossing is that seed might have resulted from self-fertilization. Growth habit and flower color were used as morphological markers to identify cross-fertilizations in the  $BC_1F_1$ s (Table B1). NSL has two characteristics that are fixed at homozygosity and dominant in nature; indeterminacy (GG) and purple flowers (FF) (Figure B2). The other parents, Redhawk and C97407, are homozygous recessive in both loci (ggff) and show a determinate growth habit. Redhawk has white flowers while C97407 has pink flowers due to epistatic interactions. All of the  $F_1$ s (GgFf) expressed indeterminacy and purple flowers. Cotyledon color was also recorded, yet didn't provide full proof that a cross was made.

Only five plants from two different pods were determined to be self-pollinations from a total of 62  $BC_1F_1$  seeds from 24 pods that were planted. The other 22 pods were confirmed to be crosses due to plant characteristics of indeterminacy, purple flower color or dwarf lethality. Variation in RNB 1-8 (Table B1) was recorded by a determinate plant, an indeterminate plant and a dwarf lethal plant all coming from the same pod. One  $BC_1F_1$  individual, RNB 1-9, yielded only one seed. This seed grew into a determinate plant with white flowers. It was thought to be a self until its seed was examined. The  $BC_1F_2$  seed from RNB 1-9 was smaller than the seed of the recurrent parent, Redhawk,

having blunt ends and a darker seed color. Based on these seed characteristics, RNB 1-9 was determined to be a cross.

Two projects were developed in the second backcrossing scheme (Table B2). The genetics project continued using the recurrent parents, Redhawk and C97407, to create BC<sub>2</sub>F<sub>1</sub> individuals. A second project was devised to introgress the root rot genes from BC<sub>1</sub>F<sub>1</sub> plants into other commercial kidney and cranberry seed types. BC<sub>1</sub>F<sub>1</sub> plants were crossed into eleven diverse large seeded elite lines (Table B3). An average of ten individual plants were planted for each of the 680 BC<sub>2</sub>F<sub>4</sub> line.

### **RAPD Analysis**

Molecular markers conferring root rot resistance were screened on the parents (Schneider et al., 2001). Only 11 of the 16 RAPD markers (Schneider et al., 2001) associated to root rot resistance were tested. Polymorphisms between NSL and the two susceptible parents, Redhawk and C97407 were expected to show the same absence/presence as FR266 and Montcalm, the parents used to discover the markers associated to root rot resistance. Six RAPDs showed identical marker phenotypes as in FR266 and Montcalm (Table B4). RAPD markers P7<sub>700</sub> and G6<sub>1100</sub> were previously mapped to chromosome B2 on the bean core linkage map and were shown to encompass the *PvPR2* locus (Schneider et al., 2001). The PR proteins translated from this locus were suggested to aid in root rot resistance. These markers can be used in the current populations to identify potentially resistant lines. Advanced Backcross QTL analysis can also be initiated to further characterize and identify durable QTL for root rot resistance in Andean bean germplasm.

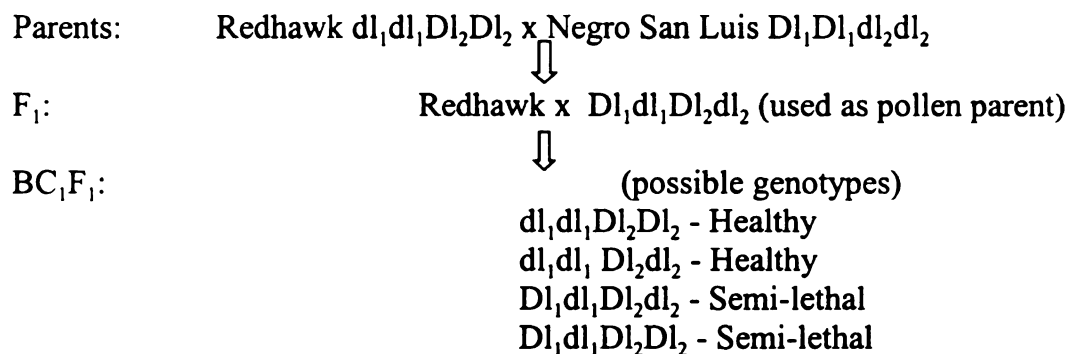


Figure B1. Diagram of inheritance of dwarf lethal genes  $Dl_1$  and  $Dl_2$  from the initial cross to the BC<sub>1</sub>F<sub>1</sub>.

