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This thesis ☐ is ☒ is not to be regarded as confidential. Gelusa Ejeta (Major Professor)

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HERITABILITY ESTIMATES, GENETIC CORRELATION, AND IDENTIFICATION OF RAPD MARKERS LINKED TO SEEDLING VIGOR AND ASSOCIATED AGRONOMIC TRAITS IN SORGHUM

A Thesis

Submitted to the faculty

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This dissertation is dedicated to my family,

for their support and encouragement and to all those who have made it possible.

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ABSTRACT

Cisse, Ndiaga PH.D., Purdue University, December, 1995. Heritability estimates, genetic cor-relations, and identification of RAPD markers linked to seedling vigor and associated agronomic traits in sorghum. Major professor: Gebisa Ejeta.

Seedling vigor in sorghum (*Sorghum bicolor* (L.) Moench), is important for improving stand establishment. These studies were conducted to investigate the heritability of the difference in seedling vigor observed between SRN39 and Shanqui red, to assess the genetic relationships of seedling vigor with field performance and phenolic compounds concentrations, and to identify QTLs associated with these characters. One hundred recombinant inbred lines and their parents were evaluated for seedling vigor, crop performance and phenolic compounds concentrations. Percent seed germination at 12" C and at 22" C, seedling emergence, seedling height and shoot dry weight were determined. under controlled environments. Significant genetic correlation between different estimates of seedling vigor were observed. Germination at 22" C, emergence, seedling vigor scores, and the rate of seedling dry matter accumulation were signlficantly correlated with grain yield. High phenolic concentrations were associated with vigorous seedlings, high percent germination at 22" C, high emergence, and taller seedlings. Only total phenols were significantly associated with grain yield. Lines with a red coleoptile

tended to be more vigorous and more productive than lines with a green coleoptile. RAPD markers on linkage groups D and F were significantly associated with seedling vigor scores. Germination at low and at optimum temperatures were mostly under different genetic control. The marker analysis showed that the visual scoring system used was effective in integrating germination, emergence, and seedling height. It was concluded that Shanqui red could be a valuable parent for the development of early maturing varieties that have improved stand establishment, and adapted to environments where low temperatures at planting in spring and early frost in the fall prevail. The identification of markers associated with seedling vigor and field performance should make breeding for the improvement of these traits more efficient, by minimizing the amount of genotype by environment interaction effect.

INTRODUCTION

Early vigor is considered an essential component of a crop plant ideotype for all enviromnental conditions (Ludlow et al., 1990). In arid environments, varieties with early vigor and good seedling establishment tend to enhance transpiration at the expense of direct soil evaporation, resulting in high level of dry matter accumulation and improved grain yield.

In temperate environments early planting and use of minimum tillage accentuate germination and seedling growth problems, because low soil temperature and high moisture often prevail at planting time. Seedling tolerance to low temperature is enhanced by rapid germination, high percentage germination, and vigorous seedling growth (Keim et al.,, 1984).

The plant characteristics that are responsible for differences in early vigor among and within plant species have not been fully characterized (Acevedo et al., 1991). Some simple characteristics, such as kernel weight, percent germination, emergence and seedling growth (shoot height, dry weight and growth rate) may be important. Differences in percent germination and emergence can influence plant population density. Since crop yield is a function of density (Willey et al., 1992), it follows that seedling vigor can influence crop yield through germination and emergence. Seedling vigor may have a direct effect on the ability of the plant to accumulate dry matter. The direct effects of seedling vigor on yield greatly depends upon the crop species. With grain crops such as sorghum which are harvested after they have completed their life cycle (full reproductive

maturity), it is believed that a yield response to seedling vigor traits occurs only when plant densities are lower than the density required to maxirnize grain yield (Tekrony et al., 1991).

Sorghum plants produce large amounts and a great diversity of phenolic compounds (Butler, 1989). Many of these phenols determine plant color, appearance, nutritional quality, and host defenses. Sorghum phenolic compounds can be divided into five basic groups; phenolic acids, lignins, quinones, flavonoids and tannin (Butler, 1989). It was reported that the percent germination in sorghum cultivars with high and low tannin content was the same (Chavan et al., 1981). However both root and shoot growth were markedly suppressed in high tannin as compared to low tannin seedlings. The rates of germination were also the same, but the subsequent rates of root and and shoot growth were much lower in high tannin seeds. It was concluded that tannins in sorghum seeds retard seedling growth due to inhibition of starch degradation by inactivating the hydrolytic enzymes during germination.

Until recently, polymorphisms have been detected with phenotypic assays of genotypes. However genetic analysis based on phenotype is a function of the heritability of the trait where factors such as the environment and quantitative inheritance often confound the expression of a genetic trait (Dudley, 1993). Plant breeders routinely find that genotypes which perform well in one environment are not as well suited to another environment. DNA markers (RFLPs, RAPDs) have advantages in that they do not have these interactions observed in phenotype-based assays. The identification of DNA

markers associated with seedling vigor, grain yield, plant height and maturity should allow more efficient breeding activities for the improvement of these traits.

Recombinant inbred (RI) lines of a cross between 'SRN39' and ' Shanqui red' (SQR), obtained through the Single Seed Descent method of plant breeding, were used to investigate the genetic basis of the differences in seedling vigor of the two parents and to determine the inter-relationships among different estimates of seedling vigor. The results of these studies are presented in chapter 1. In chapter II the genetic relationships of seedling vigor traits with yield, plant height, and maturity at high plant density recommended for commercial production are reported. In chapter III the effects of different phenolic compounds including tannins on seedling vigor traits, yield, plant height, maturity, and the relationship between the different phenolic compounds and their estimates of variance components and heritability are presented. Finally, in chapter IV of this thesis the Quantitative Trait Loci (QTLs) associated with seedling vigor, grain yield, plant height and maturity are presented.

LITERATURE REVIEW

Seedling vigor

Major constraints for dryland sorghum [(*Sorghum bicolor* (L.) Moench] production in the semi-arid regions are lack of sufficient water in the seed zone and variation in soil temperature at planting. Stand establishment is particularly difficult in hot dry weather. According to Radford et al. (1989) only 55 % of sorghum seed planted in the field in Australia resulted in successful plant establishment. They estimated that sorghum growers were losing 30 % of potential yield because of inadecluate plant density. In sub-Saharan Africa and in India , the temperature of the soil surface in farmers fields commonly exceeds 45°C and temperatures as high as 60°C have been recorded (Ougham et al., 1988). However soil temperatures above 45°C inhibit the germination and emergence of grain sorghum seedlings. Peacock (1982) indicated that optimum germination and emergence of grain sorghum occurs at soil temperatures of 21 to 35°C while the lethal temperature ranged from 40 to 48°C.

Grain sorghum traditionally has not been grown in cool regions as has maize (Zen *mays* L.). Partial credit for a broader range of adaptation for maize can be given to breeding efforts for improved seedling cold tolerance. Similarly improved cold tolerance in sorghum would allow expansion of this crop into higher elevation and. more temperate latitudes. Improved cold tolerance would also benefit production where planting is usually

delayed due to danger of stand reductions from late cold periods or in cool soils associated with minimum tillage (Bacon et al., 1986).

For decades, the studies of plant cold- tolerance and cold-stress injury had two primary goals (Guy, 1990). The first was to describe the mechanisms during a period of cold that ïead to cell injury and death. These mechanisms included ice formation within the cells and in the extracellular regions of plant tissue. The second and parallel goal, was to catalog and understand the biochemical and physiological changes oçcurring during cold acclimation. These studies have revealed that plant and algal cells can rapidly begin to alter their membrane lipid composition (Lynch et al., 1984), RNA (Cattivelli et al., 1989) and protein content (Gilmour et al., 1988) within hours of exposure to low non-freezing temperatures. These changes suggest a molecular basis for the adjustment of metabolisms to low temperature. Several cDNAs for genes upregulated at low temperature have been isolated (Schaffer et al., 1988). Guy (1990) indicated that in the characterization of the molecular-genetic basis of cold tolerance, major opportunities exist in targeting genes that encode cold-labile enzymes and proteins, enzymes in the biosynthesis of lipid metabolism and key regulatory enzymes of the respiratory pathway. Breeders have exploited the genetic variability present in many crops to develop cultivars with improved cold tolerance.

Early vigor is considered an essential component of a crop plant ideotype in all environmental conditions (Ludlow, 1990). Important advantages have been attributed to early vigor. First, the soil surface , which is usually moist during vegetative development, would be shaded more by a vigorous crop and this would result in less evaporation of

water from the soil surface and therefore, more water for transpiration and growth (Condon et al., 1987; Cooper et al., 1987; Gregory et al., 1992). Second, a greater leaf area and growth when vapor pressure deficit is low result in greater carbon assimilation per unit transpirational water loss than if growth occurred later when temperatures are higher (Tanner and Sinclair, 1983). Third, a greater crop biomass by anthesis results in a higher yield potential. In addition to increased production, more vigorous crops inhibits the growth of weeds resulting in the use of less chemical herbicides, and may also reduce the emerging problem of herbicide resistance in weed species (Lopez-Castaneda et al., 1995). These reasons have led to the investigation of the factors which are responsible for variation in early vigor (leaf area development and biomass accumulation) within crop plants to identify ways to improve their vigor and hence water-use efficiency and yield. A small improvement in growth rate early in the crop development can lead to a considerable increase in biomass at anthesis, as growth follows an exponential pattern (Richards, 1987).

The plant characteristics that are responsible for differences in early vigor among and within plant species have not been fùlly characterized (Acevedo et al., 1991; Regan et al., 1992). Some simple characteristics may be important. Selecting sorghum cultivars for rapid and uniform germination under a wide range of temperatures is important for early seedling establishment in the field (Brar, 1994). Radford and Henzell (1990) recommended screening commercial cultivars of sorghum for germination and seedling elongation at temperatures occurring in seed beds at planting. At high temperature, rapid germination can establish the crop before soil drying. Genetic variability has been shown

to exist for high temperature tolerance for germination and emergence (Wilson et al., 1982). Rapid germination permits the seminal root system to access wet soil ahead of the drying front (McCovan et al., **1985**). Using protrusion ofthe radicle as a measure of germination, Thomas and Miller (1979), and Mann et al. (1985) reported variation in the response of germination to temperature among diverse lines of sorghum. Lines considered higher yielding in more tropical environments had lower base temperatures for germination, while other lines considered higher yielding in more temperate environments had higher base temperature. Mann et al. (1985) also reported that the response to temperature is influenced by the environment experienced during seed maturation. More recent studies by Lawlor et al. (1990), however, have demonstrated that the attributes chosen as a measure of germination may influence the magnitude of any response to seed maturation environment. When germination was defined to include protrusion of the coleoptile, rather than the earlier protrusion of the radicle alone, no effect of the seed maturation environment was found. Then lines no longer differed in base temperature, but differed in the responsiveness of germination rate to temperature. Those genotypes considered better adapted to more tropical environments had greater responsiveness to tetnperature. Base temperature for germination, when defined as coleoptiie protrusion, was higher than for radicle protrusion, and corresponded with values of about 10°C in sorghum (Monk, 1977; Angus et al., 198 1). Defining germination to include protrusion of the coleoptile is therefore more appropiate to field emergence, and consequently to crop adaptation (Wade et al., 1993). Monteith (1987) proposed a linear mode1 for considering the response of germination to temperature. In this model, germination did

not occur below a base temperature, and the rate increased linearly with temperature to an optimum, above which it decreased linearly again to a maximum temperature above which seeds would not germinate.

Partitioning variance components showed both base temperature for germination defined as protrusion of the coleoptile and responsiveness of germination rate of sorghum hybrid were subject to strong genetic control (Wade et al, 1993). Narrow-sense heritability was low and specific combining ability high, indicating that response of germination to temperature is hybrid specific but not constrained to hybrid groups. Sc.reening sorghum hybrids for variation in base temperature or temperature responsivness of germination rate was thus considered unlikely to provide a valid basis for identifying tropically adapted germplasms. However these attributes may have value in assisting the identification of germplasms which are desirable for crop establishment . Cold tolerance is enhanced by rapid germination and high percentage germination. The improvement of germination under cold temperatures was attempted in maize by combining tests in both controlled and field environment (McConnell et al., 1979). After four cycles of selection, cold germination at 7.2°C was improved by about 9 % but little improvement was realized in field emergence and seedling vigor. In an inheritance study of cold tolerance in parent and selected populations of a Nebraska derivative of the Iowa Stiff Stalk Synthetic, Keim et al. (1984) found that estimates of additive genetic variance for total number of plants emerged were not significative in the parent population. Negative additive genetic variances were obtained for seedling vigor (visual scores) and weight per seedling. Variance component estimates can be negative due to sampling errors when the true

components are zero or small positive numbers (Nyquist, 199 1). Dudley and Moll (1969) argued that repeated experimentation, involving the same trait in related populations, will give estimates which approach a "true" value, when averaged together. If negative estimates are not reported, an unbiased average cannot be computed from accumulated results in the literature (Nyquist, 1991). In the selected population, all estimates of additive genetic variance were positive with smaller standard errors (Keim et al., 1984). Increased additive genetic variance may have resulted from the four cycles of selection. Alleles for cold tolerance may have been at extremely low frequencies in the parent population. In such a case the parent population would contain few individuals exhibiting appreciable cold tolerance. With selection, the frequency of alleles for cold tolerance would increase toward the value of 0.707, where additive genetic variance would be at a maximum for complete dominance (Palconer, 1989). Additive genetic correlations in the selected population between total number of seedlings emerged and seedling vigor, and between emergence and weight per seedling were highly significant. The cor-relation between seedling vigor and weight per seedling was not significant. Significant additive genetic correlations are not surprising, since the traits all measured an expression of cold tolerance.

Seedling vigor was significantly correlated with cold germination in rice (*Oriza sativa* L.) (Jones et al., 1976). Phenotypic recurrent selection in sorghum, under early spring planting, resulted in a 15 % increase in cold germination after four cycles (Bacon, 1986). The sorghum seedlings emerge in the same way as maize, oats (*Avena sativa* L.) and rice seedlings, by elongation of the mesocotyl and coleoptile, in contrast with barley

(*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) in which the mesocotyl does not elongate (Hosikawa, 1969). The mesocotyl and coleoptile of sorghum are joined by a node which appears as a slight bulge in the shoot (Mandoli and Briggs, 1984). Above this node, the primary leaf grows within the coleoptile. A positive correlation has been found between the coleoptile length of sorghum and its emergence (Wanjari and Bhoyar, 1980). Wilson et al. (1982) found genetic diversity in seedling length and its response to temperature. They also mentioned the coleoptile of sorghum, but not the mesocotyl, in discussing the effects of temperature on emergence. However, the coleoptile generally constitutes only a small portion of the total length of a sorghum seedling, but the proportion increased at high and low temperatures (Radford et al, 1990). The percentage of coleoptile in the seedling length varied from 6 to 53 % in this study with eight sorghum genotypes at seven constant temperatures (15, 20, 25, 30, 35, 40, 45°C). The optimum temperature for coleoptile elongation was 15 to 20°C. Both coleoptile and mesocotyl elongation were sensitive to temperature, but mesocotyl elongation showed the largest absolute and proportional changes between 15 and 45°C. The presence of a large coleoptile tiller was also found to contribute substantially to early vigor in wheat (Liang and Richard, 1994a). The Mexican dwarfwheat fails to germinate when sown in the field as deep as the indigeneous cultivars in India mainly due to their shorter coleoptile (Swaminathan et al., 1956). Significant differences in sorghum were found in coleoptile length which ranged from 1.35 to 4.18 cm (Wanjari et al., 1980).

Dry matter production at the early and late vegetative stages and at anthesis was significantly correlated in wheat (Whan et al., 1991). Similarly dry matter production was

also significantly correlated with grain yield. Good early vigor and high vegetative biomass would then improve yield. The average heritabilities for the early vegetative, late vegetative and anthesis stages were 72, 73, and 69 %, respectively. Fresh and dry weight measurements showed positive combining ability effects in maize. Additive gene effect were more important. Female effects were also significant (Barla-szabo et al., 1990). Ram et al. (1991) estimated broad-sense heritability at 50.5 % for seedling dry weight in pigeonpea *(Cajanus cajan* L.) Millsp.. Significant differences in seedling dry weight were found in a subset of 5 12 genotypes of the sorghum world collection (Maiti et al., 198 1). Seedling dry weight was highly correlated to leaf area, leaf number and plant height.

The faster rates of leaf and tiller production make barley more vigorous in early stages compared to wheat , triticale (*x Triticosecale*) or oats *(Avenu saliva* L.) (Lopez-Castaneda et al., 1995). The inter-val between germination and the appearance of the first two seedling leaves was found responsible for the substantially greater vigor of barley. With this early vigor, barley establishes a larger leaf area and biomass faster. This has been given as a possible reason for the success of barley compared with other cereals. Barley is usually the highest yielding temperate cereal in low rainfall areas where there is a Mediterranean-type climate (Gregory et al., 1992). The yield advantage of barley is particularly evident under dry conditions, but it may disappear when water is not limiting (Fischer and Wood , 1979). However, the size of the first seedling leaf was more important than the rate of leaf appereance, rate of tiller appearance, or partitioning of C between different plant organs in contributing to vigor among a group of Australian and Chinese wheat genotypes (Liang et al., 1994b). Anda et al., (1994) did not find significant

difference in leaf area of sorghum seedlings below 12°C. But raising the temperature above 12°C changed the leaf area significantly. With an increase from 13 to 16°C in soil temperature, seedling leaf area increased by 12.5 % at low soil water and increased by 55.5 % at high soil water treatment. Increased leaf area was correlated with increased shoot dry matter.

Relative growth rate is defined as the rate of dry mass accumulation per unit of existing dry mass. Lopez-Castaneda et al. (1994) did not find relative growth rate important in explaining the difference in seedling vigor between barley, oats, and triticale. They concluded that difference in mass established early were maintained until physiological maturity.

Seedling vigor is assessed using different estimates and screening techniques. Maiti et al. (198 1) stated that seedling vigor is best assessed by direct measurement of dry weight. However a major limitation in the assessment of dry matter production is the difficulty in detecting clear differences due to sampling errors and the amount of work involved in taking adequate samples. Clearly, this is a limitation if selection were to be carried out on a large breeding populations. The use of photographs to measure ground cover as a possible indicator of dry matter production has been attempted. This technique was sensitive to differences in growth habit between wheat genotypes (Whan et al., 1991). Maiti et al. (198 1) used a visual scoring system to evaluate a set of sorghum genotypes for seedling vigor on a scale of 1 (more vigorous) to 5 (least vigorous) at 7 and 14 days after emergence. The system was a relative one, based on the range of variability for seedling size in the material being used. The scoring system took into account the height of plant,

spread of leaf canopy and/or the length and width of the individual leaves. Highly significant correlations were found between visual scoring and dry weight and leaf area.

Laboratory experiments have proven to be adequate in estimating germination. Abdullahi et al. (1972) and Brar et al. (1994) reported a significant positive correlation between germination in the laboratory and field emergence. Also Mendoza-Onofre et al (1979) reported a good correlation between performance in growth chamber and field conditions with S 1 progenies of two sorghum populations. However McConnell et al. (1979) attributed the lack of correlation between laboratory and field results for cold emergence and seedling vigor to the mild spring weather during the two years of evaluation.

Seedling vigor and correlated traits.

Agronomic traits

Seedling vigor can be measured by many variables including kernel weight, germination, emergence and seedling growth (shoot height, dry weight and growth rate). Many studies emphasized the relationship of laboratory germination and vigor to field emergence. There is less published information relating seedling vigor to other aspects of crop performance (Tekrony et al., 199 1). However there are possibilities that seedling vigor can influence crop yield (Ellis, 1992). Differences in percent germination and emergence can influence plant population density. Since crop yield is a function of density (Willey et al., 1969), it follows that seedling vigor can influence crop yield through germination and emergence. If seed quality (size, percent germination) only affected

percent emergence, then growers could theoretically, overcome such effects by adjusting seed sowing rate. It has been shown that the effect of differences in laboratory germination on field emergence in different seedbeds can be quantified (Khah et al., 1986; Wheeler et al., 1992). However, in practice, adjustments in seed sowing rates are hampered by difficulties in forecasting the particular seedbed environment (Ellis, 1992).

Seedling vigor also affects crop performance through effects on the plant growth processes involved in the production of yield. Yield of any crop is determined by the solar radiation intercepted by the plant community, the effrciency with which intercepted insolation is converted to dry matter, and the proportion of the biomass that is economic yield (Charles-Edwards, 1982). Most of the plant tissues involved in the production of dry matter and yield are formed after seedling emergence, and it seems unlikely that seed quality (weight, percent germination) would influence their ability to carry out physiological processes and accumulate dry matter. Seed quality did not affect the relative growth rate of soybean seedlings, provided they were free of physiological injury or necrotic lesions (Egli et al., 1990). Priming or natural variation in seed quality have been reported to have no effect on the relative growth rate of orrions (*Allium cepa* L.; Ellis, 1989). However genetic aberrations, which may occur in long term storage, could cause impaired physiological function in later formed plant tissue (Roberts, 1972; Harrison, 1966).

Seedling vigor may have a direct effect on the ability of the plant to accumulate dry matter. The direct effects of seedling vigor on yield greatly depends upon the crop species. Crop harvested during vegetative growth are frequently planted at low

population densities and harvested on an individual plant or area basis as vegetative mass of aboveground [Lettuce *(Lactuca sativa* L.), cabbage (*Brassica oleracea* var *capitata*)] or underground [sugarbeet *(Beta vulgaris* L.), radish (*Rafanus sativua* L.), carrot (*Daucus carota* L.)] structures. The effects of seedling vigor can be specially critical in these crops, where delayed emergence or missing plants may reduce yield and uniformity at harvest (Tekrony et al., 1991).

