

GENETICS OF TASSEL MORPHOLOGY IN MAIZE

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ABSTRACT

My research focused on tassel branching in maize and it encompassed studies of genetic and environmental factors affecting this trait. The maize tassel provides indispensable pollen in the process of reproduction, but it also competes with ears for photosynthetic products and can produce significant shade to leaves below. Thus newly developed corn hybrids have drastically reduced numbers of tassel branches compared to the ancient races of maize and tropical populations. My initial study involved analysis of branch numbers in 215 indigenous races of maize from ten countries through its regions of origin. Branch numbers ranged from 2.8 to 58.2 with an overall average of 27.0. In contrast a survey of 73 modern inbred lines, grown at Waimanalo Research Station, ranged from 2.5 to 36.5 branches with an overall average of 14.4. The difference was concluded to reflect long-term selection by maize breeders favoring smaller tassels.

Genetic studies of variation in near-isogenic lines of Hawaii's Hi27 revealed a major co-dominant gene, named *Brta* ("Branched tassel"). This gene essentially doubled branch numbers from 11 to 20. The *Brta* locus was found to be linked closely to the *floury 1* and *virescent 4* genes on Chromosome 2. Diallel analyses of several sets of progenies revealed high heterosis (>30%) for F1 hybrids and extensive variation in advanced progenies. A high ratio of 15.7 was observed for GCA:SCA (General:Specific combining ability), indicating that genetic control was largely due to additive gene action.

Three sets of recombinant inbred lines (RILs) were evaluated for tassel branch numbers, each having one parent with about 5 branches and the other about 20 branches.

The RILs averaged about at the midpoint between these parents, ranging widely with minimal transgressive segregation. The data were almost normal in distribution and were best interpreted as involving four QTLs (quantitative trait loci) acting additively.

Environmental effects on branching were great from the low-light, cool winter to the warm, high-light summer, and these appeared to correlate directly with plant growth. Tassel branch numbers of one series of inbreds increased from 14.3 in December to 15.2 in February and 15.6 in April. A single significant GxE interaction involved the mutant *ra2* (ramose tassel), which had more branches in winter than in summer, but it was clearly an exception.

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CHAPTER 1

TASSEL MORPHOLOGY AND MUTANTS THAT AFFECT IT

1. 1 Morphology and Ontogeny of the Maize Tassel

Maize (*Zea mays* L.) is a monoecious plant normally having unisexual male and female flowers in physically separated organs of the plant. The tassel (male inflorescence) arises from the shoot apical meristem while the ear (female inflorescence) originates from axillary bud apices. Both inflorescences initially contain bisexual flowers. During the course of development they become unisexual through abortion of gynoecia in tassel flowers and abortion of stamens in ear flowers. The tassels thus normally develop only male flowers and the ears develop female flowers. Exceptions to this pattern are common and under genetic control, often creating inflorescences more similar to those of other members of the large grass family, Gramineae.

The tassel is a branched inflorescence located at the tip of the main stem. It consists of a central spike with widely varying numbers of lateral branches (about 5-50). The paired spikelets occur in many ranks around the central spike, but are arranged in only two rows on the lower surface of the lateral branches. Each spikelet has a pair of leaf-like glumes which subtend florets. Each floret is characterized by a pair of thin scales representing the petals of a flower. One of these, the lemma, is adjacent to the glume. The other petal, called the palea, is located opposite to the lemma. There are three anthers in each floret. Two of the three anthers are located adjacent to the palea; the third is located adjacent to the lemma and is flanked by two lodicules. These lodicules swell at anthesis allowing

extrusion of anthers by elongation of the filaments. Following extrusion, anthers dangle downward and shed pollen from opercula (openings) at the anther tips.

1.2 Ontogeny and Differentiation of the Tassel

The development of the tassel precedes that of the ear, and normally occurs within one month of planting in Hawaii (Chong Hee Lee 1982). Following the initiation of all leaf primordia the apical meristem of the shoot elongates and is transformed into a reproductive (tassel) meristem. The tassel differentiates in an acropetal sequence (Figure 1-1). The first primordia to arise develop into primary lateral branches on the central spike. This is followed by the initiation of rows of spikelet-pair primordia, which form first on the central spike and subsequently on the lateral branches.

There are about 500-1000 spikelets produced on each tassel, depending on the genotype and growth environments. Spikelet development begins with the initiation of the outer glume and this is followed by the initiation of the inner glume and of the two lemmas. Subsequently, basally and abaxial to the outer lemma, the lower floret is initiated. The development of the upper floret precedes that of the lower floret. The extent of this developmental difference varies between genotypes and with the stage of development, and is maintained through to anthesis (Hsu et al. 1988; Cheng et al. 1983).