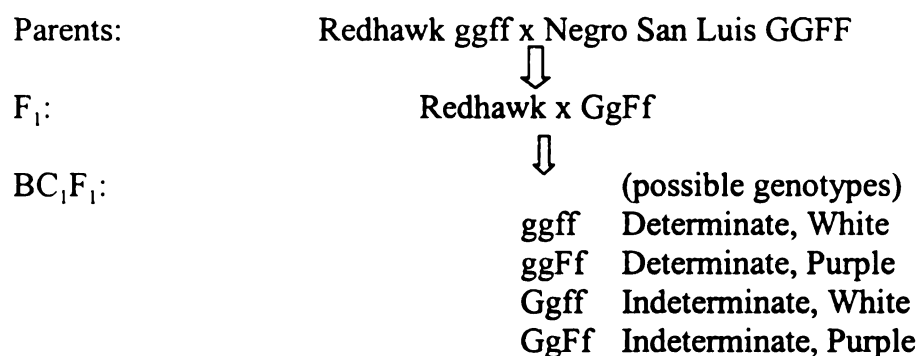


Figure B2. Diagram of the inheritance of morphological markers between kidney and black bean parents.

Table B1. Morphological characteristics of BC<sub>1</sub>F<sub>1</sub> cranberry and kidney plants.

Cranberry	Cotyledon	flower color	determinate	indeterminate	dwarf lethals	total plants	Results!
CNB 2-1	green	purple	3	0	0	3	cross
CNB 2-2	green	-	0	0	3	3	cross
CNB 2-3	green	pink	2	1	0	3	cross
CNB 2-4	purple	purple	0	2	0	2	cross
CNB 3-1	green	pink	0	2	0	2	cross
CNB 3-2	purple	pink	0	2	1	3	cross
CNB 3-3	purple	purple	1	2	0	3	cross
CNB 3-4	green	-	2	0	0	2	self
CNB 3-5	purple	purple	0	2	0	2	cross
RNB 1-1	green	-	0	0	3	3	cross

Kidney	Cotyledon	flower color	determinate	indeterminate	dwarf lethals	total plants	Results!
RNB 1-2	purple	white	1	2	0	3	cross
RNB 1-3	green	white	0	1	2	3	cross
RNB 1-4	green	-	0	0	3	3	cross
RNB 1-5	green	-	0	1	2	3	cross
RNB 1-6	green	-	1	1	1	3	cross
RNB 1-7	green	-	0	0	1	1	cross
RNB 1-8	purple	white	2	1	1	3	cross
RNB 1-9	green	white	1	0	0	1	cross
RNB 1-10	purple	-	0	0	3	3	cross
RNB 2-1	purple	white	2	1	2	3	cross
RNB 2-2	purple	purple and white	2	0	1	3	cross
RNB 2-3	green	white	3	0	0	3	self
RNB 3-1	green	-	0	0	1	1	cross
RNB 3-2	green	purple	1	2	0	3	cross

**Table B2: Summary of seed increase methods.**

<u>Season</u>	<u>Genetics and Commercial Projects</u>
Summer 2000	BC <sub>2</sub> F <sub>1</sub> seed planted for increase in Montcalm. Harvested by plant.
Fall 2000	BC <sub>2</sub> F <sub>2</sub> seed increased in Greenhouse
Spring 2001	BC <sub>2</sub> F <sub>3</sub> seed increased in Greenhouse. Harvested one plant per pot
Summer 2001	Over 680 BC <sub>2</sub> F <sub>3,4</sub> lines planted in Montcalm

**Table B3: Commercial varieties and breeding lines crossed with BC<sub>1</sub>F<sub>1</sub> individuals.**

<u>Variety Name</u>	<u>Seed Type</u>
Chardonnay	Light Red Kidney
Chinook 2000	Light Red Kidney
Red Kanner	Light Red Kidney
Montcalm	Dark Red Kidney
K99968	White Kidney
K99973	White Kidney
K99974	White Kidney
K99983	White Kidney
Hooter	Cranberry
T. Hort	Cranberry
I99134	Cranberry

Table B4: Presence or absence of RAPD markers present in Negro San Luis (NSL), Redhawk and C97407 compared to the check varieties, FR266 and Montcalm.