Crops harvested at an early stage of reproductive development usually are planted at higher population densities than crops harvested during vegetative growth. The quantity (fresh weight), uniformity, and quality of reproductive structures determine the yield. Perry (1969) evaluated high and low-vigor seed lots of two pea cultivars and reported significantly lower yield for low-vigor seed lots tbinned to similar plant population as high-vigor lots. Abdalla and Roberts (1969) stored pea seeds in various artificial environments, and reported that seed lots with lower viability had lower early plant growth rates (O-35 days after planting) and reduced plant height and leaf number at 45 and 59 days after planting. At later stages of growth, no significant differences in relative growth rate were recorded, and reductions in dry seed yield occurred only for those seedlots where viability had declined below 50%. Unfortunately, measurements of fresh seed and fruit weight at an early stage of reproductive development were not taken. A non significant relationship between germination and fresh pod weight was reported in lima beans (*Phaseolus linensis* L.; Bennett and Waters, 1984).

Grain crops are harvested after they have completed their life cycle (full reproductive maturity) and only the seeds are harvested for yield. Work with soybean

(Tekrony et al., 1987), bean (Spilde, 1987), and corn (Abegbuyi et al., 1989) suggest that there is no relationship between germination, seedling dry weight and seedling growth rate with yield. However significant increases in yield with seedling growth rate were shown for corn (Burris, 1975). Significant increases in yield with high percent, germination seed were also shown for corn when grown at low population densities compared with seed that had been stored for 5 to 7 years (Funk et al., 1962). It was demonstrated that seedling growth rate of spring barley showed an advantage only at lower plant density, while no association existed at normal population (Perry, 1980). It was found that low percent-germination seeds of spring wheat produced lower yields only in lower than normal populations or later than normal plantings. Yield of soybean was reported to be related to accelerated aging, cold test, seedling and seedling growth rate in hill plots but not in row plots planted at normal populations. In sorghum a significant relationship between yield and speed of germination, and root and shoot growth was reported (Camargo et al., 1973), but, the plant density (66,000 plants / ha) was lower than normally recommended (Vanderlip, 1972). No significant differences were found between kernel weight with days to 50 % bloom, plant height, and grain yield in sorghum (Suh et al., 1974). It was also found that kernel weight has no influence on grain yield of sorghum (Maranville et al., 1977).

Phenolic compounds

Sorghum plants produce large amounts and a great diversity of phenolic compounds (Butler, 1989). Many of these phenols determine plant color, appearance,

nutritional quality, and host defenses. Polyphenols are secondary metabolites. Their amount and nature vary greatly with genotypes and environmental conditions under which plants are grown.

Chemically, phenolic acids are the simplest polyphenols of sorghum. Hahn et al., (1983:) identified eight different phenolic acids in grain sorghum. Furrilic acid was the most abundant of these compounds. In addition, 12 other unidentified peaks were separated by high-performance liquid chromatography.

Anthocyanidins are the major pigments in most plants. In sorghum the dominant pigments are the 3-deoxyanthocyanidins. The color of sorghum kernels is influenced by pericarp color, mesocarp thickness, presence of testa, and by endosperm texture and color (Hahn and Rooney, 1986). The pericarp color is determined by two genes, (Kambal et al., 1976) and can be white, lemon-yellow or red. Kambal et al. (1976) did not find any visible pigments in white-seeded kernels but considerable amounts ofp-coumaric, caffeic and ferrulic acids were detected. The pigment in the yellow kernels was identified as eriodictyol chalcone, a deep yellow pigment. The red seeds contained the anthocyanidins, luteolinidin and apigeninidin. Doggett (1988) classified sorghum seedlings in two groups, red and green. Red coleoptile color is controlled by a single dominant gene over green.

Flavan-4-ols, also called leucoanthocyanidins since they are converted to anthocyanidins when heated in acid with the loss of a water molecule (W'atterson and Butler, 1983), include monomers of flavanols such as flavan-3,4 diols and flavan-4-ols. The concentration of flavan-4-ols in sorghum seeds is highly dependent on the seed maturity (Jambunathan et al., 1990). Grain at early stages of maturity (10 and 14 days

after flowering) contained the highest flavan-4-ol concentrations, followed by a drastic decrease with increased maturity. It was suggested that flavan-4-ols could be degraded, converted or incorporated into other molecules such as 3-deoxyanthocyanidins or tannins.

Tannins are a group of phenolic compounds found in plants, which convert animals hides to leather during the tanning process (Butler, 1989). There are two classes of tannins. Hydrolysable and condensed tannins. Only condensed tannins, which are oligomers of flavan-3-ols have been found in sorghum. These oligomers are now referred to as procyanidins, because the red anthocyanidin pigment cyanidin is released when treated with minera1 acids. Tannins are the most abundant phenolic coumpound extractable from the seed of brown, bird-resistant sorghum (Hahn et al., 1984). Tannins bind to and precipitate proteins, reducing the nutritional value of the grain. High tannin sorghums have different kernel structures from other sorghums (Hahn et al., 1984). High tannin sorghums have a prominent pigmented testa located beneath the pericarp. The pigmented testa is purple or reddish-brown and varies in thickness. The presence of a pigmented testa is controlled by the complementary B1 and B2 genes. The S gene controls the presence of pigments and tannins in the epicarp. When S is dominant, more phenols and tannins are in the pericarp.

It was reported that the percent germination in sorghum cultivars with high (3.4 %) and low (0.5 %) tannin content was the same. However both root and shoot growth were markedly suppressed in high tannin as compared to low tannin seedlings. The rates of germination were also the same, but the subsequent rates of root and and shoot growth were much lower in high tannin seeds (Chavan et al., 1981). The tannin content decreased

markedly during germination. Tannins are located in the seed coat of the sorghum grain (Jumbunathan et al., 1973). The loss of tannin was attributed to leaching in growth medium and penetration into the endosperm with imbibed water during germination. Starch content decreased, and the rate of formation and total accumulation of reducing sugars and free amino acids were lower in high tannin seeds. The interpretation was that starch and protein degradation were inhibited or lowered in high tannin seeds during germination, leading to suppressed seedling growth. This inhibition would result from the portion that enters the endosperm. Such tannins are likely to form complexes with seed protein reserve and enzymes, and inactivate them (Chavan et al., 1981).

During germination, reserves of nutrients like star-ch and proteins are degraded to soluble sugars and amino acids, respectively, to meet the seedling requirements. Any depression of starch and protein degradations would indicate interference with the metabolic systems operating on reserve starch and protein, mainly enzymes like amylases and proteases (Dalvi, 1974). Tannins are reported to form complexes with hydrolytic enzymes and inactivate them (Tamir et al., 1969; Milic et al., 1972). A marked suppression of seedling root growth was also observed with a low tannin (0.1 %) sorghum cultivar, germinated at 1 %, 2 % and 3 % tannin acid concentrations. The inhibition increased with concentration and time (Chukwura et al., 1982). A decrease in starch content in the control sample (distilled water) and 1 % tannic acid solution, but not at higher concentrations was also noted. A concommitant increase of soluble carbohydrate content at low concentration of tannic acid, and in distilled water , and a decrease at higher concentrations were also observed. The fact that high concentrations of tannic acid

reduce the level of soluble carbohydrate falls germination, below its original level, was viewed as an indication that tannic acids directly inhibited the production of these carbohydrates. Alpha and beta-amylase activity were also observed to be inhibited by an increase in the concentration of tannic acid. It was concluded that tannins present in sorghum seeds retard seedling growth due to inhibition of starch degradation by inactivating the hydrolytic enzymes during germination.

Quantitative Trait Loci

Improvement of crop species for quantitative traits is difficult because the effects of individual genes controlling the traits cannot be readily identified. Mather and Jinks (197 1) summarized several cases where simply inherited markers were associated with variation in quantitative traits, Sax (1923) first reported association of a simply inherited genetic marker with a quantitative trait in plants when he observed segregation of seed size associated with segregation for a seed coat color marker in beans (*Phaseolus vulgaris* L.). Rasmusson (1935) demonstrated linkage of flowering time (a quantitative trait) in pea (*Pisum sativum* L.) with a simply inherited gene for flower color. Everson and Schaller (1955) found morphological markers which flanked a chromosomal region affecting yield in barley. In maize, translocations have been used to assoçiate segregation for quantitative traits such as European corn borer resistance with individual chromosome segments (Burnham, 1966). In wheat, monosomics have been used to identify associations of quantitative traits with individual chromosomes (Law, 1967).

Though these markers have served well in various types of basic and applied research, their use in many areas of plant breeding has been very limited. Major limitations of these studies included the limited number of markers available, undesirable effects of many of the morphological markers, and in the case of translocation or whole chromosome effects, the extreme size of the chromosome being compared.

The development in recent years of protein and DNA markers offers the possibility of developing new approaches to breeding procedures. The greater utility of molecular markers derives from five inherent properties that distinguish them from morphological markers (Stuber, 1992): (1) Genotypes of molecular loci can be determined at the whole plant tissue and cellular levels. Phenotypes of most morphological markers can only be distinguished at the whole plant level, and frequently, the mature plant is required. (2) A relatively large number of naturally occuring alleles can be found at molecular loci. Distinguishable alleles at morphological marker loci occur less frequently and often must be induced through the application of exogenous mutagens or the construction of special genetic stocks. (3) Deleterious effects are not Usually associated with alternate alleles of molecular markers. This is not the case with morphological markers, which are often accompanied by undesirable phenotypic effects. (4) Alleles of some molecular markers (RFLP, SSR) are codominant, allowing all possible genotypes to be distinguished in any segregating generations. Alleles at morphological marker loci usually interact in a dominant-recessive manner, prohibiting their use in many crosses. (5) Unfavorable epistatic interactions frequently occur among loci encoding morphological marker-traits thereby limiting the number of segregating markers that can be tolerated or unequivocally

scored in a single population. Most molecular markers appear to be free of epistatic effects, Thus the number of loci that can be monitored in a single population is theoretically unlimited. Limitations are however dictated by the number of polymorphic markers for which assay procedures are available or by the limitations associated with the facilities and resources available to the researcher or breeder.

Isozymes were the first molecular markers used to identify QTL in maize, tomato (*Lycopersicon spp.*), wild oats *(Avena fatua* L.) and soybeans [*Glycine max (* L.) Merr.], (Stuber, 1992). The effectiveness of marker-assisted selection in maize was demonstrated with the use of isozyme markers (Stuber and Edwards, 1986). Although isozyme markers likely have no phenotypic effects, the numbers of such markers available are small.

The large numbers of restriction fragment length polymorphisms (RFLP) and random amplified polymorphic DNA (RAPD) markers in many species has allowed the development of linkage maps with a high degree of resolution. A primary genetic linkage map, consisting of easily scored polymorphic marker loci uniformly distributed throughout a genome, is an essential prerequisite for detailed genetic studies and marker-facilitated breeding approaches in any crop plant (Stuber, 1992).

Construction of a linkage map involves following the inheritance of the markers in appropiate pedigrees. Either the F2 produced from crossing two lines or the backcross of the F1 to one of the parents provides an appropiate mapping population. Although more complex to analyse, an F2 provides almost twice as much information as a backcross because markers are segregating in both the male and female gametic populations

generating the F2 (Lander et al., 1987). Recombinant inbred lines (RI) also are useful for generating linkage map (Burr et al., 1988).

Recombinant inbred lines are produced by continually selfing or sib-mating the progeny of individual members of an F2 population until homozygosity is achieved.. Each Rl line is fixed for a different combination of linked blocks of parental alleles, so an RI family constitutes a permanent population in which segregation is fixed. If the original parents of the F2 were inbred lines, then only two alleles at a given locus will be segregating in the population. RI populations offer two major advantages over F2 or backcross populations. First, once homozygosity has been attained, RI lines may be propagated indefinitely without further segregation. This is beneficial because in any major mapping effort, DNA is eventually exhausted when either an F2 or backcross population is used. In order to resume mapping, the allelic distribution of all markers must be redetermined in a new segregating population. Second, RI lines undergo multiple rounds of meiosis before homozygosity is reached. As a result, linked genes have a greater probability of recombination. Linkage beyond 20 cM is frequently not detected because of the extensive recombination characteristic of RI lines (Burr, 1991).

Several sorghum linkage maps have been published [Hulbert et al. (1990); Whitkus et al., (1992); Melake Berhan et al. (1993); Ragab et al. (1994); Chittenden et al., (1994); Pereira et al., (1994) and Weerasuriya (1995)]. These studies have shown that single-copy maize sequences hybridized well to sorghum DNA, and detected low copy number bands in sorghum. However maize repetitive DNA sequences hybridized poorly, or not at all, to sorghum. This suggests that the larger size of the maize

genome is due not to the number or types of genes, but rather to the differences in the amount of repetitive DNA (Bennetzen et al., 1993). The linkage relationships of polymorphic loci in maize and sorghum were usually conserved. Probes that were linked in maize were often linked in sorghum, with large regions of colinearity between the genomes. However, several rearrangements were also detected. Whitkus et al., (1992) suggested that the primary process involved in the divergence of maize and sorghum genomes were duplications, inversions and translocations. Chittenden et al., (1994) have published the most "extensive" genetic map of sorghum to date. Cosegregation of 276 RFLP loci revealed a genetic map comprised of 10 linkage groups putatively corresponding to the ten gametic chromosomes of *S. bicolor* and *S propinquum*. This map spanned a genetic distance of 1445 cM with an average of 5.2 cM between markers and was estimated as an 93 % coverage of the sorghum genome.

The underlying assumption of using marker loci to detect polygenes is that linkage disequilibrium exists between alleles at the marker locus and alleles of the linked polygene(s). Linkage disequilibrium can be defined as the nonrandom association of alleles at different loci in a population. While a recombinant inbred populations have less linkage disequilibrium compared to backcross or F2 populations, due to more opportunity for meiotic recombination, they have the advantage of homozygous lines that can be replicated and retested for more accurate measurement of the quantitative trait (Burr, 1991).

There are several statistical procedures for determining whether a polygene is linked to a marker gene, and they all share the same basic principle: To partition the

population into different genotypic classes based on genotypes at the marker locus and then to use cor-relative statistics to determine whether the individuals of one genotype differ significantly compared to individuals of other genotype(s) with respect to the trait being measured (Tanksley, 1993). If the phenotypes differ significantly, it is interpreted that a gene(s) affecting the trait is linked to the marker locus used to subdivide the population. The procedure is then repeated for additional marker loci throughout the genome to detect as many loci as possible. Normally, it is not possible to determine whether the effect detected with a marker locus is due to one or more linked genes affecting the trait. For this reason, the term quantitative trait locus (QTL) was coined to describe a region of a chromosome (usually defined by linkage to a marker gene) that has a significant effect on a quantitative trait.

The simplest approach for detecting QTL is to analyse the data using one marker at a time. This approach is often referred to as single point analysis and does not require a complete molecular marker linkage map. The disadvantages of point analysis have been summarized by Tanksley (1993) as: (a) The farther a QTL is from the marker gene, the less likely it is to be detected statistically due to crossover events between the marker and QTL that result in misclassification. (b) The magnitude of the effect of any detected QTL will normally be underestimated, due also to recombination between the marker locus and QTL. Both problems are, however, minimized when a large number of segregating molecular markers are used, covering the entire genome (usually at inter-vals less than 15 cM). Under these conditions any potential QTL would be closely linked to at least one molecular marker. For progenies resulting from crosses between homozygous lines, only

two marker genotypes are available for comparison in backcross or recombinant inbred populations. Thus F tests in the analysis of variance or t test between marker genotyge means are appropriate. Alternatively, linear regression of marker genotype means on genotype can be used to estimate the additive effect associated with the marker locus (Dudley, 1993).

Distributional extremes or trait-based analysis (Lebowitz et al., 1987) is a modification of the single point approach. In this analysis, individuals in the tails of an F2 or backcross distribution are sampled for marker frequencies. Those markers differing in frequency between the tails are assumed to be associated with a QTL affecting the trait. The method is effective only for only one trait at a time because different individuals will likely be in tails for different traits. Likewise, it is likely to be effective only for traits controlled by a small number of QTL which show little interaction.

Lander and Botstein (1989) proposed a method called interval analysis to take full advantage of linkage maps for quantitative studies. Instead of analysing the population one marker at a time, sets of linked markers are analysed simultaneously with regard to their effects on quantitative traits. By using linked marker analysis, it is possible to compensate for recombination between the markers and the QTL, increasing the probability of statistically detecting the QTL and also providing an unbiased estimate of the QTL effect on the character. The major benefit of inter-val analysis over the point approach is tealized when linked markers are fairly far apart (>20 cM). Under these conditions there are likely to be many crossovers between the markers and QTL, which can be compensated for with interval analysis (Tanksley, 1993). When the marker

density is higher (markers < 15 cM apart) point and interval analysis give nearly identical results. When marker loci are very far apart (e. g. > 35 cM), even inter-val analysis is inefficient in detecting QTL in the interval between the marker loci. Because maximum likelihood estimates reduce to least squares estimates when data are normally distributed (Snedecor and Cochran, 1989), Paterson et al. (1988) and Bubeck et al. (1993) showed that both analytical methods gave virtually the same results in detecting QTL.

Haley and Knott (1992); Martinez and Curnow (1994); Wright and Movers (1994) used inter-val mapping by the regression approach and obtained similar results as Lander and Botstein (1989) with the log-likelihood analysis. The relative simplicity and computational rapidity of the regression method made it easier to fit models for two or more linked and / or interacting QTL, and gave good estimates of QTL effects. However the efficiency of flanking-marker methods decreases as the number of incompletely-genotyped individuals increases. For this reason Kearsey and Hyne (1994) proposed a simple "marker-regression"approach. This approach produced estimates of QTL locations and effects comparable to interval mapping based on log-likelihood or multiple regression. The method is simple to understand, easy to implement and offers unique features: First the residual mean square can be used to test the adequacy of the simple one-QTL mode1 on a given chromosome in a single test. Second, it provides a simple test for whether the QTL, located on a given chromosome in different populations, are the same and this is achieved by standard joint regression analysis. Different populations typically segregate at different marker loci and hence create difficulties for flanking-marker methods. The method is applicable to F2, backcrosses, doubled haploid lines or recombinant inbred lines.

Dudley (1993) stated that with large numbers of markers and a single factor analysis of variance for each marker locus, a certain proportion of the effects will be declared significant when in fact there is no association between the marker and QTL (false positive or type 1 errors). For example, if the 0.05 probability level is used, five out of 100 independent F tests are expected to be significant even if none of the marker loci is significantly associated with a QTL. Because large numbers of markers are often used (e.g > 100) and a number of traits may be analysed, an appreciable number of type l en-or is likely. Lander and Botstein (1989) suggest using a significant level equivalent to 0.001 in order to reduce the number of false positives. Their argument is on reducing the probability of finding a type 1 error any place in the genome to 0.05 when analysing a species with 12 chromosomes and markers uniformly spaced 20 centiMorgans apart. But reducing the probability of type 1 error increases the probability of type 2 errors (rejection of the hypothesis of no association between a marker and a QTL when in fact such an association exists). The problem of determining appropriate threshold values has several other sources: There is the question of determining (or approximating) the distribution of the test statistic under an appropriate null hypothesis. Also the sample size, the genome size of the organism under study, the genetic map density, segregation ratio distortions, the proportion and pattern of missing data and the number and magnitude of segregating QTL also contribute to the problem.

Churchill and Doerge (1994) described a procedure for estimating a threshold value and thus detecting significant QTL effects which is valid for any continuously distributed trait. The method gives the correct type 1 error level and has power to detect

QTL effects under the alternative hypothesis. The method is based on the concept of permutation tests (Fisher, 1935). It involves repeated "shuffling" of the quantitative trait value and the generation of a random sample of the test statistic from an appropiate null distribution.

Recently Kruglyak and Lander (1995) extended interval mapping to any quantitative trait regardless of its distribution, through the use of another nonparametric method. The basic statistic Zw is a generalization of the Wilcoxon rank-sum statistic to the situation of interval mapping. The efficiency of this test relative to the t-test is 96 %, if the distribution is normal and is never less than 86 % for any other distribution (Lindgreen, 1968). The efficiency is defined as the ratio of the sample sizes required for the two tests to achieve the same power. This approach has been incorporated in MAPMAKER / QTL (version 2), allowing robust mapping of QTL without concern about the precise distribution of the trait.

CHAPTER ONE

ESTIMATES OF SEEDLING VIGOR AND THEIR GENETIC RELATIONSHIP IN A RECOMBINANT INBRED POPULATION OF SORGHUM

ABSTRACT

Seedling vigor in sorghum (*Sorghum bicolor* (L.) Moench) is important for improving stand establishment in arid regions, and where low soil temperatures prevail at planting time. This study was conducted to evaluate different methods of estimating seedling vigor, and to assess the genetic basis of the difference in seedling vigor observed between two cultivars, SRN39 and Shanqui red. One hundred recombinant inbred lines generated from the above parents, and five controls, were evaluated for seedling vigor in the field for two years. A visual score of 1 (most vigorous) to 9 (least vigorous) was used. The experimental materials were also evaluated in an incubator for percent germination at 12" C and at 22" C, and in the greenhouse for emergence, seedling height and shoot dry weight. The two-year field scores were highly correlated (r = 0.64, P = 0.000 1), indicating that visual scoring was a reliable method of estimating seedling vigor. Shanqui red had a mean score of 1.8 in 1993 and 1 in 1994, while SRN39 had respectively as scores 7.9 and 7 for the two years. the inbred line distribution varied between 1.6 and 7.4 in 1993, and between 1 and 9 in 1994. The 1993 data fit a duplicate dominant model ($\chi 2 = 0.053$, $0.9 < P < 0.75$), while a triplicate dominant mode1 was appropiate for 1994 ($\chi 2 = 1.12$, $0.5 < P < 0.25$). Thus seedling vigor in this sorghum population is controlled by only a few genes, probably only two or three. Significant genetic and additive variances were observed for the visual scores and the traits measured in controlled environments. The genetic correlation coefficients of seedling scores with the different estimates of vigor were significant except with100-seed weight. Signiticant genetic interrelationships were

also revealed between the traits measured in the greenhouse and incubator. It was concluded that kernel weight has little effect on stand establishment. The relatively simple inheritance of the visual scores, combined with the identification of molecular markers associated with this trait, indicates that seedling vigor can be used efficiently by breeding programs geared towards improving stand establishement.