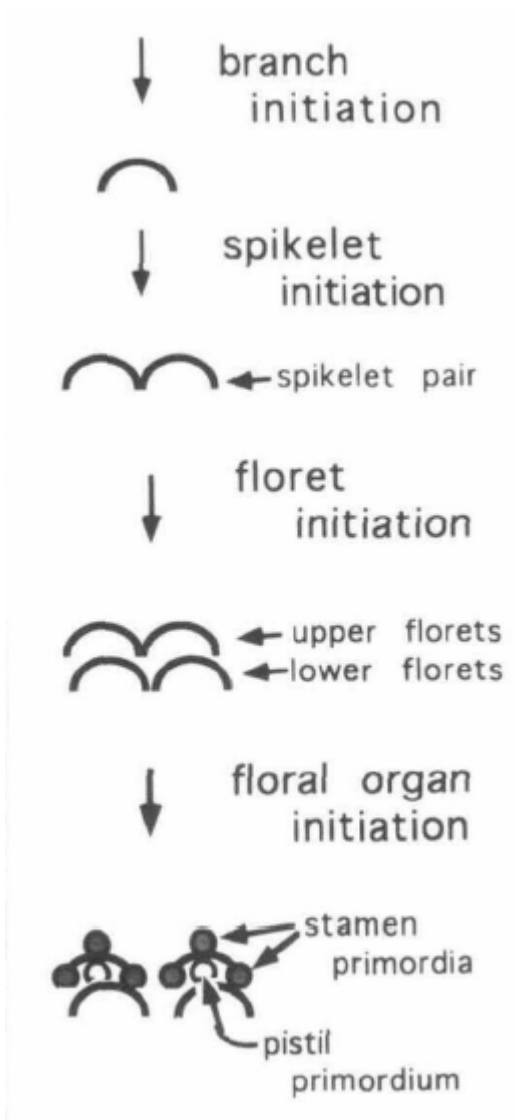


Figure 1-1 Ontogeny and differentiation of maize tassel

1.3 Mutations that affect Tassel Development

Many mutations have been described that affect the morphology of the maize tassel with a host of mutants affecting pollen development and sterility (Neuffer, Coe and Wessler, 1997). Some of the most prominent of the morphological changes are described here in an alphabetical sequence.

1.3.1 Adherent tassel

Adherent tassel (*ad1*) mutant is located on chromosome 1L-192. Adherence is a variation in which leaves, bracts and inflorescences coalesce. In this case, the tassels are greatly altered, being compressed into a solid structure rather than the familiar branched panicle. In our *ad*^{Hi27} near-isogenic line the adherence of tassel branches was observed but rarely involved leaves (Figure 1-2). Plant heights may be slightly less but grain yields are normal.



Figure 1-2 Tassel of adherent tassel mutant

1.3.2 Barren stalk1

Barren stalk1 (*bal*) mutant is located on chromosome 3L-109. The recessive mutant has no tassel branches, spikelets, tillers, or ears. The typical *bal* tassel is completely unbranched and devoid of any spikelets on the main tassel rachis. Ritter (2002) conducted a study of the interaction of *bal* with a branched-tassel mutant *tb1*

(*teosinte-branched*), described later in this section. The *ba1* gene completely repressed axillary meristem development and was epistatic to *tb1*.

1.3.3 Bif1, Bif2

Barren inflorescence 1 (*Bif1*) (located on 8S-54) and Barren inflorescence 2 (located on 1L-121.5) are dominant mutations that greatly reduce tassel branching (McSteen, 2000). The homozygote of *Bif1* is more extreme, having a completely barren tassel (no pollen) and nearly barren ear. Tassels of *Bif1* in the Hi27 NiLs have very few branches and spikelets are generally unpaired (Figure 1-3). Very little pollen is shed because of reduced number of spikelets.



Figure 1-3 The tassel of *Bif1* mutant

1.3.4 Branched silkless

Branched silkless (*bd*) Location: 7L-137.05. This mutant regulates the inflorescence transition from spikelet meristem to floret meristem, especially during the development of the female part of the maize, leading to the branched ears and no silk (Figure 1-4). The tassel itself appears very thick due to a great abundance of spikelets especially on central spike, but tassel branch number is normal.