LG†	RAPD‡	FR266	Montcalm	NSL	Redhawk	C97407
1	D3.600	+	-			
	P7.1550	+	-	+	-	-
2	P7.700	-	+	-	+	+
	P10.1600	-	+	-	+	+
	G6.1100	-	+	-	+	+
3	I18.1800	-	+			
	I18.1700	-	+	+	-	-
5	AG2.800	+	-			
	G17.900	+	-	+	+	+
6	G3.800	+	-	+	-	-
	G3.2000	+	-	-	-	+
	P9.1550	+	-	+	+	-
4	Y11.600	-	+			
	O12.800	-	+	-	+	+
7	S8.500	+	-			
	V12.1100	+	-	+	-	-

† LG = Linkage Group

‡ RAPD markers previously identified (Schneider et al., 2001).

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APPENDIX C

PRESENCE OF TWO SCAR MARKERS LINKED TO RESISTANCE FOR COMMON  
BACTERIAL BLIGHT IN POPULATION L91

## INTRODUCTION

Common bacterial blight (CBB), caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye., is a serious seed-borne disease endemic to common bean production in Michigan and The only effective means of control is by planting disease-free seed. Development of genetic resistance to CBB will combat the negative impact on bean production in Michigan.

Quantitative trait loci regions conferring resistance to CBB have been identified and localized on the bean core map (Miklas et al., 2000). SCAR markers tightly linked to QTLs on bean linkage groups, B8 and B10, have been developed. The SAP6 marker from common bean is located on linkage group B10, whereas SU91 on B8 has tepary bean ancestry. These markers can be used to screen for CBB resistance in breeding programs where phenotypic screening for CBB is not routinely used. VAX 5, parent of population L91, has been bred with pyramided CBB resistance and carries both SCAR markers (Singh and Munoz, 1999). SCAR markers linked to QTL for resistance to CBB were tested in population L91 to identify RILs possessing CBB resistance.

## MATERIALS AND METHODS

DNA was extracted from leaf tissue of each F<sub>3:4</sub> RIL and parental genotype was harvested, lyophilized and granulated. Lyophilized and granulated tissue was allocated into 100 ml samples and DNA was extracted following the mini-prep procedure (Afanador et al., 1993). The DNA concentration of each sample was quantified using a fluorometer (Hoefer TKO100, Hoefer Scientific, San Francisco, CA). This stock sample was diluted to a 10 ng/ml working solution for amplification by PCR.

SCAR protocol for one reaction in PCR, totaling 30.0µl was as follows: 17.85µl H<sub>2</sub>O, 3.0µl 10X Buffer (Gibco), 2.25µl MgCl<sub>2</sub> (Gibco), 0.60µl dNTP mix, 0.30µl Gibco Taq Polymerase, 3.0µl 10 ng/µl Primer mix, 3.0µl 10 ng/µl DNA Template. The dNTP mix consisted of 10µl of 100mM of each dinucleotide (4) diluted in 60µl H<sub>2</sub>O. The primer mix included 10 ng/µl for the forward and reverse primers added in a 1:1 mixture. This protocol was modified so that both markers could be amplified in the same PCR reaction. This multiplexing step consists of halving the primer mix so that in each reaction 1.5µl of SAP6 and 1.5µl of SU91 were added to make the standard 3.0µl of primer mix. SAP6 and SU91 were multiplexed in the following thermocycler regime: 34 cycles of 10s at 94°C, 40s at 57°C, and 2 min at 72°C followed by one cycle of 5 min at 72°C. Samples were ran by electrophoresis on a 1.4% agarose gel and viewed by ultra-violet fluorescence. SAP6 and SU91 have fragment sizes of 820 and 700 bp respectively.

## RESULTS AND DISCUSSION

VAX 5 was crossed to B98311, to generate population L91. Among the 69 RILS from population L91, only 11 had both SCAR markers (Table C1). Some bands varied in brightness of the fluorescence. This could be explained by the collection of the DNA samples. Tissue from three plants of each line was collected. Variation for the SCAR marker might exist between plants in the  $F_{3,4}$  generation such that one two or three plants may or may not have had the markers.