INTRODUCTION

Early vigor is considered an essential component of a crop plant ideotype for all environmental conditions (Ludlow et al., 1990). In arid environments, varieties with early vigor and good seedling establishment tend to enhance transpiration at the expense of direct soil evaporation, resulting in high level of dry matter accumulation and improved grain yield. In temperate environments early planting and use of minimum tillage accentuate germination and seedling growth problems, because low soil temperature and high moisture often prevail at planting time. Seedling tolerance to low temperature is enhanced by rapid germination, high percentage germination, and vigorous seedling growth (Keim et al., 1984).

The plant characteristics that are responsible for differences in early vigor among and within plant species have not been fully characterized (Acevedo et al., 1991; Regan et al., 1992). Some simple characteristics may be important. Evans et al. (1977) reported that wheat seedlings grown from large seeds accumulate more dry matter than seedlings grown from small seeds. More recently, Lafond et al., (1986) reported that seed size and speed of emergence contributed to differences among nine wheat cultivars in seedling vigor measured as shoot dry weight. Seed size accounted for 50 % of the variation in seedling shot dry weight. It was concluded that improvement of seedling vigor could be done by selecting for seed size, speed of emergence and / or rate of plant development. Ram et al., (1991) also reported that seed weight in pigeonpea was significantly correlated with germination percentage, field emergence, seedling dry matter and two indices of

seedling vigor. Kernel weight of sorghum has also been shown to influence the percentage of germination (Abdullahi and Vanderlip, 1972; Maranville and Clegg, 1977), seedling weight (Swanson and Hunter, 1936), and stand stablishment (Abdullahi and Vanderlip, 1972), while other studies have found kernel weight to be poorly related to field establishment (Vanderlip et al., 1973). Selecting sorghum cultivars for rapid and uniform germination under a wide range of temperatures is important for early seedling establishment in the field (Brar, 1994). Rapid germination permits the seminal root system to access wet soil and establish the crop before soil drying (McCovan et al., 1985). Radford and Henzell (1990) recommended screening commercial cultivars of sorghum for germination and seedling elongation at temperatures occuring in seed beds at planting. Genetic variability has been shown to exist for high temperature tolerance for germination and emergence (Wilson et al., 1982). Using protrusion of the radicle as a measure of germination, Thomas and Miller, (1979), and Mann et al. (1985) reported variation in the response of germination to temperature among diverse lines of sorghum.

In tropical maize populations, the rate of cold emergence was positively correlated with seedling vigor as a result of genetic recombination and selection for adaption to their environment (Blum, 1988). The improvement of germination under cold temperatures was attempted in maize by combining tests in both controlled and field environments (McConnell et al., 1979). After four cycles of selection, cold germination at 7.2°C was improved by about 9 % but little improvement was realized in emergence and seedling vigor in the field. In rice, seedling vigor was significantly correlated with cold germination and was common in varieties adapted to high altitude (Jones et al., 1976). Phenotypic

recurrent selection in sorghum under early spring planting resulted in a 15 % increase in cold germination after four cycles (Bacon, 1986). Laboratory experiments have proven to be adequate in estimating germination, and establishing a significant positive correlation between germination in the laboratory and field emergence (Abdullahi et al., 1972, Mendoza-Onofre et al., 1979, and Brar et al., 1994). Sorghum seedlings emerge by elongation of the mesocotyl and coleoptile, the same way as in maize, oats and rice, in contrast with barley and wheat in which the mesocotyl does not elongate (Hosikawa, 1969). Stand establishement of grain sorghum is often a problem in crusted soils which are common in semi-arid climates. Emergence potential in crusted soils was found to be correlated with coleoptile diameter and potential germination (Mason et al., 1994).

There have been few reports on the genetics of seedling vigor. Fresh and dry weight measurements of maize seedlings showed positive çombining ability effects. Additive gene effects were important. Female effects were also significant (Barla-szabo et al., 1990). Ram et al., (1991) estimated broad-sense heritability at 50.5 % for seedling dry weight in pigeonpea *(Cajanus cajan* L.) Millsp. A positive correlation has also been found between the coleoptile length of sorghum and its emergence (Wanjari and Bhoyar, 1980). A narrow-sense heritability of 0.3 1 for coleoptile length was reported in wheat (Nykaza, 1983). Significant additive genetic effects for coleoptile length in durum wheat (*Triticum durum,* Desf.) was also found (Ouassou et al., 1991). Soman et al., (1991) found significant differences among pearl millet genotypes for coleoptile and mesocotyl length, Genetic variation for coleoptile length was found in two pearl millet populations Tift #2 S 1 (Tift2) and Nebraska Early Dwarf Synthetic (M'Ragwa et al., 1995). There

were significant responses to selection in Tift2 population for coleoptile length evaluated in germination towels. Realized heritabilities of TiR2 population for long and short coleoptile length were 0.21 and 0.55. For the Nebraska population realized heritabilities for long and short coleoptile were 0.00 and 0.90, respectively. Wilson et al. (1982) found genetic diversity in seedling length and its response to temperature. They also mentioned the coleoptile of sorghum but not the mesocotyl in discussing the effects of temperature on emergence. However, the coleoptile generally constitutes only a small portion of the total length of a sorghum seedling but the proportion increased at high and low temperatures (Radford et al, 1990). The percentage of seedling length contributed by the coleoptile varied from 6 to 53 % in this study with eight sorghum genotypes at seven constant temperatures (15, 20, 25, 30, 35, 40, 45°C). The optimum temperature for coleoptile elongation was 15 to 20°C. Both coleoptile and mesocotyl elongation were sensitive to temperature, but mesocotyl elongation showed the largest absolute and proportional changes between 15 and 45°C. The presence of a large coleoptile tiller was also found to contribute substantially to early vigor in wheat (Liang and Richard, 1994a). The Mexican dwarf wheat fails to germinate when sown in the field as deep as the indigeneous cultivars in India mainly due to their shorter coleoptile (Swaminathan et al., 1956). Significant differences in sorghum were found in coleoptile length ranging from 1.35 to 4.18 cm (Wanjari et al., 1980).

Crop growth rate or simply growth rate (GR) is defined as the increase of plant material per unit of time, while relative growth rate (RGR) is the rate of dry mass accumulation per unit of existing dry mass. Growth rate (GR) can always be applied

without becoming involved in any assumption about the form of the growth curves; it also has as advantage that meaningful values can be obtained even when it is possible to take only two harvests (Radford, 1967). Lopez-Castaneda et al., (199417) did not find relative growth rate important in explaining the difference in seedling vigor between barley, oats, and Triticale. They concluded that differences in mass established early were maintained until physiological maturity.

Seedling vigor is best assessed by direct measurement of dry weight. Seedling dry weight was highly correlated to leaf area, leaf number and plant height in sorghum (Maiti et al.,1981). However a major limitation in the assessment of dry matter production is the difficulty in detecting clear difference due to sampling errors and the amount of work involved in taking adequate samples. Maiti et al., (1981) used a visual scoring system to evaluate sorghum genotypes for seedling vigor on a scale of 1 (more vîgorous) to 5 (least vigorous) at 7 and 14 days after emergence. Highly significant correlations were found between the visual scores and dry weight and leaf area.

Our studies were prompted by field observations at West Lafayette, Indiana, that Shanqui red sorghum excelled over other lines in its ability to initiate vigorous seedling growth in the spring. The objectives of the study reported in this chapter were to determine if the difference were genetic and to establish the interrelationships among different estimates of seedling vigor in a recombinant inbred population derived from the cross between 'SRN39' and 'Shanqui red'.

MATERIALS AND METHODS

Plant materials

Recombinant inbred (RI) lines were obtained, through the Single Seed Descent method of plant breeding from a single cross between SRN39, an African caudatum genotype, and Shanqui red (SQR), a Chinese Kaoling line. These two parents differ in seedling vigor with Shanqui red being more vigorous. One hundred random F2 plants of this cross were selfed and advanced by Single Seed Descent to the F5 generation. Seeds from these plants were then used to grow F6 progeny rows in Puerto Rico. Several plants within a row were selfed and bulked to produce F7 seeds that were used for a two-year experiment in the field. Selfed F8 seeds obtained in the summer of 1993 were used for the greenhouse and incubator experiments.

Field experiment

An experiment employing a randomized complete block design with five replications was conducted in 1993 and repeated in 1994 at the Purdue University Agronomy Research Center in West Lafayette, Indiana. The experiment included one hundred recombinant inbred lines and five other cultivars as checks. A single row plot, 5 m long and 75 cm between rows, was used. Each plot was drill seeded and hand-thinned to a spacing of six plants per foot.

The entries were visually scored for seedling vigor on a scale of 1 (most vigorous) to 9 (least vigorous). The scores were assigned at seedling stage (2-3 leaves), when the

differences between entries were observed to be large; this stage corresponded to 29 and 15 days after planting respectively for the 1993 and 1994 experiments. The visual scoring system was a relative evaluation based on the range of variation of seedling size in the population under study . The estimates visually integrate emergence percentage, plant height, thickness of leaf canopy and the length and width of individual leaves.

Greenhouse experiment

The experiment was conducted on a sand bench during June-July 1995. The experiment included ninety-nine recombinant inbred lines and the two parents, with Iine number two not included because of inadequate amount of seeds. Additionally data for lines five and six were not included in the analysis because of mixture. A t-andomized complete block design with three replications was employed. A single row plot, 30 cm long and 10 cm between rows was used. Fifty kernels per row were placed at 2 cm depth and watered daily. One week after planting, the number of emerged plants per row was recorded. The first seedling height was also measured at this time, and each plot was thinned to 20 plants. Seedling height was again taken at 2 and 3 weeks after planting. These measurements were taken from the soil surface to the tip of the leaves. Ten seedlings were harvested at 2 and 3 weeks after planting, dried at 120" C for 5 days, and shoot dry weight was recorded.

Three growth rates were calculated from the seedling height and shoot dry weight measurements. The first (GR1) and second (GR2) growth rates were obtained respectively between seedling height 1 and seedling height 2 and between seedling height 2 and

seedling height 3. Similarly growth rate 3 (GR3) were obtained from shoot dry weight 1 and shoot dry weight 2.

Laboratory experiments

Two experiments with two replications each were conducted in an incubator. The temperature in the incubator was 12° C for the first experiment and 22" C for the second, both were at 100 % relative humidity. Fifty seeds of each entry were placed on a filter paper in a 100 x 15 mm (diameter by height) petri dish, and moistened once every two days. Eaçh experiment included one hundred recombinant inbred lines and their two parents. One week after the beginning of each experiment., the seeds were placed at -70° C to stop all physiological processes. The number ofgerminated seeds was subsequently recorded as determined by radicle protrusion through the seed coat, in accordance with the Association of Officia1 Seed Analysts (AOSA, 1970) definition of germination.

Data analysis

The field visual scores were used to determine inheritance and the number of genes controlling seedling vigor in sorghum. At the F7 generation of a line derived through the Single Seed Descent method, nearly all loci are expected to be homozygous. We hypothesized a duplicate or triplicate dominant model, in which the presence of at least one dominant homozygous locus of either gene resulted in a vigorous phenotype. With a duglicate model, three genotypic classes with at least one homozygous dominant locus are

expected, to give a vigorous phenotype and one with both loci homozygous recessive and non-vigorous plants. It would be expected that the 100 recombinant inbred lines population be divided into 75 vigorous and 25 non-vigorous lines. A triplicate mode1 would give seven classes with at least one homozygous dominant locus and one with all loci homozygous recessive. It would then be expected that, the seven classes would result in 87.5 vigorous lines, while the remaining class would consist of 12.5 non-vigorous genotypes. Chi-square analyses were computed to test the goodness-of-fit of the data to the above hypothesized genetic ratios. Heritability estimates were calculated for the different variables measured in the field, greenhouse and laboratory experiments. The mean squares for family [MS(fam)] and for the experimental error [MS(error)] were obtained from an analysis of variance and were used to obtain the genotypic variance. The additive variance was calculated by adjusting for inbreeding using the formula $\sigma^2_A = 2 - (1/2)^{t-1}$ where t is the generation of evaluation, so that at F7, the genotypic variance contains 3 1/16 of the additive variance in the F2 generation. Broad-sense heritability on a family mean basis was calculated as follows:

H_f = MS(among lines)- MS(YxL) / MS(among lines) for field traits.

H_f = MS(among lines)- MS(error) / MS(among lines) for greenhouse and incubator experiments.

The analyses of variance and covariance provided the sum of squares and cross products so that the genetic correlations could be obtained, using the formula;

$rg = (\sigma_{F(xy)}) / (\sigma^2_{F(xx)} . \sigma^2_{F(yy)})^{1/2}$.

Where: rg = the genetic cor-relation between x and y.

$\sigma_{F(xy)}$ = family covariance component between x and y.

$\sigma^2_{F(xx)}$ = family variance component for variable x

$\sigma^2_{F(yy)}$ = family variance component for variable y.

The analysis of covariance between traits measured in the field and controlled environments was calculated assuming that the expected environmental covariance was zero, so that the mean cross product for family MCP (fam.) = $\sigma^2_{F(xx)}$

Standard errors for estimates of genetic cor-relations were calculated according to the procedure given in Mode and Robinson (1959) using the computer program SPHENGE (Santini, Nyquist et al., 1992 at Purdue University). Significance at the a = 0.05 and 0.01 levels for genetic correlations was declared if the coefficient exceeded its standard error by two and three times respectively.

RESULTS AND DISCUSSION

Highly significant differences for seedling visual scores between genotypes were observed (Table 1.1). The performance of the sorghum lines for the visual scores was fairly consistent from year to year. A given line tended to have the same phenotype (vigorous or non-vigorous) in both years, as shown by the high cor-relation between 1993 and 1994 scores (R = 0.64, P = 0.001). Visual scoring has been found to be an effective method for estimating seedling vigor (Maiti et al., 1981). However, they considered the relative nature of the scores to be an obvious limitation of the method that would not allow comparisons between experiments or generations of breeding materials. The highly significant correlation of 1993 and 1994 scores in our study confirm the reliability of the method for evaluating and comparing experiments and therefore contradicts the latter statement by Maiti et al. (198 1).

The differences in seedling vigor between the two parents were clearly reflected in their visual scores. Shanqui Red had a mean score of 1.8 and 1 respectively in 1993 and 1994, while the corresponding scores of SRN39 were of 7.9 and 7 (Table 1.2). Among the recombinant inbreds, lines with a seedling vigor score between 1 and 4.5 were considered vigorous, while those with a rating of 4.6 to 9 were regarded as non-vigorous. Considerable variation in the scores among the different inbred lines were observed. In 1993, the mean scores (average of five observations) among inbred lines ranged from 1.6 to 7.4 (Fig. 1. 1), while in 1994 the mean values were between 1.0 and 9.0 (Fig. 1.2). In both years the frequency distribution was skewed toward the vigorous parent, Shanqui

The significant additive genetic variance observed for the visual scores and for the different measures of seedling vigor in controlled environments, indicate that there is adequate variation to allow improvement for germination under cold temperature, for germination and emergence at normal temperatures, and for seedling growth and development. Cold tolerance is enhanced by rapid germination and high percentage germination. The parental line Shanqui red originated in a temperate environment in China, and we hypothesize that the genes controlling seedling vigor may also be valuable for cold tolerance. The germination percentage of Shanqui red at 12" C was high (87 %) while that of SRN39 was low (24 %). The significant genetic variance and high heritability of percentage of germination at low temperature indicate that Shanqui red can be effrciently used in breeding programs for improving stand establishment of grain sorghum in environments where low soil temperatures prevail at planting time. McConnell et al. (1979) improved cold germination at 7.2" C in maize after four cycles by about 9 %. A 15 % increase in cold germination was obtained after four cycles of phenotypic recurrent selection in sorghum, under early spring planting (Bacon, 1986). The high heritability estimates for germination at 22° C, as well as for emergence and seedling height in our study also indicate that attempts to improve these traits would be successfùl. But progress in breeding for improved seedling dry weight is expected to be slow as suggested by the low to moderate heritability estimate.

Estimates of genetic correlation among different measures of seedling vigor in the greenhouse experiment indicated significant positive relationhips between percent emergence and seedling dry weight at 2 weeks after planting, and between seedling height

and seedling dry weight (Table 1.8). The high genetic cor-relation between seedling height and seedling dry weight indicate that efficient selection for seedling height would improve seedling dry weight. Genetic correlation coefficients between emergence and seedling height and weight were relatively small, suggesting that a simultaneous selection scheme could be used to achieve both high percentage emergence and taller / heavier seedlings. Germination at 12" C was significantly associated only with shoot dry weight I (r_g = .49) and with none of the other estimates of seedling vigor in the greenhouse. Germination at 22"' C was highly significantly correlated with percent emergence (r_g = 1.04) and was also associated with seedling height 1, II and III and shoot dry weight I and II and growth rate 1. No association was observed between germination at 12° C and germination at 22" C (Table 1.9). Significant associations were revealed between the visual field seedling vigor scores and germination at 22" C, as well as with emergence, seedling height, shoot dry weight and growth rate 1 (table 1.10). Germination at 12" C was weakly but significantly related with the seedling visual scores (r_g = -0.12). (The negative sign in the genetic correlations, results from the fact that a score of 1 designated the vigorous phenotypes and and a score 9 represented the non-vigorous lines). There were high genetic cor-relation between visual scores and percent germination at 22° C and and between emergence, similarly significant genetic cor-relation was also found between germination at 22° C and emergence. These results suggested that these two traits are controlled by the same gene(s) in this population. The non significant genetic correlation between percent germination at 12" C and germination at 22° C and emergence suggests however, that germination under cold temperature is controlled by a different set of genes than those

controlling germination and emergence at normal temperatures. These results may explain the non significant association between seedling vigor estimated as visual scores in the field with germination at 12° C. McConnell et al. (1979) made the same observation, and attributed the lack of cor-relation between laboratory cold emergence and field seedling vigor results to the difference in conditions between laboratory and the mild spring during the two years of evaluation. Mock and Eberhart (1972) also found environmental influences to be large when selecting for cold tolerance. They later reported that improved seedling vigor accompanied cold tolerance (Mock et al., 1976). Kernel weight was poorly associated only with percent emergence, and with none of the other estimates of seedling vigor (table 1.9). This was in agreement with previous observations (Vanderlip et al., 1973). Others studies have concluded that kernel weight has little effect on stand establishment (Radford et al., 1990; Mian et al. 1992).

Our results indicate that the visual scoring system used was effective in integrating germination and emergence at hight temperatures, seedling height, shoot dry weight and growth rate 1. This indicates that the visual scoring system can be efficiently used in a breeding program to improve seedling vigor. There is an apparent limitation to the improvement of seedling cold tolerance, due to the dependence on direct selection under the prevailing environmental conditions (McConnell et al., 1979; Mock et al., 1979). This can be overcome with the identification of molecular markers associated with seedling vigor and their use in breeding programs. Molecular markers, unlike phenotype-based assays, are not a function of the environment, which can confound the expression of a genic trait (Stuber, 1992). Sorghum cultivars with a satisfactory level of cold tolerance

could eventually be suitable for early spring planting, when low soil temperatures restrict germination and stand establishment of intolerant genotypes.

Table 1.1. Expected mean squares and analysis of variance for seedling vigor on visual scores combined across years.

Source	DF	Expected mean squares	Mean squares
Year (Y)	y-l = 1	$\sigma_\varepsilon^2 + l\sigma_r^2 + r\sigma_{yl}^2 + rl\sigma_y^2$	726.785294””
Rep. / Y	y (r-l) = 8	$\sigma_\varepsilon^2 + l\sigma_r^2$	9.936274**
Entries (E)	L-l = 101	$\sigma_\varepsilon^2 + r\sigma_{yE}^2 + ry\phi_E$	17.480072””
P vs L	1	$\sigma_\varepsilon^2 + r\sigma_{yl}^2 + ry\phi_{PvsL}$	37.612254””
Between P	P-l = 1	$\sigma_\varepsilon^2 + r\sigma_{yp}^2 + ry\phi p$	180.00000””
Among L	L-l = 99	$\sigma_\varepsilon^2 + r\sigma_{yl}^2 + ry\sigma_L^2$	15.635101""
Y x E	(y-l) (L-l) = 101	$\sigma_\varepsilon^2 + r\phi_{yE}$	3.704106""
Y x PvsL	1	$\sigma_\varepsilon^2 + r\phi_{yPvsL}$	4.0237""
Y x P	(y-l) (P-l) =1	$\sigma_\varepsilon^2 + r\phi_{yP}$	0.0000"
Y x L	L - l = 99	$\sigma_\varepsilon^2 + r\sigma_{yl}^2$	3.3783""
Error	y (r-l) (L-l) = 808	σ_ε^2	0.69

Table 1.2. Performance of parental and recombinant inbred lines for seedling vigor traits.