Figure 1-4 The ear of *bd* mutant

1.3.5 Corngrass

Corngrass1 (*Cg1*) Location: 3S-24.4 Corngrass1 is a dominant mutant that keeps maize from making the transition to adult growth. It makes more leaves that have reduced lignin, which possess the potential value to develop the biofuels. In the Hi27 background, plants are grassy and have tillers and flag leaves (Figure 1-5). They produce many 2 to 4 rowed ears and variably incomplete tassels. Selves of corn-like segregants produced 3:1 ratio

differing widely in expression, with homozygotes assumed to be the most severe and sterile, confirming views that gene is partially (or co-) dominant.



Figure 1-5 Image of corngrass mutant

1.3.6 Few Branched

Few branches (*Fbr*) is a dominant allele, unmapped, in which the tassel consists of single spike or few branches. Leaf bracts replace the second tassel branch (from base) with single functional tassel branch just below it. Irregular silk-like awns form at the tips of the glumes of the more extreme single spike homozygotes. It was reported for the first time by M.G. Neuffer in 1989.

1.3.7 Fasciated ears

Fasciated ear2 (*fea2*) located on chromosome 4L-75.5. The *fea2* gene of maize functions to limit stem cell proliferation in the inflorescence. Mutants in *fea2* display

severe fasciation of the ear and an increase in the number of vertical rows of seed produced. It also affects the transition from shoot apical meristem to inflorescence meristem and can lead to more tassel branches. Byung II Je (2012) recently reported a new mutant *fea3* that causes the over-proliferation of the inflorescence meristem. (Figure 1-6)



Figure 1-6 Images of tassel mutant from left to right: wild type, *fea2*, *fea3*, *fea2/fea3* (photos by Byung II Je, 2012)

1.3.8 Ramosa Mutants

Ramosa mutants have highly branched inflorescences. Three mutant genes (*ra1*, *ra2* and *ra3*) have been identified and mapped (Figure 1-8). The term “ramose” comes from the Latin “ramus” meaning “branch”. The architecture of grass inflorescences was thoroughly investigated in maize and other grasses by Vollbrecht et al. (2005) with special attention to the role of gene *ramosa-1* in maize. This gene occurred as a mutant in a corn field 100 years ago, and is known to create highly branched tassels and ears. The authors

argue that this gene basically controls tassel architecture by affecting branch length as meristems are initiated. Limited studies of mutants of the *ramosa-2* locus also suggested that it regulates *ra1* activity by acting upstream. The studies were performed using mutants induced in the background of inbred B73, which has a tassel with extremely suppressed branching. Few tropical races or inbreds have a similar suppression of tassel branching. This infers that there are genes or QTLs in B73 that act well upstream from the *ramosa* loci.

A significant model for meristematic activity and thus branching in corn was provided by Troll (1964) in his book on inflorescences of grasses. A basal region associated with tillering (genes such as *gt*, *tb* and *tlr*) was recognized. We have provided evidence that enhanced meristematic activity basally is also accompanied by secondary meristematic activity at the tips of husk leaves (Brewbaker and Josue, 2007). Another major region of meristematic activity is in the flowering branch (shank) of the ear, where multiple ears are very common in corn. Also in this region can be increased meristematic activity in the ear itself from genes such as *ra1* and *li1* (lineate), but also in the *dbcb* (double-cob) mutant discovered in our program (Brewbaker and Huang, 2009). A third and most significant meristematic region is that at the base of the male inflorescence that creates tassels as different as B73 and the *ramosa-2* mutant in Hi27.

The *ramosa 1* mutant (Located on chromosome 7L-63) has branched conical inflorescences both on tassel and ear. It is fully fertile but often fails to produce seeds. The *ramosa1* allele was found to code for a zinc finger transcriptional factor. Paula McSteen

and her co-workers (McSteen et al., 2000) argue that it plays a role in inflorescence structure by making long branches instead of switching to make short branches (spikelet pairs). Therefore, *ra1* either promotes short-branch identity or suppresses the long-branch.

The *ramosa 2* (located at 3S-32.76) was first reported in 1935 (Brewbaker, 2012). This mutant is available as a near-isogenic line *ra2*^{Hi27}. It has a very similar tassel branching phenotype as *ra1*. Differences include its upright arrangement of tassel branches, not conical like *ra1* (Figure 1-7), and irregular kernel placement on a normal ear.

The gene *ramosa3* was suspected to work as a regulator upstream of *ra1* and *ra2*. The loci *ramosa1* (*ra1*), *ra2*, and *ra3* have been cloned and form part of a network of genes suggested to control the production of lateral branching in all grasses (Kellogg, 2007). The *ra1* and *ra3* mutants have been found only in Andropogoneae according to Kellogg.