Field data from Saginaw 2000 and SCAR marker data were compiled for evaluation and selection of the elite eleven lines (Table C2). Two lines, L91-47 and L91-45, were selected based on the presence of both SCAR markers, the band intensity and agronomic characteristics. Each was used into the crossing program with other elite black lines. Segregation for seed brightness occurred in the L91 population. B98311 has a dull seed coat while VAX 5 has a shiny seed coat. Further selection for dull seed coat was made in L91-45 because it segregated for seed coat appearance. Resistance to CBB has not been confirmed in selected lines through direct field or greenhouse screening. Marker technology has allowed indirect selection for CBB resistance as the MSU Bean Breeding Program is not routinely testing for resistance directly in the greenhouse or field.

Table C1. Presence/absence of the SAP6 and SU91 SCAR markers for 69 RILs in population L91.

RIL	SAP6	SU91	RIL	SAP6	SU91
L91 - 1	-	-	L91 - 40	+	-
L91 - 2	-	+	L91 - 41	-	-
L91 - 3	+	-	L91 - 42	+	+
L91 - 4	-	-	L91 - 43	+	-
L91 - 5	+	-	L91 - 44	-	+
L91 - 6	+	+	L91 - 45	+	+
L91 - 7	+	-	L91 - 46	+	-
L91 - 8	-	+	L91 - 47	+	+
L91 - 9	+	+	L91 - 48	+	-
L91 - 10	+	-	L91 - 49	-	-
L91 - 11	-	-	L91 - 50	+	-
L91 - 12	-	-	L91 - 51	+	-
L91 - 13	-	-	L91 - 52	-	-
L91 - 14	+	-	L91 - 53	-	-
L91 - 15	-	+	L91 - 54	-	+
L91 - 16	-	-	L91 - 55	+	-
L91 - 17	-	-	L91 - 56	-	-
L91 - 18	+	-	L91 - 57	-	-
L91 - 19	-	-	L91 - 58	-	-
L91 - 20	+	-	L91 - 59	+	+
L91 - 21	-	-	L91 - 60	-	+
L91 - 22	+	+	L91 - 61	-	-
L91 - 23	-	+	L91 - 62	-	-
L91 - 24	+	-	L91 - 63	+	-
L91 - 25	+	-	L91 - 64	-	-
L91 - 26	+	-	L91 - 65	+	-
L91 - 27	+	-	L91 - 66	+	+
L91 - 28	-	+	L91 - 67	-	-
L91 - 29	+	-	L91 - 68	+	+
L91 - 30	+	-	L91 - 69	-	+
L91 - 31	-	-			
L91 - 32	+	-			
L91 - 33	-	-			
L91 - 34	+	+			
L91 - 35	+	+			
L91 - 36	-	-			
L91 - 37	+	-			
L91 - 38	-	-			
L91 - 39	-	-			

Table C2. Marker and agronomic characteristics of eleven genotypes possessing both SCAR markers along with the parents.

Genotype	Intensity of SAP6	Intensity of SU91	flor† d	hght cm	lodg	matr d	DS	Seed brilliance
L91-6	2‡	1	52	49	2	103	3	Dull
L91-9	3	2	51	50	2	102	4	Shiny
L91-22	2	2	50	33	3	99	3	Dull
L91-34	2	1	51	45	3	102	3	Shiny
L91-35	2	1	49	42	2	102	3	Dull
L91-42	4	4	50	52	2	102	5	Shiny
L91-45	4	4	52	59	3	104	3	Mixed
L91-47	4	4	51	44	2	99	5	Dull
L91-59	3	2	54	49	3	106	3	Shiny
L91-66	5	5	53	44	3	107	3	Shiny
L91-68	4	3	53	46	2	105	3	Shiny
VAX 5	5	5	-	53	2	110	5	Shiny
B98311	0	0	51	46	3	103	3	Dull

† flor - days to flowering, hght - height, lodg - lodging (1-5), matr - days to maturity, DS - desirability score (1-9).

‡ Fluorescence of band is characterized by 1 = very faint to 5 = very bright and 0 = no amplification.

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