Traits	Parental		Recombinant inbreds	
	sRN39 mean	SQR mean	Mean	Range
93 scores	7.8	1.8	3.87 ± 0.07	1.6-8.4
94scores	7	1	2.16 ± 0.07	1-9
Emerg. (%)	58	97	69.64 ± 1.65	29-100
Height1 (cm)	9.3	16	13.08 ± 0.16	9.3-17
Height2 (cm)	17.3	30.3	27.73 ± 0.34	17-34
Height3 (cm)	28.3	41.6	37.13 ± 0.39	26-45.6
Weight 1 (g)	244.6	375	339.27 ±15.28	185-443
Weight2 (g)	630.6	1030.6	857.96 ±153.18	493-1182
seed weight (g)	3.475	2.650	2.86 ± 0.04	1.97-4.04
Growth rate 1	0.9	1.8	1.9 ± 0.04	0.9-2.65
Growth rate II	1.35	1.65	1.21 ± 0.03	0.6-2.1
Growth rate III	44.35	71.05	82.44 ± 17.46	23.8-1783
Germ. 12°C (%)	24	87	59.66 ± 2.24	9.5-96
Germ.22°C (%)	43.25	98	74.63 ± 1.24	43.25-98

Table 1.3. Chi-square (χ^2) analyses of seediing vigor scores for two genetic models.

	Expected number of lines		Observed number of lines							
			Vigorous			Non-vigorous				
Genetic mode1	Vigorous	Non-vigorous	1993	1994	mean	1993	1994	mean	χ^2	P
Duplicate dominant	75	25	74			26			0.053	0.9-0.7
Triplicate dominant	87.5	12.5		91			9		1.12	0.5-0.2
					87			13	0.023	0.9-0.7

Table 1.4. Analyses of variance for seedling vigor traits measured in the greenhouse.

		Emergence	Seedling height 1	Seedling height II
Source	DF	MS	M S	M S
Reps (r)	1	0.020202	3.1565	1612.2475
Entries	98	527.610390**	5.9128""	30.8371""
Par. vs Lines	1	227.78726"	0.9597"	77.5595**
Bet. Parents	1	1521.0000””	49.0000**	169.0000""
Among Lines	96	520.385739**	5.5156""	28.9113""
Error	98	63.04061	1.5749	8.1352

		Seedling. height III	Shoot weight 1	Shoot weight II
Source	DF	MS	M S	M S
Reps (r)	1	3033.4595	85147.6566	2602704.0455
Entries	98	37.9591""	7965.9499**	62693.0103**
Par. vs Lines	1	22.2268"	10018.3976"	40271.9870"
Bet. Parents	1	225.0000**	27060.25""	123552.2500**
Among Lines	96	36.1747**	7745.6713””	62292.6122””
Error	98	13.1494	*4805.7382*	33120.9332

Table 1.4 cont. Analyses of variance for seedling vigor traits measured in the greenhouse

Source	DF	Growth rate 1 MS	Growth rate 11 MS	Growth rate III MS
Reps (r)	1	30.0556	4.5454	35639.2820
Entries	98	0.26””	0.2491”	782.3607”
Par. vs Lines	1	1.2503””	0.3418”	206.4852”
Bet. Parents	1	0.7347””	0.0816”	713.6645”
Among Lines	96	0.2441””	0.2491”	782.3607”
Error	98	0.1133	0.2319	530.3407

Table 1.5. Analyses of variance for 100-kernel weight and percent germination at 12 °C and 22" C

		100-kernel weight	germination 12° c	germination 22" C
Source	DF	MS	MS	MS
Reps (r)	1	0.7464	706.4444	18.7929
Entries	98	0.2698""	1066.7518**	335.1590**
Par. vs Lines	1	0.1519**	51.1716"	46.6761"
Bet. Parents	1	0.6806""	3969.0000**	3080.2500"
Among Lines	96	0.2667""	1066.7518**	309.5694""
Error	98	0.2316	353.4240	102.9766

Table 1.6. Genetic, additive and environmental variance estimates of seedling vigor traits.

Trait	σ^2_G	σ^2_A	σ^2_E
Visual scores	1.2256	0.6326	0.6947
% Germination at 12°c	356.6639	184.0846	353.4240
% Germination at 22°c	103.2964	53.3 127	102.7660
Emergence	228.6725	118.0245	63.0406
Seedling height 1	1.9703	1.01169	1.5749
Seedling height 2	10.3880	5.3616	8.1352
Seedling height 3	11.5126	5.9420	13.1494
Shoot dry weight 1	1469.9665	758.6924	4805.7382
Shoot dry weight 2	14585.8395	7528.1552	33 120.9332
1 00-seed weight	0.1218	0.0628	0.023 12
Growth rate 1	0.0654	0.0337	0.1133
Growth rate II	0.0086	0.0044	0.23 19
Growth rate III	126.0100	65.0374	530.3407

Table 1.7. Estimates of broad-sense heritability for seedling vigor traits of F7 recombinant inbred Family means.

	Heritability	Environment
Visual scores	0.7839	Field
100-seed weight	.9121	Field
% Emergence	0.8774	Greenhouse
Seedling height 1	0.7144	Greenhouse
Seedling height 2	0.6089	Greenhouse
Seedling height 3	0.3670	Greenhouse
Seedling dry weight 1	0.3979	Greenhouse
Seedling dry weight 2	0.45780	Greenhouse
Growth rate 1	.5274	Greenhouse
Growth rate II	.0796	Greenhouse
Growth rate III	.3219	Greenhouse
% germination at 12" c	0.6565	Incubator
% germination at 22" c	0.6604	Incubator

Table 1.8. Genetic correlation coefficients and their standard errors (in parenthesis) in SRN39 x SQR recombinant inbred population.

Traits	Height 1	Height 2	Height 3	weight 1	weight 2
Emergence	0.1711 (0.1266)	0.1649 (0.1269)	0.1457 (0.1388)	0.4995* (0.1744)	0.1606 (0.1606)
Height 1	-	1.0144** (0.0332)	0.9868** (0.0646)	0.8797** (0.1666)	0.8171** (0.1248)
Height 2	-	-	0.9809** (0.0545)	0.8769** (0.1444)	0.7668 **(0.1237)
Height 3	-	-	-	0.8967 ** (0.1613)	0.7778 * (0.0950)
Weight 1	-	-	-	-	1.1255** (.3488)
GR I	-	-	-	0.9052**(.1772)	0.7399** (.1697)
GR II	-	-	-	0.2108 (.7600)	.1539 (.6068)
GR III	.8195** (.1992)	.7446** (.1998)	.7519** (.1440)	-	-

Table 1.9. Genetic correlation coefficients and their standard errors (in parenthesis) of seedling vigor traits measured in the greenhouse and incubator.

Traits	Germination 12" C	Germination 22" C	100-kernel weight
Emergence	.0544 (. 1358)	1.0376** (.0696)	-.2407* (.1084;)
Seedling height I	.0939 (. 1558)	.3689** (. 1481)	.2019 (. 1195)
Seedling height II	.0452 (.1543)	.3067* (. 1465)	1904 (. 1196)
Seedling height III	.1532 (.1683)	.2289* (. 1636)	.0722 (. 1375)
Shoot dry weight I	.4992* (.2411)	.8210** (2193)	.3525 (.1782)
Shoot dry weight II	.2286 (.2008)	.3872* (.1890)	.2526 (. 1575)
Growth rate I	.0079 (.1856)	.2738* (. 1774)	-.9150 (1.0727)
Growth rate II	.5345(.7878)	-.3314(.6556)	-.9150(1.0727)
Growth rate III	.1080 (.2442)	-. 1939 (.2403)	.2144 (. 1937)
Germination 12" c		.2641 (. 1591)	-.1620 (.1303)
Germination 22" c			-. 1166 (-1302)

Table 1.10. Genetic correlation coefficients and standard errors (in parenthesis) of seedling visual scores, and traits measured in greenhouse and incubator.

Traits	Scores
1 00-seed weight	.1078 (.1222)
Emergence	-.5595** (.0956)
Seedling height 1	-.3530** (.1330)
Seedling height II	-.4398** (.1294)
Seedling height III	-.5380** (.1424)
Shoot dry weight 1	-.6717** (.2047)
Shoot dry weight II	-.4082* (. 1826)
Growth rate I	-.5220** (. 1588)
Growth rate II	-.5328 (.7752)
Growth rate III	.2676 (.23 19)
Germination at 12” c	-1224 (. 1490)
Germination at 22” c	-.6003** (. 1140)

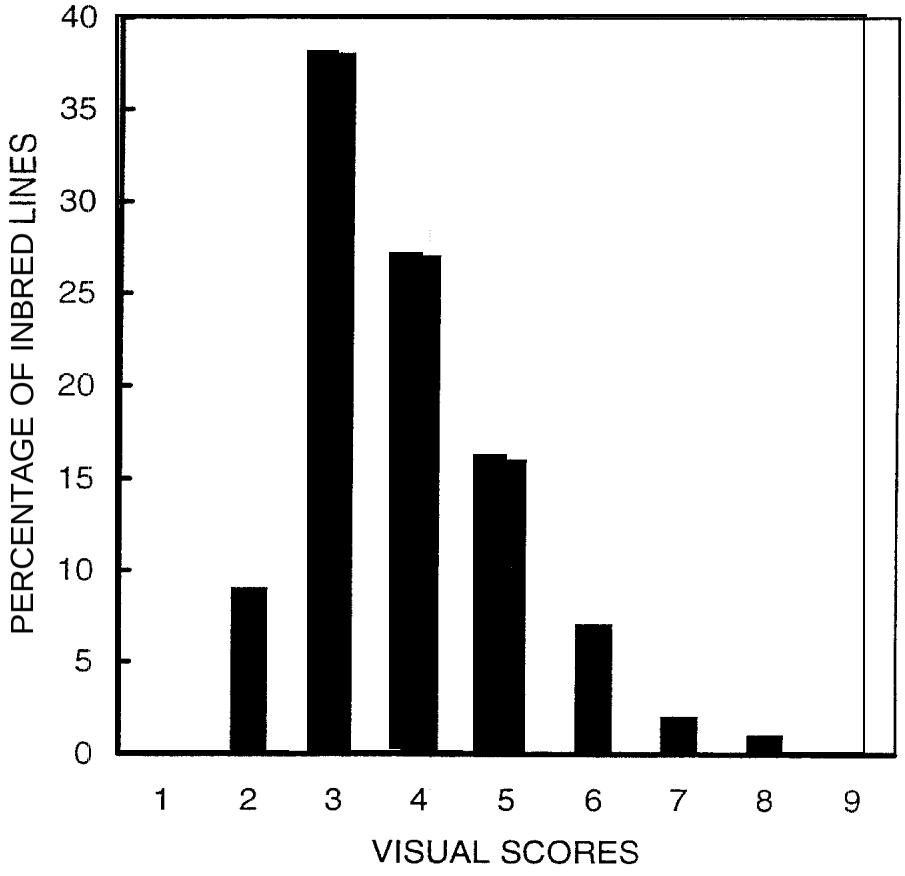

Figure 1.1. 1993 inbred line distribution for seedling vigor scores.

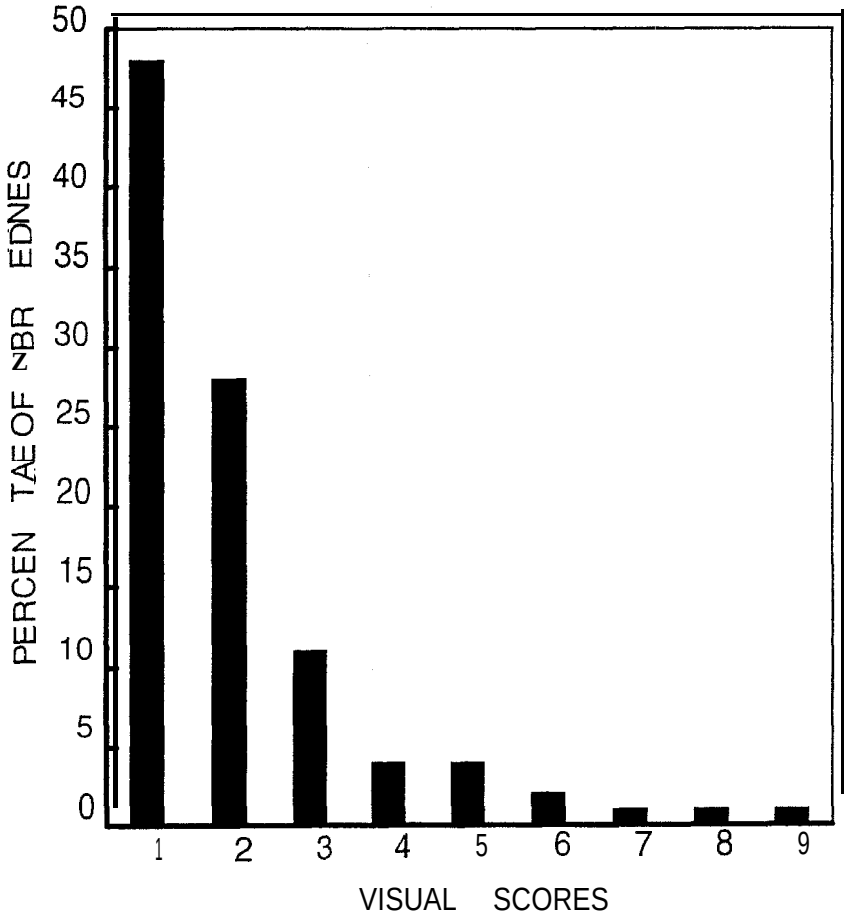

Figure 1.2. 1994 inbred line distribution for seedling vigor scores.

CHAPTER TWO

RELATIONSHIP OF SEEDLING VIGOR ESTIMATES WITH AGRONOMIC TRAITS IN SORGHUM

ABSTRACT

Crop yield is a function of plant density, the efficiency of dry matter production, and the proportion of the biomass that is economic yield. Germination and emergence can influence plant population density. However the direct effects of seedling vigor traits on subsequent plant performance are more difficult to discern. The objective of this study was to evaluate the genetic relationships of seedling vigor traits with traits in later stages of plant development in sorghum (*Sorghum bicolor* L. Moench). Field and controlled-environment experiments were conducted to obtain estimates of seedling vigor parameters, grain yield, plant height, and maturity in a recombinant inbred population. Germination at 22" C, emergence, seedling vigor scores and the rate of dry matter accumulation at seedling stage were significantly correlated with grain yield. However no association was found between kernel weight, shoot height and grain yield. The association of early maturity with high percent germination and emergence, shoot growth (height and weight) and the rate of dry matter accumulation was believed to result from tight linkage in the parental line 'Shanqui red'. It was concluded that this cultivar could be a valuable parent for the development of early maturing varieties, with improved stand establishment, and adapted to environments where low ternperatures prevail at planting in spring and early frost in the fall.

INTRODUCTION

Seedling vigor can be measured by many variables including kernel weight, germination, emergence and seedling growth (shoot height, dry weight and growth rate). Many studies emphasized the relationship of laboratory germination and vigor to field emergence. There is less published information relating seedling vigor to other aspects of crop performance (Tekrony et al., 1991). However there are possibilities that seedling vigor can influence crop yield (Ellis, 1992). Differences in germination and emergence can influence plant population density. Since crop yield is a function of density (Willey et al., 1969), it follows that seedling vigor can influence crop yield through germination and emergence. If seed quality (size, percent germination) only affected percent emergence, then growers could, theoretically, overcome such effects by adjusting seed sowing rate. It has been shown that the effect of differences in laboratory germination on field emergence in different seedbeds can be quantified (Khah et al., 1986; Wheeler et al., 1992). However, in practice adjustments in seed sowing rates are hampered by difficulties in forecasting the particular seedbed environment (Ellis, 1992).

Seedling vigor also affects crop performance through effects on the plant growth processes involved in the production of yield. Yield of any crop is determined by the insolation intercepted by the plant community, the efficiency with which intercepted insolation is converted to dry matter, and the proportion of the biomass that is economic yield (Charles-Edwards, 1982). Most of the plant tissues involved in the production of dry matter and yield are formed after seedling emergence, and it seems unlikely that seed

quality (weight, percent germination) would influence their ability to carry out physiological processes and accumulate dry matter. Seed quality did not affect the relative growth rate of soybean seedlings, provided they were free of physiological injury or necrotic lesions (Egli et al., 1990). Priming or natural variation in seed quality have been reported to have no effect on the relative growth rate of onions (*Allium cepa* L.; Ellis, 1989). However genetic aberrations which may occur in long term storage could impair physiological function in later formed plant tissue (Roberts, 1972; Harrison, 1966).

Seedling vigor may have a direct effect on the ability of the plant to accumulate dry matter. The direct effects of seedling vigor on yield greatly depend upon the crop species. Crops harvested during vegetative growth are fi-equently planted at low population densities and harvested on an individual plant or area basis as vegetative mass of aboveground [Lettuce (*Lactuca sativa* L.), cabbage (*Brassica oleracea* var *capitata)]* or underground [sugarbeet *(Beta vulgaris* L.), radish (*Rafanus sativus* L., carrot (*Daucus carota* L.)] structures. The effects of seedling vigor can be specially critical in these crops, where delayed emergence or missing plants may reduce yield and uniformity at harvest (Tekrony et al., 1991).

Crops harvested at an early stage of reproductive development usually are planted at higher population densities than crops harvested during vegetative growth. The quantity (fresh weight), uniformity, and quality of reproductive structures determine the yield. Perry (1969) evaluated high and low-vigor seed lots of two pea cultivars and reported significantly lower yield for low-vigor seed lots thinned to similar plant population as high-vigor lots. Abdalla and Roberts (1969) stored pea seeds in various

artificial environments, and reported that seed lots with lower viability had lower early plant growth rates (O-35 days after planting) and reduced plant height and leaf number at 45 and 59 days after planting. At later stages of growth, no significant differences in relative growth rate were recorded, and reductions in dry seed yield occurred only for those seedlots where viability had declined below 50%. Unfortunately, measurements of fresh seed and fruit weight at an early stage of reproductive development were not taken. A non significant relationship between germination and fresh pod weight was reported in lima beans (*Phaseolus linensis* L.) (Bennett and Waters, 1984).

Grain crops are harvested after they have completed their life cycle (full reproductive maturity) and only the seeds are harvested for yield. Work with soybean (Tekrony et al., 1987), bean (Spilde, 1987), and corn (Abegbuyi et al., 1989) suggests that there is no relationship between germination, seedling dry weight and seedling growth rate with yield. However significant increases in yield with seedling growth rate were shown for corn (Burris, 1975). Significant increases in yield with high percent germination of seed were also shown for corn when grown at low population densities compared with seed that had been stored for 5 to 7 years (Funk et al., 1962). It was also demonstrated that seedling growth rate of spring barley showed an advantage only at lower plant density, while no association existed at normal population density (Perry, 1980). It was found that low percent-germination seeds of spring wheat produced lower yields only in lower than normal populations or later than normal plantings. Yield of soybean was reported to be related to accelerated aging, cold test, seedling and seedling growth rate in hill plots but not in row plots planted at normal populations. In sorghum a significant

relationship between grain yield and speed of germination, root and shoot growth was reported (Camargo et al., 1973); however the plant density (66, 000 plants / ha) was lower than normally recommended (Vanderlip, 1972). No significant association was found between kernel weight and days to 50 % bloom, plant height and grain yield in sorghum (Suh et al., 1974). It was also reported that kernel weight had no influence on grain yield of sorghum (Maranville et al., 1977).

From these studies, it was concluded that a yield response to seedling vigor traits occurs only when plant densities are lower than the density required to maximize yield. However no report is available in sorghum on the genetic correlation of seedling vigor variables with the performance of the crop. The objective of this study was to evaluate the genetic relationships of seedling vigor traits with yield, plant height and maturity at high plant density recommended for commercial production.

MATERIALS AND METHODS

Recombinant inbred (RI) lines of a cross between 'SRN39' and 'Shanqui red' (SQR) were obtained through the Single Seed Descent method of plant breeding. These two parents differ in seedling vigor with Shanqui red being the more vigorous. An experiment employing a randomized complete block design with five replications was conducted in 1993 and repeated in 1994 at the Purdue University Agronomy Research Center in West Lafayette, Indiana. The experimental materials and designs are the same as described in chapter one. The entries were visually scored for seedling vigor on a scale of 1 (most vigorous) to 9 (least vigorous). The number of days from planting until anthers had extruded half way down the panicles of at least 50 % of plants in a row was used as an estimate of maturity. Plant height was measured centimeters from the soil surface to the top of the panicles after maturity. Grain yield (kg / ha) was estimated from the center 3 m of a single row plot in 1993, and in 1.994 from the entire 5 m of a single row plot. Grain samples taken from replications 1 and III at harvest in 1994 were used to estimate 100-seed weight. Because of the high heritability of grain size (0.91) this seed-weight value was also used as an estimate of kernel size at planting.

The laboratory experiments conducted in an incubator during February-March and the greenhouse experiment conducted on a sand bench during June-July 1995 had the same entries, design and procedures for data collections as described in chapter I.

Heritability estimates were calculated for the visual scores, grain yield, plant height and maturity. These estimates were also calculated for the different variables measured in

the greenhouse and laboratory experiments. The mean squares for family [MS(fam)] and for the experimental error [MS(error)] were obtained from an analysis of variance and were used to obtain the genotypic variance. The additive variance was calculated by adjusting for inbreeding using the formula $\sigma^2_A = 2 - (1/2)^{t-1}$ where t is the generation of evaluation, so that at F7, the genotypic variance contains 3 1/16 of the additive variance in the F2 generation. Broad-sense heritability on a family mean basis was calculated as follows:

H_f = MS(among lines)- 1/2 MS(YxL) / MS(among lines) for traits measured in the field.