Figure 1-7 The tassels of *ra1* (left) (Vollbrecht, 2005) and *ra2* (right)

1.3.9 Teosinte branched

Teosinte branched (*tb1*) located on chromosome 1L-220. This gene is a recessive mutation that causes the plant to produce excess tillers that are often terminated by unbranched tassels. The ear is also replaced by elongated branches that can terminate as unbranched tassels. The locus is believed to have been a critical gene in the evolutionary process of cultivated corn from its progenitor teosinte.

1.3.10 Thick tassel dwarf

Thick tassel dwarf1 (*td1*) is located on chromosome 5L-70.3. The mutation of maize affects both male and female inflorescence development. The ear is fasciated with extra rows of kernels, while tassel shows an increase in spikelet density.

1.3.11 Tillered

Tillered (*tlr1*) is located on chromosome 1L. The mutant features extra tillers. Heterozygote has smaller ear with staminate tip and long shank. Homozygote more extreme, like *tb1*, with grassy tillers, many small primitive ears, and often single-spiked tassels (Figure 1-8)



Figure 1-8 Photo of *tlr1*^{Hi27} mutant

1.3.12 Teopod

Teopod (Tp1) Location: 7L-76. Tp1 is a semi-dominant mutation with pleiotropic effects on both vegetative and reproductive structures. Heterozygotes (Figure 1-9) are identical in the two stocks, with unbranched tassels, prolificacy, large ears and the characteristic long glumes especially around basal kernels similar to those described by discoverer E. W. Lindstrom in 1925 (Brewbaker, 2012). Homozygous Tp/Tp plants have no tassels and are highly tillered, grassy, and very prolific with long husk covers.



Figure 1-9 The tassel of *Tp1* heterozygotes

1.3.13 Tassel seeds

Tassel seed mutants are common in maize and have female flowers in the tassel. Six genes have been reported to create this effect and are denoted *ts1* (2S-75.3), *ts2* (1S-69), *ts3* (1L-217), *ts4* (3L-78), *ts5* (4S-48) and *ts6* (1L-260) (Figure 1-10). In the formation of unisexual flowers derived from an initially bisexual floral meristem, abortion of pistil primordia in staminate florets is controlled by some tasselseed-mediated cell death process.

Acosta (2009) positionally cloned tasselseed1 gene and revealed its role as a lipoxygenase for jasmonic acid in male flower development in maize. Irish (1997) classified the development of tassel into four types of reproductive meristems, found that *ts4* mutants fail to form spikelet meristem and *Ts6* mutant are delayed in the conversion of certain spikelet meristem into floret meristem.



Figure 1-10 Photos of *ts2*, *ts3*, *ts4* (top) and *ts5* and *ts6* (bottom) from left to right

1.3.14 Tunicate

This dominant gene locates on chromosome 4 (4L-118). Heterozygotes are classical in appearance, having ears with coarse glumes covering all kernels. Tassels are not easily distinguished from wild type in Hi27. Homozygotes are dwarfed with no ears but have a single complex branched tassel with long glumes and occasional kernels. (Figure 1-11)



Figure 1-11 Photo of tassels of Tu1 heterozygote (left) and homozygote (right) of Tu^{Hi27}

1.3.15 Waxy kernels, Floppy Tassels

Waxy (*wx1*) gene locates on chromosome 9S-47.9. This mutant produces opaque endosperm of smooth, firm, non-corneous starch, consisting almost entirely of amylopectin (stains red with iodine) instead of amylose (blue staining) in endosperm. Homozygous *wx* lines are identified as having unusual lax tassel branches (Figure 1-12), labeled “floppy tassel” (Brewbaker and Huang 2009). Trait is inherited as a gene tightly linked to waxy and is interpreted as co-dominant, with intermediate expression in heterozygotes.



Figure 1- 12 The image of (*wx flta*)^{Hi27} with floppy tassel

1.4 Tassel Branching in Near-Isogenic Lines of Hi27

Many mutants of maize affect tassel development (Neuffer et al., 1997). Our data make clear that quantifying these effects requires near-isogenicity, due to the significant effect of heterosis. The creation of near-isogenic lines (NILs) in Hawaii inbred Hi27 (Brewbaker 2012) thus permits more precise assessment of any gene's impact on branch numbers. In 2012 we recorded tassel branch numbers and characteristics for 169 NILs with at least 6 backcrosses to Hi27. Many of these NILs had been studied for branch numbers during the 45 years of their development, and several were known from the literature to affect branching. Data for the most unusual of these mutants is presented in Table 1-1. The eight reps of Hi27 (ten plants per rep) in this trial averaged 9.25 tassels, and the overall average of the 169 mutants was 10.4 ± 5.1 . The tabular data are divided among the 13 NILs