Hf = MS(among lines)- MS(error) / MS(among lines) for greenhouse and incubator experiments.

The analyses of variance and covariance provided the sum of squares and cross products so that the genetic cor-relations could be obtained, using the formula;

$rg = (\sigma_{F(xy)}) / (\sigma^2_{F(xx)} . \sigma^2_{F(yy)})^{1/2}$.

Where: rg = the genetic correlation between x and y.

$\sigma_{F(xy)}$ = family covariance component between x and y.

$\sigma^2_{F(xx)}$ = family variance component for variable x.

$\sigma^2_{F(yy)}$ = family variance component for variable y.

The analysis of covariance between traits measured in the field and controlled environments was calculated assuming that the expected environmental covariance was zero, so that the mean cross product for family MCP (fam..) = $\sigma^2_{F(xx)}$

Standard errors for estimates of genetic correlations were calculated according to the procedure given in Mode and Robinson (1959) using the computer program

SPHENGE (Santini, Nyquist et al., 1992 at Purdue University). Significance at the a = 0.05 and 0.01 levels for genetic correlations was declared if the coefficient exceeded its standard error by two and three times respectively.

RESULTS AND DISCUSSION

Significant differences were found with all the traits (visual scores, 100-seed weight, plant height, maturity and grain yield) measured in the field (Table 2.1). The parental line Shanqui red had higher yield, and was earlier and taller than SRN39 (Table 2.2). The recombinant inbred population tended to have intermediary values for these traits except for 1994 yield when it was lower.

Seedling vigor scores were highly correlated with grain yield and plant height, but the association with days to maturity was not significant (Table 2.3). Acevedo et al. (199 1) also found a highly significant cor-relation coefficient between seedling vigor scores and grain yield in barley. It was also reported in maize that improved seedling vigor at low temperatures was accompanied by lower plant height (Mock and Bakri, 1976).

Kernel weight was not associated with yield or maturity. This is in agreement with observations made by Suh et al., (1974); Vanderlip et al. (1973); Maranville et al. (1977). The significant association of kernel size with plant height is also in agreement with a previous finding (Ibrahim et al., 1985), but is contrary to another report in sorghum (Suh et al., 1974).

Germination at 22" C and emergence were significantly associated with yield (Table 2.4). Such associations can arise because yield is a function of plant density (Ellis, 1992); and because percent germination and emergence can influence plant population. This result is contrary to earlier reports that percent germination and emergence have no effect on yield at high density in sorghum (Vanderlip, 1972) and other crops (Tekrony et

al., 199 1). Percent germination at 22" C and emergence were also significantly associated with plant height, and negatively correlated with maturity. These genetic correlations may result fi-om the associations of these traits in the parental line, 'Shanqui red', which has higher percent germination and emergence, and is taller and earlier than SRN39. The single cross between the parental lines, followed by selfing, may not have allowed sufficient breakage of linkage blocks (Hanson, 1959).

No genetic association was revealed between germination at 12" C with yield, plant height and maturity. It was also reported that separate genetic systems control cold tolerance and maturity in corn, allowing selection within adapted material without mating to unadapted and early types (Mock and Eberhart, 1972). However following selection for germination at 14° C in sorghum, an increase in grain yield of 198 kg / ha was observed, plant height showed an irregular response, and days to half bloom decreased by 0.55 days per cycle of phenotypic recurrent selection (Bac:on et al., 1986). Selection in corn for phenotypes which germinated and grew at temperatures below 10" C, gave lines which flowered in 60 days (Brown, '1968). Mock and Bakri (1976) found that improved seedling vigor , lower plant height, lower harvest moisture and early flowering accompanied increased cold tolerance.

Shoot growth (height and dry weight) was not associated with yield and plant height, in agreement with observations made in corn (Adegbuyi et al., 1989), and would support the statement that in situations where vegetatitve growth is adequate to maximize yield, it is unlikely that the effect of seedling vigor on vegetative growth will carry over to affect yield (Tekrony et al., 1991). Camargo et al., (1973) obtained different results and

reported a significant relationship between sorghum yield and shoot grooth, but in lower than normally recommended plant density. In small grain cereals, differences in mass established early were maintained until physiological maturity and contributed to the better performance of barley compared to oats and triticale (Lopez-Castaneda et al., 1994b). Shoot growth (height and dry weight) was also not associated with plant height, but a negative genetic cor-relation was observed with maturity (Table 2.4).

Growth rates 1 and II (rate of height increase) were not associated with yield, plant height and maturity. The rate of shoot dry matter accumulation (growth rate III) was significantly correlated with yield and maturity. Conflicting results have been reported with different crops. Burris (1975) reported a positive association between seedling growth rate and grain yield in corn, while no correlation was found between these two traits in another study in corn (Tekrony et al., 1989) and in bean (Spidle, 1987). The result of our study suggest, then, that the rate of shoot dry matter accumulation is related to grain yield at high plant density in sorghum. Growth rate III was also negatively associated with maturity, as were shoot growth (height and weight), germination and emergence. Findings by Allard (1988) state that natural selection favors the development of multilocus clusters conferring adaptation. Linkage between seedling vigor traits and early maturity in Shanqui red would favor adaptation in its original environment (North China) where low temperatures at planting and early frost are likely.

The significant genotypic and additive variance of germination, emergence, shoot growth and maturity (Table 2.5) indicate that Shanqui red could be a valuable source material for the development of early maturing varieties with improved stand

establishrnent, and adapted to environments where low temperature prevails at planting in spring and early frost in the fall. The high heritability estimates (Table 2.6) of maturity and seedling vigor traits suggest that such a breeding objective is feasible. However, separate genetic systems control maturity and grain yield, as indicated by a nonsignificant genetic correlation (Table 2.3). This makes it a unique population, because yield and maturity are correlated in most others (Ibrahim et al., 1985; Bacon et al., 1986)

Table 2.1. Expected mean squares and analysis of variance for seedling vigor visual scores combined across years.

Source	DF	Expected mean squares	Mean squares
Year (Y)	y-l = 1	$\sigma_\varepsilon^2 + l\sigma_r^2 + r\sigma_{yl}^2 + rl\sigma_y^2$	726.785294**
Rep. / Y	y (r-l) = 8	$\sigma_\varepsilon^2 + l\sigma_r^2$	9.936274””
Entries (E)	L-l = 101	$\sigma_\varepsilon^2 + r\sigma_{yE}^2 + ry\phi_E$	17.480072””
Par. vs L	1	$\sigma_\varepsilon^2 + r\sigma_{yl}^2 + ry\phi_{PvsL}$	37.612254””
Bet. Par.	P-l = 1	$\sigma_\varepsilon^2 + r\sigma_{yp}^2 + ry\phi p$	180.00000””
Among L	L-l = 99	$\sigma_\varepsilon^2 + r\sigma_{yl}^2 + ry\sigma_L^2$	15.635101””
Y x E	(y-l) (L-l) = 101	$\sigma_\varepsilon^2 + r\phi_{yE}$	3.704106””
Y x PvsL	1	$\sigma_\varepsilon^2 + r\phi_{yPvsL}$	4.0237””
Y x P	(y-l) (P-l) =1	$\sigma_\varepsilon^2 + r\phi_{yP}$	~0.0000”
Y x L	L - l = 99	$\sigma_\varepsilon^2 + r\sigma_{yl}^2$	3.3783””
Error	y (r-l) (L-l) = 808	σ_ε^2	0.69

Table 2.1 Cont. Expected mean squares and analysis of variance combined across years for grain yield, plant height, maturity and 1 00-kernel weight

Source	DF	M S	M S	M S	M S
		Grain yield	Plant height	Maturity	1 00-kernel w
Year (Y)	1	169167170.26	301.9853	33185.4157	
Rep. / Y	8	3907293.53	793.6544	42.0603	0.7564
Entries (E)	101	7469531.480””	9958.7045**	123.2014**	0.2698**
P vs L	1	237.466321”	224.803””	1.8722””	0.8326””
Between P	1	24557712.20”	5746.0500””	884.4500**	0.0000”
Among L	9 9	7132508.44””	10099.578””	116.7376””	0.2667
Y x E	101	3753431.980””	279.3912**	12.7642	
Y x PvsL	1	3446876.2	46.2607””	0.4833”	
Y x P	1	10512500.00””	42.0500””	6.0500””	
Y x L	99	3688255.090**	284.1435**	12.9561**	
Error	808	1897631.80	102.25	4.1663	0.0234

Table 2.2. Field performance of parental and recombinant inbred lines.

Traits	Parental		RI lines	
	sRN39 mean	SQR mean	mean	Range
93 visual scores	7.8	1.8	3.87 ± 0.07	1.6 - 8.4
94 visual scores	7	1	2.16 ± 0.07	1 - 9
93 yield (kg/ha)	3002	6668	4170 ± 107.34	1825 -6668
94 yield (kg/ha)	6088	6854	4975 ± 98.45	2619-7323
93 maturity (d)	93	78	85.32 ± 0.40	78-94
94 maturity (d)	80	68	73.83 ± 0.33	67-84
93 height (cm)	160	196	182.63 ± 3.08	107-246
94 height (cm)	165	196	183.86% 3.37	107-254

Table 2.3. Genetic correlation coefficients and their standard errors (in parenthesis) Among field performance traits.

Traits	Yield	Height	Maturity	1 00-kernel wght
Vigor	-0.8165** (0.173)	-0.2268** (0.113)	0.0322 (0.124)	0.1078 (0.122)
Yield		0.5461** (0.141)	0.2325 (0.161)	-0.1735 (0.165)
Height			-0.0954 (0.107)	0.3539** (095)
Maturity				0.1577 (0.109)

Table 2.4. Genetic correlation coefficients and their standard errors (in parenthesis) of seedling vigor traits and field performance.

Trait	Yield	Plant height	Maturity
Vigor scores	-0.8165** (0.173 1)	-0.2268** (0.1130)	0.0322 (0.1248)
1 00-seed weight	-0.1735 (0.1658)	0.3539** (0.0952)	0.1577 (0.1097)
Emergence	0.5126** (0.1595)	0.1279 (0.1081)	-0.2822* (0.1075)
Shoot height 1	0.0523 (0.1953)	0.1154 (0.1214)	(-0.4392)** (0.1134)
Shoot height II	0.0023 (0.1948)	0.0948 (0.1212)	-0.3741** (0.1158)
Shoot height III	0.0448 (0.2166)	0.1106 (0.1317)	-0.4541** (0.1248)
Shoot. dry weight 1	0.1010 (0.2905)	0.2437 (0.1707)	-0.6131** (0.1755)
Shoot dry weight II	0.1947 (0.2569)	0.1507 (0.1524)	-0.5716** (0.1491)
Growth rate 1	-0.0561 (0.2215)	-0.0641 (0.6079)	0.2476 (0.3059)
Growth rate II	0.0985 (0.1357)	0.0972 (0.3598)	0.1242 (0.1799)
Growth rate III	-0.3594** (.1330)	-0.4526 (0.5759)	-0.591 o* (0.1994)
Germination 12” C	-0.1565 (0.2098)	0.0047 (0.1272)	-0.1780 (0.1334)
Germination 22” C	0.6718** (0.1990)	0.2194* (0.1217)	-0.2728** (0.1255)

Table 2.5. Genotypicc, additive and environmental variance estimates of seedling vigor and other field performance traits.

Trait	σ^2_G	σ^2_A	σ^2_E
-Grain yield	344425.335	177767.9148	189763 1.8
Plant height	981.5434	506.60307	102.25
Maturity	10.3781	5.3564	4.16
Visual scores	1.2256	0.6326	.6947
Emergence	228.6725	118.0245	63.0406
Seedling height 1	1.9703	1.0169	1.5749
Seedling height 2	10.3880	5.3616	8.1352
Seedling height 3	11.5126	5.9420	13.1454
Shoot dry weight 1	1465.9665	758.6924	4805.7382
Shoot dry weight 2	14585.8395	7528.1552	33 120.9332
1 00-kernel weight	.1218	.0628	.0216
Growth rate 1	.0654	.0337	.1133
Growth rate II	.0086	.0044	2319
Growth rate III	126.01	65.0374	530.3407
% Germination at 12°C	356.6639	184.0846	353.4240
% Germination at 22°C	103.2964	53.3 127	102.766

Table 2.6. Broad-sense heritability estimates of seedling vigor and field performance traits.

Traits	Heritability	Test environment
Visual scores	0.7839	Field
100-kernel weight	.9121	Field
% Emergence	0.8774	Greenhouse
Seedling height 1	0.7086	Greenhouse
Seedling height 2	0.7144	Greenhouse
Seedling height 3	0.6089	Greenhouse
Seedling dry weight 1	0.3670	Greenhouse
Seedling dry weight 2	0.4578	Greenhouse
Growth rate 1	.5274	Greenhouse
Growth rate II	.0796	Greenhouse
Growth rate III	.3219	Greenhouse
% germination at 12" C	0.6565	Incubator
% germination at 22° C	0.6673	Incubator
Height	0.9754	Field
Maturity	0.8890	Field
Yield	0.4829	Field

CHAPTER THREE

EFFECT OF PHENOLIC CONCENTRATIONS IN *SORGHUM* KERNELS ON SEEDLING VIGOR AND PLANT PRODUCTIVITY

ABSTRACT

Sorghum (*Sorghum bicolor* L. Moench.) plants produce large amounts of phenolic compounds which constitue a defense mechanism against fùngi , insects, and birds, but also have antinutritional effects.The role of phenols on seedling vigor and stand establishment are not well understood. One hundred recombinant inbred (RI) lines derived from two parental lines that differ for an array of phenolic compounds were evaluated. Highly significant genotypic and additive components of variance, and high broad-sense heritability estimates were found for pigments, flavan-4-ols, tannin, and total phenols content of the grain. High phenolic compound contents wet-e associated with vigorous seedlings, high percent germination at 22" C, emergence, and taller seedlings. Kernel weight was negatively associated with concentrations of these compounds. Only total phenols were significantly associated with grain yield. Lines with a red coleoptile, wbich had significantly higher pigment and total phenol contents, tended to be more vigorous at seedling stages than lines with a green coleoptile. No association of coleoptile color with plant height and maturity were observed. However, lines with a red coleoptile tended to be more productive. Because tannin content did not significantly contribute to total phenols (non significant genetic correlation), it was suggested that there are possibilities to maintain the positive association of total phenols with seedling vigor, and reduce at the same time reduce the antinutritional effects of tannin in grain sorghum.

INTRODUCTION

Sorghum plants produce large amounts and a great diversity of phenolic compounds (Butler, 1989). Many of these phenols determine plant color, appearance, nutritional quality, and host defenses. Polyphenols are secondary metabolites, their amount and nature vary greatly with genotypes and environmental conditions under which plants are grown. Sorghum phenolic compounds can be divided into five basic groups; phenolic acids, lignins, quinones, flavonoids and tannin (Butler, 1989). Phenolic acids, flavonoids and tannins are the most common groups in sorghum. Chemically, phenolic acids are the simplest polyphenols of sorghum. Eight different phenolic acids were identified in sorghum grains (Hahn et al., 1983). Ferulic acid was the most abundant.

Anthocyanidins are the major pigments in most plants. In sorghum the dominant pigments are the 3-deoxyanthocyanidins. The color of sorghum grains is influenced by pericarp color, mesocarp thickness, presence of testa, and by endosperm texture and color (Hahn and Rooney, 1986). The pericarp color is determined by two genes (Kambal et al., 1976) and can be white, lemon-yellow or red. Kambal et al. (1976) did not find any visible pigments in white-seeded grain but considerable amounts ofp-coumaric, caffeic and ferulic acids were detected. The pigment in the yellow grain was identified as eriodictyol chalcone, a deep yellow pigment. The red seeds contained the 3-deoxyanthocyanidins, luteolinidin and apigeninidin. Doggett (1988) classified sorghum seedlings in two groups, red and green. Red coleoptile color is controlled by a single dominant gene over green.

Flavan-4-ols also called leucoanthocyanidins since they are converted to anthocyanidins when heated in acid with the loss of a water molecule (Watterson and Butler, 1983), include monomers of flavanols such as flavan-3,4 diols and flavan-4-ols. The concentration of flavan-4-ols in sorghum seeds is highly dependent on seed maturity (Jambunathan et al., 1990). Grain at early stages of maturity (10 and 14 days after flowering) contained the highest flavan-4-ol concentrations, followed by a drastic decrease with increased maturity. It was suggested that flavan-4-ols could be degraded, converted or incorporated into other molecules such as 3-deoxyanthocyanidins or tannins.

Tannins are a group of phenolic compounds found in plants, which convert animal hides to leather during the tanning process (Butler, 1989). There are two classes of tannins: hydrolysable and condensed tannins. Only condensed tannins, which are oligomers of flavan-3-ols, have been found in sorghum. These oligomers are now referred as procyanidins, because the red anthocyanidin pigment cyanidin is released when the tannin is treated with minera1 acids. Tannins are the most abundant phenolic compounds extractable from the seed of brown, bird-resistant sorghum (Hahn et al., 1984). Tannins bind to and precipitate proteins, reducing the nutritional value of the grain. High tannin sorghums have different kernel structures from other sorghums (Hahn et al., 1984). High tannin sorghums have a prominent pigmented testa located beneath the pericarp. The pigmented testa is purple or reddish-brown and varies in thickness. The presence of a pigmented testa is controlled by the complementary B1 and B2 genes. The S gene controls the presence of pigments and tannins in the epicarp. When S is dominant, more phenols and tannins are present in the pericarp.

It was reported (Chavan et al., 1981) that the percent germination in sorghum cultivars with high (3.4 %) and low (0.5 %) tannin content was the same. However both root and shoot growth were markedly suppressed in high tannin as compared to low tannin seedlings. The rates of germination were also the same, but the subsequent rates of root and and shoot growth were much lower in high tannin seeds. The assayable tannin content decreased markedly during germination. Tannins are located in the seed coat of the sorghum grain (Jumbunathan et al., 1973). The loss of tannin was attributed to leaching in growth medium and penetration into the endosperm with imbibed water during germination. Starch content decreased, and the rate of formation and total accumulation ofreducing sugars and free amino acids was lower in high tannin seeds. The interpretation was that starch and protein degradation were inhibited in high tannin seeds during germination, leading to suppressed seedling growth. This inhibition would result fi-om the portion that enters the endosperm. Such tannins are likely to form complexes with seed protein reserves and enzymes, and inactivate them (Chavan et al., 198 1).

During germination, the reserves of nutrients like starch and proteins are degraded to soluble sugars and amino acids, respectively, to meet the seedling growth requirements (Dalvi, 1974). Any depression of starch and protein degradations would indicate interference with the metabolic systems operating on reserve starch and protein, mainly enzymes like amylases and proteases. Tannins are reported to form complexes with hydolytic enzymes and inactivate them (Tamir et al., 1969; Milic et al., 1972). A marked suppression of seedling root growth was also observed with a low tannin (0.1 %) sorghum cultivar, germinated in the presence 1 %, 2 % and 3 % tannic acid concentrations. The

inhibition increased with concentration and time (Chukwura et al., 1982). A decrease in starch content in the control sample (distilled water) and 1 % tannic acid solution, but not at higher concentrations was also noted. A concommitant increase of soluble carbohydrate content at low concentration of tannic acid, and in distilled water , and a decrease at higher concentrations were also observed. The fact that with high concentrations of tannic acid the soluble carbohydrate falls after germination below its original level was viewed as an indication that these substances were being utilized, and that tannic acids directly inhibited their production. Alpha and beta-amylase activity were also observed to be inhibited by an increase in the concentration of tannic acid. It was concluded that tannins present in sorghum seeds retard seedling growth due to inhibition of starch degradation by inactivating hydrolytic enzymes during germination.

In this study we evaluated a recombinant inbred population of sorghum that is segregating for an array of biochemical and agronomic traits to investigate possible associations. The specific objectives of this study were; i) to evaluate the effects of different phenolic compounds including tannins on seedling vigor and on field performance; ii) to assess the relationship between the different phenolic compounds and to estimate their heritability.

MATERIALS AND METHODS

Recombinant inbred (RI) lines of a cross between 'SRN39' and ' Shanqui red' (SQR) were obtained through the Single Seed Descent method of plant breeding. These two parents differ in seedling vigor with Shanqui red being the most vigorous. An ex:periment employing a randomized complete block design with five replications was conducted in 1993 and repeated in 1994 at the Purdue University Agronomy Research Center in West Lafayette, Indiana. The experimental materials and designs are the same as described in chapter one. The entries were visually scored for seedling vigor on a scale of 1 (most vigorous) to 9 (least vigorous). The number of days from planting until when anthers had extruded half way down the heads of at least SO % of plants in a row was used as an estimate of maturity. Plant height was measured as centimeters from the soil surface to the top of the panicle after maturity. Grain yield (kg / ha) was estimated fi-om center 3 m of a plot in 1993, and in 1994 from entire 5 m plot. Grain samples taken from replications 1 and III at harvest in 1994 were used to estimate 100-seed weight. Because of the high heritability of grain size (0.91) this 100-kernel weight value was also used as an estimate of kernel size at planting.

Tbe concentrations of the different phenolic compounds were determined from the grain samples used to estimate 100-seed weight. The procedures used were as follow:

The seeds were ground in a Cyclotec 1093 sample mill (Hoganas, Sweden). The assays were carried out within 2-3 days after grinding. A sample size of 250 mg was used for all analyses. Methanol containing 0.5 % HCL (15 ml) was utilized as extractant for 20

min on a Labquake mixer (LabIndustries, Berkeley, Ca.). After centrifigution, the supernatant was kept on ice until used for analyses. For pigment content, the absorbance of the 0.5 % HCL / methanol extract was determined at 485 nm. For flavan-4-ols determination a 0.5 ml aliquot of the 0.5 % HCL in methanol extraction was added to 7 ml of 30 % HCL in buthanol, incubated at room temperature for 2 hours, and the absorbance was read at 550 nm. After the flavan-4-ols reading, the mixture was heated in a boiling water bath for two hours. The solution was then cooled for 5 min, and the absorbance was read at 550 nm to determine the proanthocyanidin concentrations. The blank for flavan-4-ols and proanthocyanidins was prepared by mixing 0.5ml of 0.5 % HCL in methanol extract with 7 ml of a mixture of methanol, 0.1 N acetic acid, and n-buthanol (v/v/v/, 15; 15; 70). After two hours, the absorbance was recorded at 550 nm. Flavan-4-ol and tannin concentrations were determined after correcting for the blanks. All the above absorbances were read with a spectronic 20 d spectrophotometer (Milton Roy, Rochester, NY). For total phenols determination a 0.2 ml aliquot of the acidic methanol extract was diluted with 50 ml of distilled water and 3 ml of 0.05 M FeCL3 in 0.1 N HCL was added after 3 min, 3 ml of 0.008 M $K_3Fe(CN)_6$ was added and the solution was incubated again for 19 min. The absorbance of the solution was read at 720 nm with a Klett Summerson photoelectric colorimeter (Klett Mfg Co. Inc. NY). Total phenols are reported as Klett units; the other components are reported as absorbance units, all at the wavelengths noted above.

The laboratory experiments conducted in an incubator during February-March and the greenhouse experiment conducted on a sand bench during June-July 1995

had both the same entries, design and procedures for data collections as described in chapter I.

The statistical procedures used to estimate variance components, heritability, and genetic correlations between phenolic compound concentrations with seedling vigor traits and field performance, were the same as described in chapters 1 and II.

RESULTS AND DISCUSSION

Highly significant differences were observed for pigments, flavan-4-ols, tannin and total phenol concentrations (Table 3.1). Shanqui red had much higher concentrations of these compounds than SRN39. Few transgressive segregants with concentrations higher than those of Shanqui red were found, but no inbred had a level lower than those of SRN39 (Table 3.2).

The genotypic and additive components of variance for the phenolic compounds were significant, and their broad-sense heritabilities were high (Table 3.3) and in agreement with findings by Weerasuriya (1995). Woodruff et al., (1982) reported a moderate to high broad-sense heritability for tannin quantity in sorghum, Ma et al., (1978) also found a high broad-sense heritability for tannin content in common bean.

Significant and positive genetic correlations were found between pigments, flavan-4-ols, tannin and total phenols (Table 3.4). McMillan et., (1972) also found a highly significant and positive correlation between seed color and tannin content. It was reported that flavan-4ols are converted to anthocyanidins when heated in acid with the loss of a water molecule (Watterson et al., 1983). It was also suggested that flavan-4-ols could be degraded, converted or incorporated into other molecules such as tannins (Jambunathan et al., 1990). These facts may explain the above positive genetic correlations. It was noted that dark red or brown colored pericarps with pigmented testa were generally higher in phenolic compounds content than lighter colored varieties of sorghum (Wall et al., 1970).

Seedling vigor was significantly associated with high tannin concentration. Percent germination at 22" C and germination, seedling height and its rate of increase were also highly correlated with tannin concentration (Table 3.5). These results are in disagreement with findings by Chavan et al., (198 1); and Chukwura et al., (1982), who observed significant reduction in root and shoot growth with increased level of tannin. It was suggested that tannins present in sorghum seeds retard seedling growth due to inhibition of starch degradation, by inactivating the hydrolitic enzymes during germination. It was also reported that extracts from high tannin sericea lespedeza [*Lespedeza cuneata* (Dum. cours) G. Don.] residues decreased rye (*Secale cereale* L.) seed germination whereas extracts from low-tannin had no effect. Low-tannin residue extract reduced rye coleoptile length, and a more dramatic reduction was observed with high tannin extract. These results were explained by the reduction of cell elongation or division due to the effect of tannin (Kalburtji et al., 1993). With two ***Vicia*** *faba* pairs of near-isogenic lines, it was observed that high tannin-containing genotypes had significantly taller seedling than genotypes without tannin (Pascual et al., 1990). In their study, Chavan et al., (1981) used two unrelated lines with high or low tannin content, while in our present study we have a better control of the genetic background, as progenies of a single cross of two lines differing in tannin concentration were used. These conflicting findings may then result from difference in control of the genetic backgrounds.

Seedling vigor scores, germination at 22" C and emergence were significantly associated with pigments, flavan-4-ols and total phenol content. Seedling height up to 2 weeks after planting and growth rate 1 (rate of seedling height increase between 7 and 14

days after planting) were significantly correlated with flavan-4-ols and total phenols (Table 3.5). Phenolic compounds have been shown to counteract the inhibitory effect of abcissic acid (ABA) on *Amaranthus caudatus* seedling growth. Compounds with a flavan nucleus showed a higher activity at low concentration (Ray et al., 1980). Tt was latter shown that ABA inhibits wheat germination and seedling growth by blocking amylase activity, thus çhecking the availability of mobilizable carbohydrates essential for these processes (Sharma et al., 1986). By counteracting the inhibitory effect of ABA, phenoiic compounds would then increase the availability of mobilizable carbohydrates for germination and seedling growth. However high concentrations of phenolic compounds were shown to reduce radish *(Raphanus sativus* L.) seed germination and corn shoot growth (Storm 1982). These experiments demonstrating the inhibitory effects of phenolic çompounds on germination and seedling growth were in vitro studies. If natural inhibitors are isolated and applied exogenously, they could reach metabolic centers other than those which they attack in the native state (Kefeli et al., 1971). Thus exogeneous application of phenolic compounds on sorghum germination and seedlings growth medium as mentioned by Chukwura et (1982) may not reflect the effect of the endogeneous compounds to the seeds.

Kernel weight was negatively associated with pigments, flavan-4-ols, tannin and total phenols. These correlations may result from association of these traits in the parental line Shanqui red which has smaller seeds and higher concentrations of these products than SRN39. Only total phenols were significantly correlated with grain yield , while plant height was not associated with these phenolic compounds (Table 3.6). It was reported in

chickpea (*Cicer arietinum)* that brown, high tannin lines outyielded white, low tannin lines. The yield advantage of high tannin lines were attributed to a better stand establishment resulting from resistance to pathogenic fungi at germination and seedling stage (Knights et al., 1989). Attack of vegetative tissue by pathogenic fungi results in an increase in the total phenol content of the tissue (Mishra et al., 1980). Infection of sorghum mesocotyls by *Hilminthosporium maydis* and *Colletotrichum graminicola* resulted in rapid accumulation of the deoxyanthocyanidins, apigenidin and luteolinidin (Nicholson et al., 1987). Because these compounds were found to be fungitoxic and were formed only in response to inoculation, they were considered to be phytoalexins and their synthesis a defense response.

Red coleoptile lines tended to be more vigorous than lines with a green one, as reflected 'by the significant differences in seedling vigor scores, percent germination at 22" c, emergence and seedling height (Table 3.7). Lines with a red coleoptile also had significantly higher pigment and total phenol content in their seeds than green coleoptile lines (Table 3.8). Doggett (1988) has classified sorghum seedlings in two groups, red and green. Red coleoptile color in seedlings is controlled by a single gene with the red Rs_1 dominant to green rs_1. This study observed 66 red and 33 green coleoptile lines in, with χ^2 = 10.9, where $\chi^2_{.01}$= 6.63, showing a, discrepancy of the data with the hypothesized genetic ratio of 50 % red and 50 % green lines. Flavan-4-ols and tannin content were not different between the two groups. No significant differences also existed in plant height and maturity; however red coleoptile lines yielded significantly higher in 1993, and higher but not significantly SO in 1994 (Table 3.8).

Highly significant genetic and additive variances and high heritability estimates were found for pigments, flavan-4-ols, tannin and total phenols content in this sorghum population. High levels of phenolic compounds in the grain were associated with vigorous seedlings, high percent germination at 22" C, emergence, and taller seedlings. Tannins have been shown to have antinutritional effects; decreased weight gain in poultry and rats have been demonstrated (Price et al., 1980). The non significant association of total phenols with tannin content indicate that there are possibilities to balance the concentration of these compounds with high seedling vigor through genetic improvement.

Table 3.1. Expected mean squares, and analyses of variance of phenolic traits.

Sources	DF	M S	M S	M S	M S
		Pigments	Flavan-4-ols	Tannin	Total phenols
Reps	1	0.001033	0.0000703	0.003421	0.853535
Entries	98	0.023657””	0.0087693	0.013449””	195.536690””
P vs L	1	0.006952”	0.000265”	0.003642””	28.266270**
Retween P	1	0.160000””	0.035156””	0.038809””	676.000000**
Among L	96	0.02241105	0.008558**	0.013288””	192.274162**
Error	98	0.001398	0.000092	0.001636	1.537209

Table 3 .2. Phenolic compounds content of parental and recombinant inbred lines.

	Parental		RI lines	
Trait	sRN39 mean	SQR mean	mean	Range
Pigments	0.0185	0.4185	0.176 ±0.0267	0.001 U-O.4645
Flavan-4-01s	0	0.1875	0.068 ±0.0067	0.000 - 0.3215
Tannins	0	0.197	0.068 ho.0289	0.000 - 0.3355
phenols	4	3 0	14.314 ±0.8857	0.000 - 47.500

Table 3.4. Genetic correlation coefficients and theirs standard errors (in parenthesis) between phenolic compounds.

Trait	Pigments	Flavan-4-ols	Tannins	Total phenols
Pigments		0.4655”” (.0829)	0.4218”” (0929)	0.5092”” (.0785)
Flavan-4-ols			0.6154** (.0705)	0.5326* (.0730)
Tannins				0.9215”” (.0248)

Table 3.5. Genetic correlation coefficients and theirs standard errors (in parenthesis) of phenolic compounds and seedling vigor traits.

Traits	Pigments	Flavan-4-ols	Tannins	Total phenols
Visual scores	-.283* (. 1095)	-.22* (. 1095)	-.241* (.116)	-.331** (.102)
1 00-seed weight	-.401** (.093)	-.212* (. 1008)	-. 198 (. 108)	-. 143 (.103)
Emergence	,288" (. 1017)	.2189* (1025)	.286* (. 106)	,262" (. 100)
Shoot height 1	.1851 (.1186)	.356** (.1058)	.252* (. 121)	.253* (.111)
Shoot height II	.1394 (.1195)	.3054* (. 1086)	.290* (. 119)	.309** (. 108)
Shoot height III	.2004 (. 1276)	.243 (.1214)	.179 (.134)	.235 (. 122)
Shoot weight 1	.1579 (.1662)	.2678 (.1582)	.285 (. 172)	.446* (. 154)
Shoot weight II	.2137 (.1489)	.1625 (.145)	.156 (.157)	.239 (. 143)
Growth rate 1	.1138 (.1385)	.2798* (. 1284)	.329* (. 138)	.361* (. 124)
Growth rate II	.2849 (.4453)	-.2556 (.4168)	-.505 (.613)	-.337 (.465)
Growth rate III	.2492 (.1812)	.1168 (.1755)	.0967 (. 192)	"144 (.175)
Germin. 12° C	.0344 (. 1272)	-.0877 (. 1225)	-.314* (. 124)	-. 147 (. 121)
Germin. 22" C	.327** (.1152)	.3524** (. 1099)	.463** (.112)	,463"" (.102)

Table 3.6. Genetic correlation coefficients and theirs standard errors (in parenthesis) of phenolic compounds and field performance traits.

Traits	Grain yield	Plant height	Maturity
Pigments	.2161 (.1505)	-.0723(. 1042)	-.3657** (.0951)
Flavan-4-ols	.1723(. 1476)	-.0863 (.1013)	-.2719** (099)
Tannins	.3001(. 1602)	-.0086 (. 1085)	-.2215* (.1081)
Total phenols	.3148" (. 1432)	.0120 (.1019)	-.1609 (.1031)

Table 3.7. T test analyses and standard errors (in parenthesis) for coleoptile color differences in seedling vigor traits.

Traits	Green coleoptile	Red coleoptile	Prob > T
93 scores	4.37 (0.2576)	3.61 (0.1268)	.0096**
94 scores	2.88 (0.3638)	1.77 (. 1194)	.0052**
93-94 mean scores	3.63 (0.2907)	2.69 (.1046)	.0037**
Emergence	64.91 (2.7260)	72.27 (2.03 10)	.03*
Shoot height 1	12.69 (0.3 15 1)	13.26 (0.1957)	.11^{ns}
Shoot height II	26.67 (0.7033)	28.16 (0.3853)	.04*
Shoot height III	35.92 (0.7259)	37.68 (0.4542)	.03*
Shoot dry weight 1	324.69 (12.1698)	345.79 (6.2026)	.1235^{ns}
Shoot dry weight II	841.1 (29.5984)	865.7 (17.7916)	.45^{ns}
Germination 12” C	59.33 (4.2283)	59.69 (2.6723)	.94^{ns}
Germination 22” C	69.83 (2.296)	76.95 (1.4746)	.0076**

Table 3.8. T test analyses and standard errors (in parenthesis) for coleoptile color differences in phenolic compounds and field performances.

Tsaits	Green coleoptile	Red coleoptile	Prob > T
Pigments	.135 (.0191)	.199 (.0123)	.0045**
Flavan-4-ols	.06 (.0104)	.07 (.0074)	.24 ns
Tannin	0.49 (.0104)	.08 (.0111)	.0566 ns
Total phenols	11.57 (1.3404)	15.83 (1.3072)	.02*
93 grain yield	3757.15 (179.9963)	4399.27 (131.0333)	.0047**
93 plant height	178.41 (6.4478)	184.64 (3.2865)	.38 ns
93 maturity	85.47 (0.8059)	85.25 (0.4677)	.80 ns
94 grain yield	4822.94 (158.5308)	5096.85 (126.4629)	.19 ns
94 plant height	180.65 (6.5453)	185.39 (3.8195)	0.50"
94 maturity	74.29 (0.7207)	73.64 (0.3545)	.415 ns

CHAPTER FOUR

IDENTIFICATION OF QUANTITATIVE TRAIT LOCI ASSOCIATED WITH SEEDLING VIGOR AND CORRELATED CHARACTERS IN A RECOMBINANT INBRED POPULATION OF SORGHUM

ABSTRACT

Important advantages have been attributed to seedling vigor, resulting in greater biomass and grain yield in cold and dry environments. Quantitative trait loci (QTLs) for estimates of seedling vigor in sorghum (*Sorghum bicolor* L. Moench.) have been identified using a recombinant inbred population of a cross between SRN39 x Shanqui red. QTL analysis was carried out using the regression approach. RAPD markers located on linkage groups D and F were found signiflcantly associated with seedling vigor scores in the two years of test, explaining the high heritability estimate of this trait. Germination at low temperature (12" C) and germination-emergence at optimum temperatures were mostly under different genetic control, having only one marker in common, which explained 5 and 6% of their variation. Markers explaining most of the variation for germination and emergence at optimum temperatures are on linkage group F. Markers on linkage group C explained 75-80 % of the variation for seedling height. However, only one marker (UBC 178) on linkage group D, with effect on seedling scores, is associated with seedling height. All the markers with effect on shoot dry weight were associated with seedling height, explaining their relatively high genetic correlation. The visual scores and shoot dry weight had no marker in common. The visual scoring system used to estimate seedling vigor was then effective in integrating germination, emergence, and seedling height. Two linkage blocks (D, F) accounted for the differences in seedling vigor in this population. The high heritability estimate (0.97) of adult plant height between 1993 and 1994 is underlined by their associated markers which are similar. The high heritability estimate of days to

maturity could not be explained by common markers in the 2 years. Four markers were significantly associated with grain yield in both years. Their contribution to the variation of yield was reduced by 50 % between 1993 and 1994; this reduction may explain the moderate heritability (0.49) of grain yield. The identification of markers associated with seedling vigor and field performance should make breeding for the improvement of these traits more efficient, by minimizing the amount of the genotype by environment interaction effect

INTRODUCTION

Until recently, polymorphisms have been detected with phenotypic assays of genotypes (Dudley, 1993). However genetic analysis based on phenotype is a function of the heritability of the trait where factors such as the environment and quantitative inheritance often confound the expression of a genic trait. Newly developed DNA markers have advantages in that they do not have these interactions observed in phenotype-based assays. Various statistical procedures are employed to correlate quantitative trait loci (QTL) that control the expression of the trait in question. The simplest approach for detecting QTL is to analyse the data using one marker at a time. This simple point analysis does not require a complete molecular linkage map. Lander and Botstein (1989) proposed the method of interval mapping, to take the fùllest advantage of linkage maps for quantitative studies. By using linked marker analysis, it is possible to compensate for recombination between the markers and the QTL. However when the marker density is high (markers < 15 cM apart) point and interval analysis give nearly identical results (Tanksley, 1993). Because maximum likelihood estimates reduces to least squares estimate when data are normally distributed (Snedecor and Cochran, 1989), Paterson et al. (1988) and Bubeck et al. (1993) showed that both analytical methods gave virtually the same results in detecting QTL. Recently, Pereira et al. (1995) identified, with interval mapping and single-factor analysis of variance, the same unlinked genomic regions in four different linkage groups with significant effects on sorghum plant height. Interval mapping placed the most likely QTL position (likelihood peak) in linkage group

A, closer to the RFLP marker ISU1 16. In concordance, single factor analysis ofvariance indicated ISU116 had the strongest association ($P < 0.001$) with plant height in the same linkage group. Similar relationships could be observed for three other linkage groups containing plant height QTLs. Similar results were obtained when PROC GLM procedure of SAS and interval analysis of MAPMAKER were employed for the identification of QTLs associated with rice blast resistance (Wang et al., 1994). Haley and Knott (1992), Martinez and Curnow (1994), Wright and Movers (1994) used interval mapping by the regression approach and obtain similar results as Lander and Botstein (1989) did with the log-likelihood analysis. However the efficiency of flanking-marker methods decreases as the number of incompletely-genotyped individuals increases. For this reason Kearsey and Hyne (1994) proposed a simple "marker-regression" approach. This method produced estimates of QTL locations and effects comparable to inter-val mapping-approaches based on log-likelihood or multiple regression. Lark et al., (1994) identified QTLs associated with maturity and seed oil / protein in a recombinant inbred population of soybean with the regression analysis, which were the same as those obtained with the distributional extremes method .

Important advantages have been attributed to seedling vigor, resulting in greater biomass and grain yield in cold and dry environments (Ludlow et al., 1990). McConnell et al. (1979) attributed the lack of cor-relation between laboratory and field results for cold emergence and seedling vigor to the mild spring weather during the two years of evaluation. This lack of cor-relation between laboratory and field results is not surprising Plant breeders routinely find that genotypes which perform well in one environment are

not as well suited to another environment. Highly significant interactions of years by genotypes have been observed for seedling vigor visual scores, grain yield, plant height and days to maturity as shown in chapters 1 and II of this study. To minimize the amount of this genotype by environment interaction effect in future efforts to improve these traits, we have as the objective of this study the identification of quantitative trait loci associated with seedling vigor, plant height, maturity and grain yield.

MATERIALS AND METHODS

Recombinant inbred (RI) lines of a cross between 'SRN39' and 'Shanqui red' (SQR) were obtained through the Single Seed Descent method of plant breeding. These two parents differ in seedling vigor with Shanqui red being the most vigarous. An experiment employing a randomized complete block design with five replications of 100 RI lines was conducted in 1993 and repeated in 1994 at the Purdue University Agronomy Research Center in West Lafayette, Indiana. The experimental materials and designs are the same as described in chapter one. The entries were visually scored for seedling vigor on a scale of 1 (most vigorous) to 9 (least vigorous). The number of days from planting until anthers had extruded half way down the panicles of at least 50 % of plants in a row was used as an estimate of maturity. Plant height was measured as centimeters from the soil surface to the top of the head after maturity. Grain yield (kg / ha) was estimated from center 3 m of a plot in 1993, and in 1994 from entire 5 m plot.

The laboratory and the greenhouse experiments conducted, respectively, in an incubator during February-March, and on a sand bench during June-July 1995. Both had the same entries, design and procedures for data collections as described in chapter 1.

DNA Preparation

DNA was isolated from Shanqui red, SRN39 and 93 of their recombinant inbred line progenies. A modification of the CTAB isolation protocol (Bernatsky and Tanksley, 1986) was used. Fifty seeds were germinated at room temperature for five days in moist

paper towel. Coleoptiles of approximately 3 cm in length from 10-20 seeds of each line were harvested and placed in sterilized 1.5 ml Eppendorf tube. These samples were frozen in liquid nitrogen and crushed without thawing, with a metal rod cooled in liquid nitrogen. 0.8 ml CTAB buffer was added to each tube and the tissue samples were homogenized in the buffer solution using an Eppendorf pellet pestle. Samples were incubated at 55° C for 1 hour an.d then mixed with 0.3 ml phenol / chloroform / isoamyl alcohol (25:24: 1). The supernatant was recovered by centrifugation and the DNA precipitated using 0.750 ml of ice cold isopropanol. The DNA pellet was recovered by centrifugation and washed twice with 70 % ethanol, allowed to air dry, and resuspended in 0.5 ml TE. Aliquots of each DNA samples were run on an agarose gel with known amounts of undigested λ DNA and stained with ethidium bromide. Comparison with the λ DNA standards allowed sorghum DNAs concentration to be estimated. Working stock solutions were then prepared at a concentration of 5 µg / ml.

RAPD Reactions

Amplification reactions were performed in 25 µl volumes containing Taq buffer (50 mM KCl, 10mM Tris-HC1 at pH 8.8, 1.5 mM MgCl, 0.1 % Triton x 100), 0.2 mM dNTPs, 0.2 mM primer, one unit of taq DNA polymerase,, and 25ng of sorghum genomic DNA. The reaction mixtures were overlayed with one drop of minera1 oil. Amplification were performed in an Ericomp Twin Block System Easy Cycler, programmed for 45 cycles of two minutes denaturation at 94" c, one minute annealing at 36° c and two minutes elongation at 72" c. Amplification products were analysed by electrophoresis

through 1.6 % agarose gels for 2.5 hours at 130 volts in TAC buffer (Tris / sodium acetate / EDTA). Gels were stained in 1 μg / ml ethidium bromide solutions for 2-4 hours. A total of 72 primers previously showing polymorphisms between the two parental lines (Weerasuriya, 1995) were tested. RAPD loci were named OPX#, or UBC#, where OP and UBC are the primer from Operon and the University of British Columbia, and X and # are the primer kit letter and number respectively. When more than one band was scored for a particular primer, a letter was added at the end of the primer number to identify each locus.

Data Analysis

The recombinant inbred lines were scored for RAPD bands that were polymorphic between the two parents. Shanqui red and SRN39 were scored as 0 and 1 respectively. RI lines with the Shanqui red or SRN39 allele of a polymorphic RAPD amplification were scored alternatively as 0 or 1.

An Apple Macintosh version of MAPMAKER II was used for linkage analysis. Linkage groups were constructed with a LOD score > 8.0. A combination of Two point / Three point and Multipoint / Try commands was used to order the markers within linkage group with a LOD score > 3. The Kosambi mapping function (Kosambi, 1944) was used to convert recombination fractions to map distances.

Simple regression analysis was used to identify quantitative trait loci associated with seedling vigor, yield, plant height, and maturity.

RESULTS AND DISCUSSION

Fifty one of the 72 primers tested were polymorphic yielding 89 RAPD markers. Seventy two markers were included in 11 linkage groups, while the map location of 59 of these have been determined, and 17 markers remained unlinked (Fig. 4.1). The estimated map size was 925.81 cM, with an average interval between adjacent loci of 19.7 cM. The maximum distance between any two adjacent markers in this map was 42.9 cM on linkage group A. Although the locus order and relative genetic distances vary, most of the linkage groups identified could be integrated to those previously established in another RAPD map (Weerasuriya, 1995). Tentative correspondences can be made between the present linkage groups; D, B and H, C, A, E, 1 respectively with the groups D, P, H, K, Q, G of the previous map based on marker identification.

Seedling Traits

Several plant characteristics have been associated with seedling vigor . These include percent germination, percent emergence, seedling height, and dry weight. Visual scoring has been found to be a convenient and efficient method for estimating seedling vigor in a breeding program where large numbers of genotypes are evaluated. The relationships between visual scores and different plant characteristics that estimate seedling vigor have traditionally been studied with cor-relation techniques (Maiti et al., 198 1). Recently developed molecular techniques (RAPDs and RFLPs) offer new possibilities for the genetic study of seedling vigor and methods of estimating the

phenotype. Regression analysis in this study identified 9 RAPD markers associated with seedling vigor scores in 1993. These are located in two linkage groups; D and F which respectively account for 41.21 and 25.78 % of the phenotypic variation (Fig. 4.2). The same markers were again found significantly associated with vigor scores in 1994 except only one (OPL9v) in linkage group F. Linkage group D accounted for most of the variation for seedling vigor with 73.23 % of the total (Table 4.1). This similarity of markers controlling seedling vigor scores between 1993 and 1994 explain the high heritability estimate (0.78) of this trait.

Four markers were identified as being significantly associated with germination at 22° c. The marker OPA16 was the most strongly associated with this trait at the 0.000 1 probability level, and accounted for ‘18.93 % of the variation for germination at normal temperature. However this marker was not included in any linkage group in this study, but was mapped previously in the 1 group (Weerasuriya, 1995). Markers on linkage groups A (OPJ1x) and F (OPD16) accounted for 6.07 and 7.56 % of the variation. A second unlinked marker (OPC7y) was also associated with germination (Table 4.2).

Percent emergence was associated with the unlinked marker OPA16, and with OPD16 and OPL9v on the linkage group F (Fig. 4.3). The latter two accounted for 16.24 % of the variation for emergence. These three markers with a fourth on linkage group A (OPJ1x) explained 32.35 % of the variation for percent emergence of sorghum seedlings (Table 4.2). Thus percent germination at 22” c and emergence had most of their associated markers in common, explainig their highly significant genetic correlation.

Four markers on linkage group D were significantly associated with germination at low temperature (12° c), and accounted for 28.73 % of the variation. Two additional markers, OPA19 and OPJ1x on linkage groups C and A respectively were also associated with this trait. These 6 markers explained 38.98 % of the variation for germination at low temperatures. Germination at low temperatures and germination-emergence at normal temperatures, had thus only one marker (OPJlx) in common with effect on both of them, on linkage group A and explaining 5 and 6 % of their variation. This means that germination at low and at normal temperatures are mostly under different genetic control, explaining the poor selection results obtained, in studies for the improvement of germination at low temperatures when the environmental conditions in the field are not appropriate (McConnell et al., 1979). A breeding program for improved germination at low and normal temperatures should involve a shuttle testing under the two conditions. The marker OPD16 on linkage group F mostly associated with seedling vigor scores ($P < 0.0001$) was also found as having significant effect on germination and emergence at normal temperatures. A second marker on linkage group F, OPL9v, was also associated with seedling vigor scores and percent emergence. Two markers on linkage group **D** (OPG10v and OPI1x) with effect on seedling scores, had influences on germination at low temperatures.

Markers on linkage group C (Fig. 4.4), significantly associated with seedling height, explained most of the variation (74.91, 79.3, and 39.46 % respectively after 1, 2, 3 weeks after planting). Additional important linkages groups are A, E and D (Table 4.3). An unlinked locus (OPK1 8) was also significantly associated with seedling height. This

marker together with the present group C were previously mapped in linkage group H (Weerasuriya, 1995). Only one of the marker (UBC 178) on linkage group D, with effect on. seedling scores, is associated with seedling height. The significant genetic correlation between germination and emergence at normal temperatures with seedling height and dry weight could not be explained with any common marker. Although the genetic correlation between germination at low temperatures and seedling height was not significant, the markers OPA19 and OPG10v were associated with both traits.

Seedling dry weight 2 weeks after planting was significantly associated with five markers on linkage group C (Fig. 4.4). These markers accounted for 32.33 % of the variation for this trait. In addition, two unlinked markers; OPJ6v and OPK18 previously mapped on linkage group F and H, were also associated with seedling dry weight and accounted for 6.1 and 5.49 % of the variation respectively. Only OPJ6v and OPK17 on linkage group C were associated with seedling dry weight 3 weeks after planting and accounted for 5.05 and 5.71 % of the variation (Table 4.4). All the markers with effect on seedling dry weight were then assoçiated with seedling height explaining their relatively high genetic correlation. It can then be stated again that efficient selection for seedling height would improve seedling dry weight. Progress in breeding for improved seedling dry weiglt is expected to be slow as suggested by a low to moderate heritability estimate. There was no marker in common between seedling dry weight and the visual scores.

The visual scoring system used to estimate seedling vigor was then effective in integrating germination, emergence, and seedling height. This is consistent with the conclusion that seedling-visual scores are controlled by 2-3 genes. It appears now more

appropiate to state that two linkage blocks account for the differences in seedling-vigor scores in this population. The markers associated with germination, emergence and seedling height explained about 25 % of the variation of the scores. However, the scores did not account for the differences in seedling dry weight among lines. Most of the markers on linkage group D, with effect on the visual scores, were not associated with any of the seedling traits measured.

Plant characteristics which have been associated with seedling vigor (Lopez-Castaneda et al., 1995) and with possible relevance for the visual scores include, rate of germination, coleoptile length, and rate of leaf production. Perhaps these traits might explain the additional variation in seedling scores.

Adult Plant Traits

Most of the variation for plant height (Table 4.5) in each year could be explained by markers on linkage group H (90.4 and 88.78 % respectively). Markers on linkage groups A, B and G were also significantly associated with plant height (Fig. 4.5). Seedling height one week after planting, had one marker in common with adult plant height. This marker (OPE1) accounted for about 5 % of the variation in the two traits, explaining their low and non-significant genetic correlation. However the high heritability estimate (0.9754) of adult plant height is underlined by their associated RAPD markers which are similar. Three of these, (OPC20x; OPE1 and OPL3v) have been previously found with effect on plant height, through interval-mapping and maximum-likelihood method (Weerasuriya, 1995). These findings also suggest that the two methods of analysis give

similar results. The marker (UBC122) previously providing the fourth linked marker was not tested in this study. Pereira et al (1995) using RFLP, identified four unlinked genomic regions with significant effects on sorghum plant height. A standardization of methodology may help to establish if these four unlinked genomic regions are the same as the ones identified in the present study.

In the 1993 data, five markers on Iinkage group D explained 32.52 % of the variation for days to maturity (Fig 4.5). Two additional markers had an effect on this trait in both '1993 and 1994. These are OPL3x on linkage group H with 5.58 and 5.02 %, and OPE1 8v with 7.8 1 and 5.76 % of the variation of days to maturity in 1993, and 1994 respectively (Table 4.6). Although the heritability estimate of days to maturity was relatively high (0.8890) the linkage group D with no effect on the second year accounted for most of the variation (32.52 %) in the first one. However, they shared two associated markers, accounting for approximately 1 0- 12 % of their variation.

Grain yield was significantly associated with four markers in linkage group H, which accounted for 5 1.53 % of the variation in 1993 (Fig 4.5). Two additional markers; OPA1 6 and OPJ6v explained 8.2, and 9.71 % respectively of the variation for yield (Table 4.7). Two of these markers on linkage group H were again significantly associated with grain yield in 1994, but they explained much less of the variation (11.92 %). Markers OPD16 on linkage group F and the unlinked OPJ6v were also associated with grain yield in both years and accounted for 11.32, 5.20 and 9.71in 1993 and 1994, and 6.65 % in 1993 and 1994 respectively. Marker OPE14v on linkage group A was most closely associated with grain yield in the second year (10% of variation; $p < 0.0025$). Markers

OPC7v; OPC20x; OPD16 and OPDJ6v were significantly associated with grain yield in both years. Their contribution to the variation of yield was reduced by 50 % from 1993 to1994 i.e., in 1994 compare to 1993 from 51.53 % to 23.77 %. This reduction may explain the moderate heritability estimate of grain yield (0.4975). Days to maturity and grain yield of 1993 had one marker (OPL3x) in common which accounted for about 5-9 % of their variation , explaining the non-significant genetic correlation. The marker OPL3x was also an important contributor (23 %) to the variation of plant height; but the genetic correlation of this character and days to maturity was negative and non significant. This situation could be explained by the fact that the tall parent (Shanqui red) was also earlier.

Markers on linkage group C were found significantly associated with flavan-4-ols, pigment, tannin, and total phenols (Table 4.8). These common markers may explain the significant genetic correlations between the phenolic compounds. Three markers, OPA 19, OPB16x, and OPK17 accounted for 19.95, 26.51, 30.77 % respectivly of flavan-4-ols, tannin, and total phenol variation. Markers OPC7x and OPE8y on linkage group A and B, and mark.er OPK17 were associated with pigment content,. Flavan-4-ols, tannin, and total phenols have the same linked markers on group C as seedling height, explaining their significant genetic correlations (Fig 4.6). Although these markers were also associated with seedling dry weight, only total phenols were significantly correlated with this trait. The significant correlation of germination at 22" C and the phenolic compounds could not be explained by common markers. But the marker OPA19 which is important for phenolics was also associated with germination at 12" C.

The basis of heritability estimates and genetic cor-relation coefficients among traits have been explained with the identification of DNA markers. Markers associated with seedling vigor have been identified. This should make breeding for improved germination and enhanced seedling growth at low and normal temperatures more efficient, by minimizing the amount of the genotype by environment interaction effect on the expression of genotypes.

Table 4.1. Genetic markers that significantly cosegregate with seedling vigor scores in (SRN39 x SQR) recombinant inbred population.

	1993 scores		1994 scores		
Marker	Prob > F	R-square	Prob > F	R-square	linkage group
OPA2	0.0156	0.0684	0.0016	0.1146	D
OPC20y	0.0326	0.0539	0.0030	0.1025	D
OPD8y	0.0127	0.0726	0.0015	0.1167	D
OPD16	0.0001	0.1617	0.0165	0.0681	F
OPG10v	0.0333	0.0483	0.0008	0.1291	D
OPI1x	0.0286	0.0564	0.0041	0.0964	D
OPJ12x	0.0386	0.0506	0.0032	0.1009	D
OPL9v	0.0039	0.0961	ns		F
UBC178	0.0217	0.0619	0.0135	0.0721	D

Table 4.2. RAPD markers associated with germination and emergence in (SRN39 x SQR) RI population.

	Germination 12°c		Germination 22°c		Emergence		
Marker	Prob > F	R-square	Prob > F	R-square	Prob > F	R-square	group
OPA16	ns	-	0.0001	0.1893	0.0043	0.0912	unlked
OPA19	0.0437	0.0476	ns	-	ns		C
OPC7y	ns	-	0.0076	0.0799	ns	-	unlked
OPC20y	0.0032	0.0989	ns	-	ns		D
OPD16	ns	-	0.0095	0.0756	0.003	0.0998	F
OPG10v	0.0365	0.0510	ns	-	ns		D
OPI1v	0.0050	0.0900	ns	-	ns		D
OPI1x	0.0440	0.0474	ns	-	ns		D
OPJ1x	0.0298	0.0549	0.0118	0.0607	0.0139	0.0699	A
OPL9v	ns	-	ns		0.0191	0.0626	F

Table 4.3. RAPD markers associated with seedling height in (SRN39 x SQR) RI pop.

	height 1		height 2		height 3		
Marker	Prob > F	R-square	Prob > F	R-square	Prob > F	R-square	group
OPA19	0.002	0.1289	0.0001	0.1468	0.0027	0.1070	c
OPB16x	0.0001	0.1870	0.0001	0.1756	0.0022	0.1117	c
OPC7x	0.0429	0.0391	ns	-	ns		A
OPC10v	0.0018	0.0904	0.0021	0.0759	ns	-	C
OPE1	0.0214	0.0502	ns	-	ns		A
OPG10v	ns	-	ns		0.0404	0.0515	D
OPJ1v	ns	-	0.0180	0.0459	0.0229	0,063 1	unlinked
OPG6v	ns	-	0.0423	0.0342	ns	-	unlinked
OPJ12.v	ns	-	ns		0.0400	0.0517	D
OPJ17v	0.0460	0.0381	0.0267	0.0405	ns	-	E
OPK8	0.0011	0.0976	0.0002	0.1061	0.0218	0.0640	C
OPK17	0.0001	0.1622	0.0001	0.1858	0.0021	0.1119	c
OPK18	0.0034	0.0795	0.0010	0.0862	0.0338	0.055 1	unlinked
OPL9x	0.0305	0.0445	ns	-	ns		G
OPM2v	0.0028	0.0829	0.0035	0.0688	ns	-	C
OPM2x	ns	-	ns		0.0202	0.0656	(B)
UBC178	ns	-	0.0429	0.0340	0.116	0.0771	D

'Table 4.4. RAPD markers significantly associated with seedling dry weight in (SRN39 x SQR) RI population.

Marker	Dry weight 1		Dry weight 2		linkage group
	Prob > F	R-square	Prob > F	R-square	
OPA19	0.0426	0.0492	ns		C
OPB16x	0.0057	0.0894	ns		C
OPC7x	0.0047	0.0936	ns		A
OPC10v	0.0489	0.0465	ns		c
OPJ6v	0.0235	0.0610	0.0345	0.0509	unlinked
OPK8	0.0465	0.0475	ns	-	c
OPK17	0.0054	0.0907	0.0250	0.0571	c
OPK18	0.0320	0.0549	ns		unlinked

Table 4.5. RAPD markers significantly associated with plant height in (SRN39 x SQR) RI population. Parenthesis indicated that marker location was not determined.

	93-plant height		94-plant height		
'Marker	Prob > F	R-square	Prob > F	R-square	linkage group
OPA20	0.0002	0.0894	0.0001	0.0944	H
OPB10	ns		0.0364	0.0236	G
OPC7v	0.0001	0.1408	0.0001	0.1548	H
OPC20x	0.0001	0.1611	0.0001	0.1701	H
OPE1	0.0175	0.377	0.0153	0.03 14	(A)
OPE14x	0.0283	0.0322	0.0321	0.0247	(B)
OPJ1 8x	0.0498	0.0259	0.0137	0.0324	B
OPL3v	0.0015	0.0679	0.0020	0.0513	H
OPL3x	0.0001	0.2396	0.0001	0.2056	H
OPL3y	0.0001	0.1256	0.0001	0.1389	H
OPL19	0.0005	0.0796	0.0002	0.0727	H

Table 4.6. RAPD markers significantly associated with maturity in (SRN39 x SQR RI) population.

	93 -plant maturity		94-plant maturity		
Marker	Prob > F	R-square	Prob > F	R-square	linkage group
OPD3x	ns		0.0228	0.0575	A
OPD8y	0.0074	0.0763	ns		D
OPE8y	.00121	0.0672	ns		B
OPE18v	0.0064	0.0781	0.0228	0.0576	unlinked
OPI1x	0.0135	0.0652	ns		D
OPJ12v	0.0220	0.0558	ns		D
OPJ12x	0.0089	0.0727	ns		D
OPL3x	0.0192	0.0581	0.0338	0.0502	H
UBC178	0.0234	0.0552	ns		D

Table 4.7. RAPD markers significantly associated with grain yield in (SRN39 x SQR) population.

Marker	93 grain yield		94 grain yield		linkage group
	Prob > F	R-square	Prob > F	R-square	
OPA16	0.0062	0.0820	ns		unlinked
OPA20	0.0009	0.1186	ns		H
OPC7v	0.0001	0.1665	0.0265	0.0553	H
OPC20x	0.0003	0.1368	0.0168	0.0639	H
OPD16	0.0012	0.1132	0.0316	0.0520	F
OPE14v	ns	-	0.0025	0.1000	A
OPJ6v	0.0028	0.0971	0.0147	0.0665	unlinked
OPL3x	0.0034	0.0934	ns		H

Table 4.8. RAPD markers significantly associated with phenolic compounds.

	Flavan-4-ols		Pigments		
Marker	Prob > F	R-square	Prob > F	R-square	linkage Group
OPA19	0.0097	0.0798			C
OPB16x	0.0324	0.0553			c
OPC7x	-		0.0071	0.0860	A
OPE8y	-		0.0138	0.0725	B
OPI1y	0.0059	0.0900			unlinked
OPJ17x	0.0309	0.0562			unlinked
OPK8	0.005 1	0.0928			C
OPK1'7	0.0206	0.0644	0.0425	0.0498	C

Table 4.8 cont. RAPD markers significantly associated with phenolic compounds

Marker	Tannin		Total phenol		Linkage group
	Prob > F	R-square	Prob > F	R-square	
OPA19	0.0037	0.0993	0.0124	0.047	C
OPB16x	0.0134	0.0731	0.0014	0.1185	c
OPC10v	▪		0.0049	0.0935	C
OPK17	0.0051	0.0927	0.0004	0.1422	c
OPM2v	▪		0.0030	0.1033	C

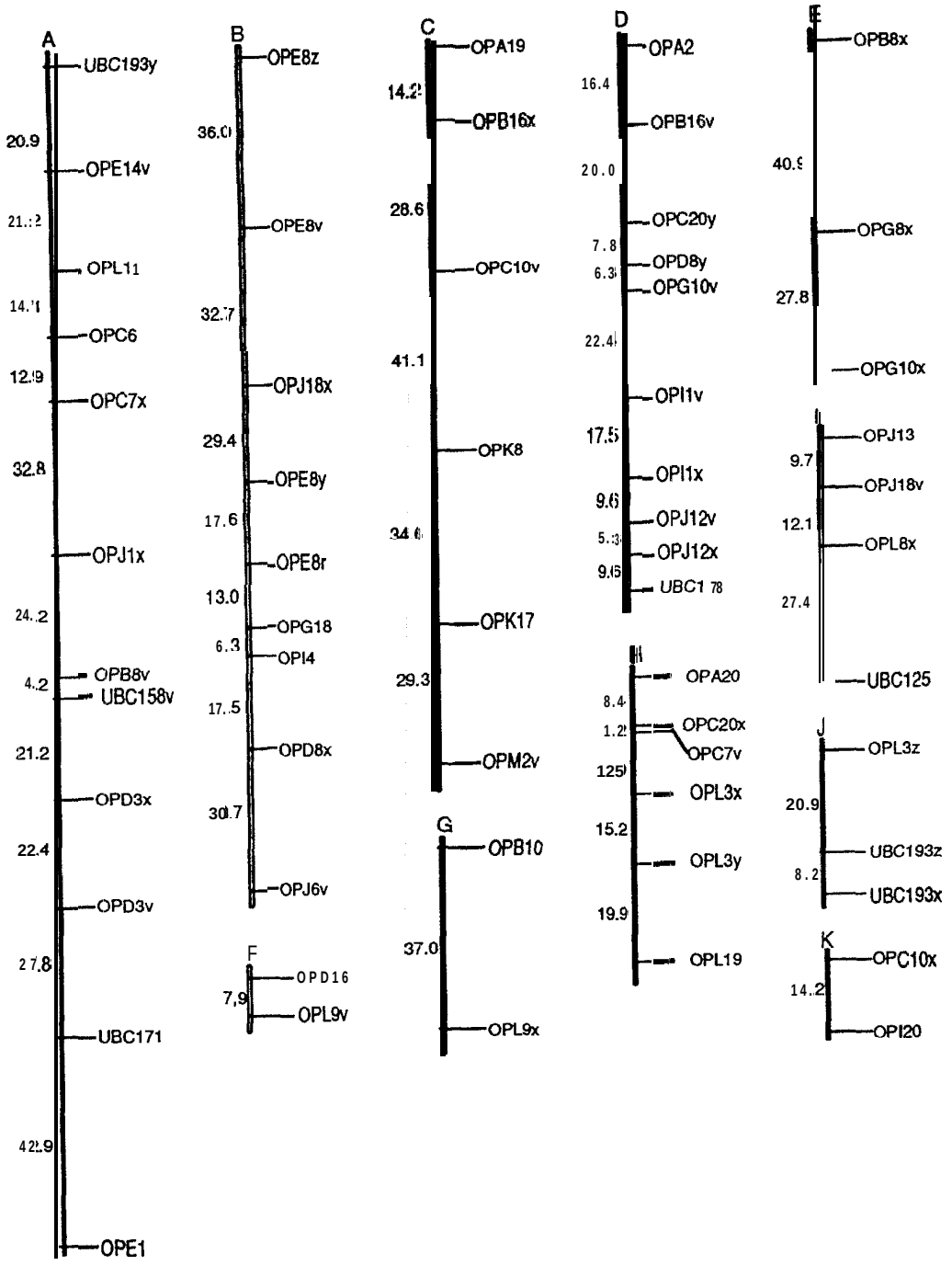

Figure 4.1. A genetic linkage map of sorghum with 59 markers in 11 linkage goups. Numbers on left of linkage groups represent map distances in centi-Morgans.

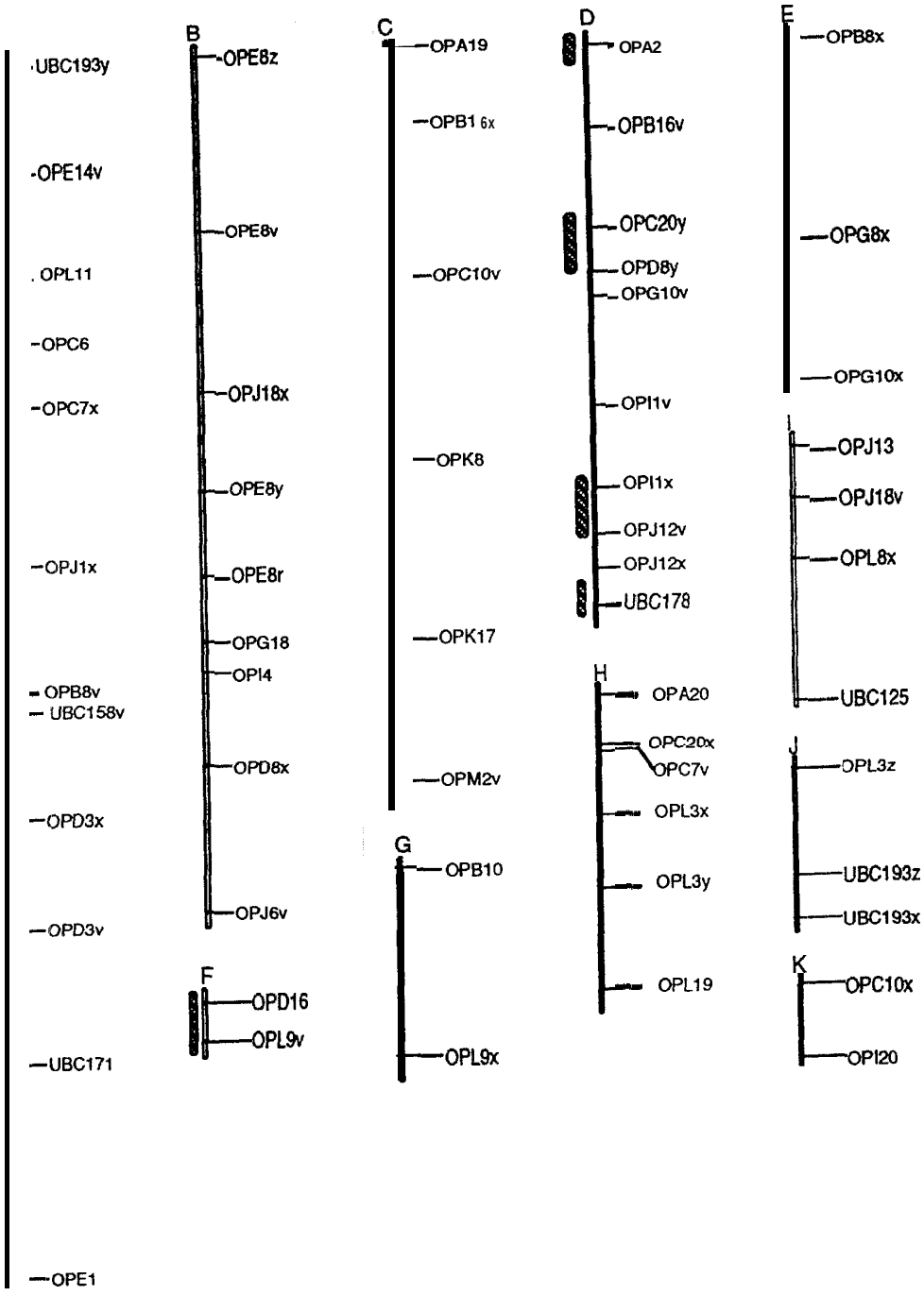

Figure 4.2. Location of QTLs for seedling vigor scores in SRN39 x SQR RI population.

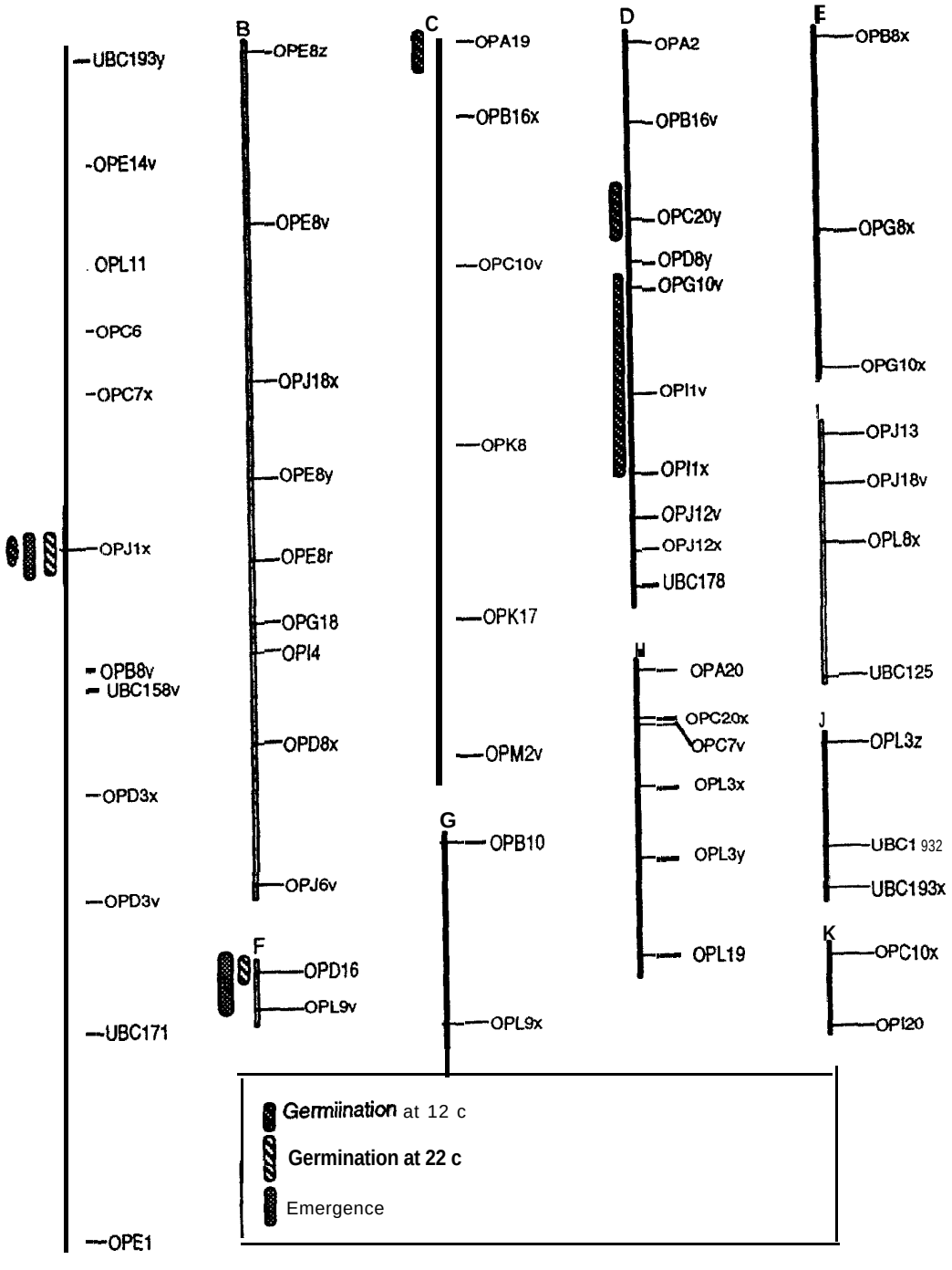

Figure 4.3. Location of QTLs for germination and emergence in SRN39 x SQR RI pop.

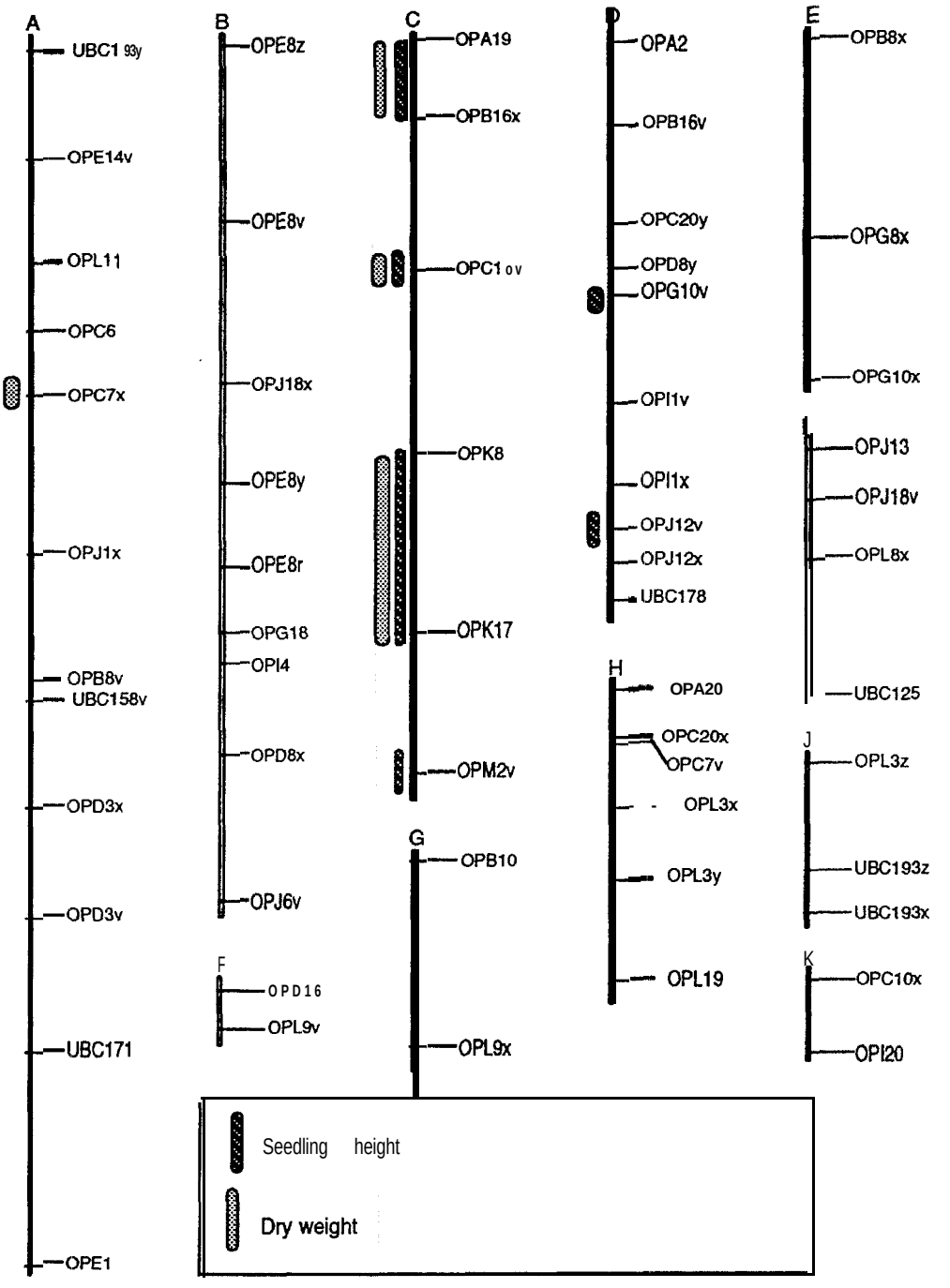

Figure 4.4. Location of QTLs for seedling height, dry matter, in SRN39 x SQR RI pop.

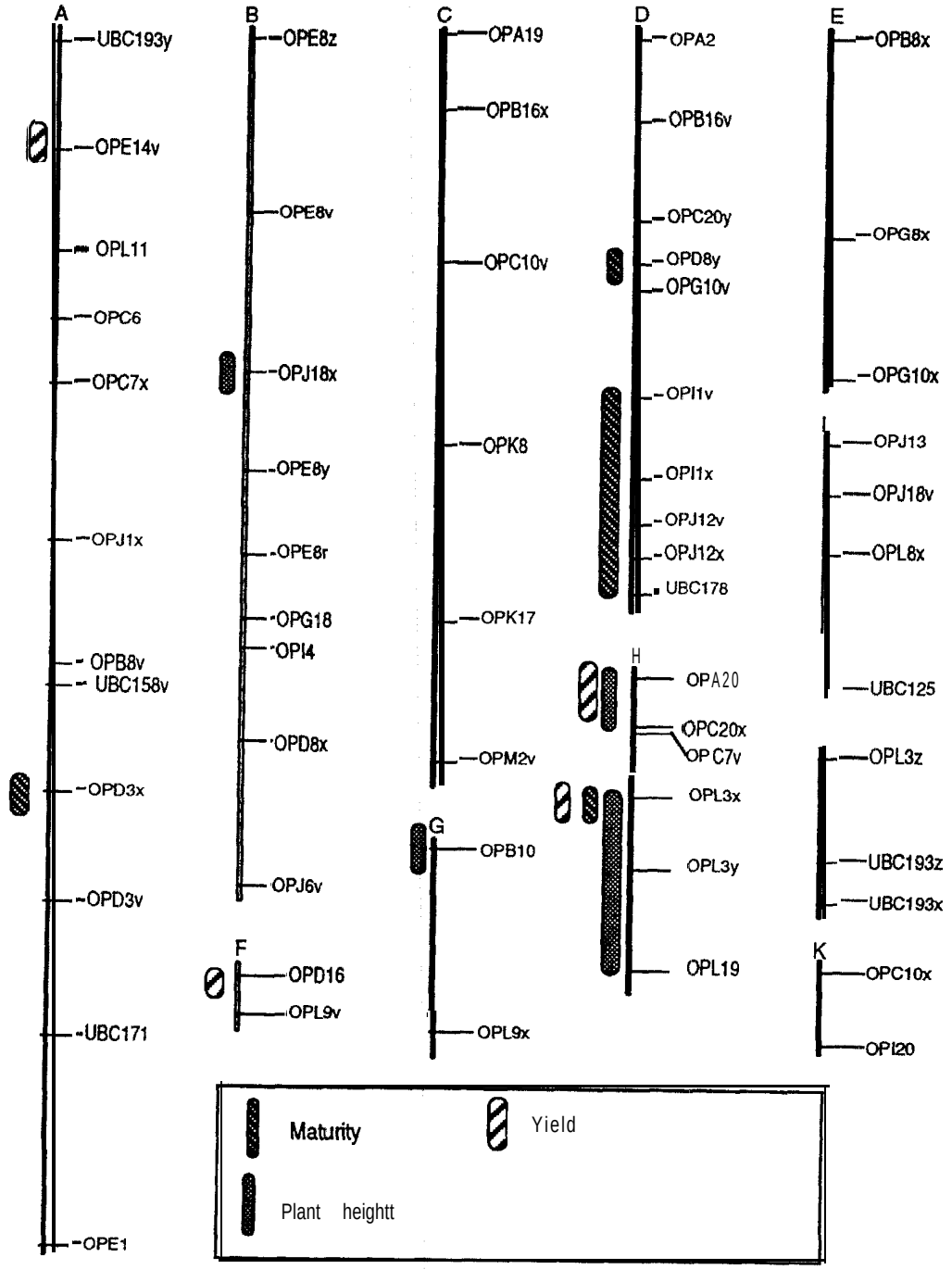

Figure 4.5. Location of QTLs for maturity, height, yield, in SRN39 x SQR RI pop.

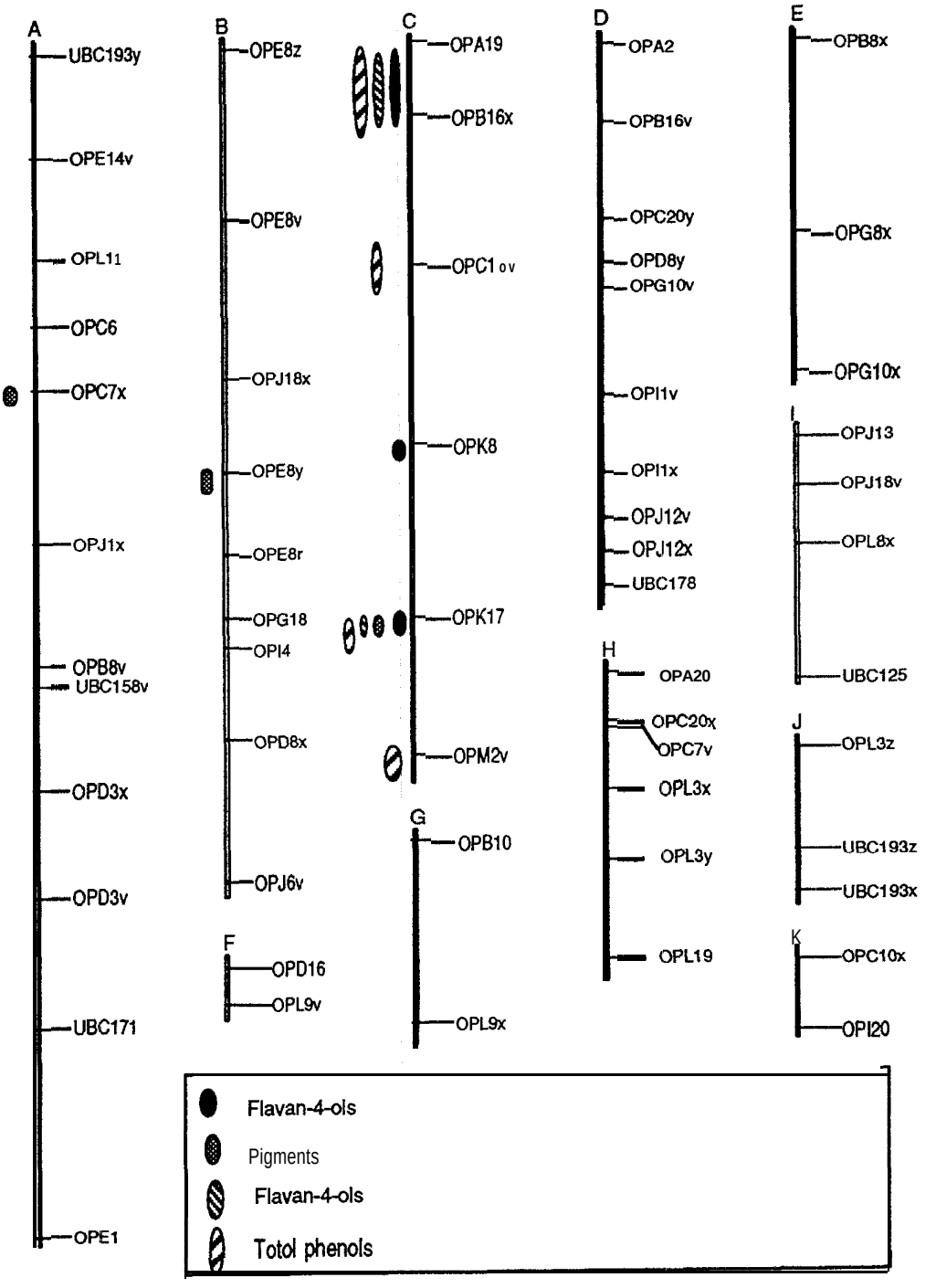

Figure 4.6. Location of QTLs associated with phenolic compounds.

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