with significantly lower branch numbers than Hi27 and the six NILs with higher numbers. Reduced branching was a common phenomenon among Hi27 NILs. It was notable for mutants like *ad* (1.4 branches), *ba* (3.3), *lg* and *lg2* (~3), and *Tp* (0.3), as noted in the literature (Neuffer et al., 1997). Reduced branches were also associated with mutants having reduced plant height and vigor (e.g., *na*, *na2*, *oy*), while the *Og* allele of *oy* was normal. Minor reductions in branching that were not statistically significant were associated with dwarf mutants like *ct* (7.9 branches), *d* (7.6) and *sdw* (7.0), while other dwarfs (e.g., *br*, *bv* (= *br3*), *cr*, *py* and *ws3*) were rather normal in branching. Many of the grassy mutants variably affect tassel development, ranging from normal to spike-like and even with tassels in the ears (e.g., *tlr*). Among these grassy types, however, only the NIL Cg^Hi27 (a heterozygote) was significantly low in branching. Mutants like *gt*, *tb* and *tlr* varied widely in tassel type but had average branch numbers similar to Hi27. Low branch numbers (3.7) also characterized our two *Rf4* NILs and were unexpected. The literature also includes notes on reduced tassel branching in *an1* (anther ear), *fbr1* (few branches) and *ub1* (unbranched), mutants not available in this set of NILs. Excessive branching has been recorded for the well-known *ramosa-2* mutant (Neuffer et al., 1997) and was confirmed here (39.8 ± 7.9 short branches in five trials) and shown to be fully recessive in hybrids. Our *brta* (branched tassel) NILs averaged 19.5 branches in the 2012 trial, and hybrids were intermediate but heterotic. Our two lazy NILs (*la fl2* and *la su fl2*) averaged 20.2 branches, with similar values for our Bf1 NIL (blue fluorescent) (19.0) and our *ts4* NIL (18.0). Tassels of Tunicate (*Tu*) heterozygotes were normally branched, but *Tu/Tu* homozygotes had a bushy and generally sterile tassel with many branches. Unusually

dense tassels have been described in the literature for *clt* (clumped tassel) and *td* (thick tassel dwarf), but branch numbers appear not to be elevated. It is clear that many monogenes affect branch number of tassels and that most of them significantly affect plant vigor also.

Table 1-1 Tassel branch averages (Avg.) in near-isogenic lines (NILs) of inbred HI27

NIL	Trait	Avg	Percent*	Locus
Mutants with significantly lower branch numbers				
<i>ad</i>	adherent tassel	0.2	3%	1L-192
<i>ba</i>	barrenstalk	3.3	34%	3L-109
<i>Cg</i>	Corngrass	4.3	44%	3S-24
<i>d</i>	dwarf	5.6	57%	3S-30
<i>lg</i>	liguleless	3.6	37%	2S-12
<i>lg2</i>	liguleless	3.1	32%	3L-103
<i>na</i>	nana dwarf	1.4	14%	3L-130
<i>na2</i>	nana dwarf	3.4	33%	5S-57
<i>oy</i>	oil yellow	2.9	30%	10S-34
<i>Rf4</i>	Fertility restorer	3.7	38%	8S-4
<i>Rg</i>	Ragged	5.3	54%	3S-69
<i>Tp</i>	Teopod	0.2	2%	7L-76
<i>ws3</i>	White sheath 3	4.2	43%	2S-2
Mutants with significantly higher branch numbers				
<i>Bf</i>	Blue fluorescent	19.0	195%	9L-151
<i>brta v4</i>	branched tassel	19.5	200%	~2L-85
<i>la fl2</i>	lazy floury	19.0	194%	4S-52
<i>ra2</i>	ramosa	40.6	417%	3S-33
<i>su lfl</i>	sugary leaf-fleck	22.6	232%	4S-64
<i>ts4</i>	tassel seed	18.0	185%	3L-78

* Data in percent of HI27 original parent inbred

CHAPTER 2

TASSEL BRANCH NUMBERS IN THE INDIGENOUS RACES OF MAIZE

2.1 Introduction

The indigenous races of maize range from the United States to Chile. Descriptive data of many types were recorded on more than 200 races in a remarkable series of 11 publications financed by the U.S. National Academy of Sciences (e.g. “Race of Maize in Mexico”, Wellhausen, 1952). The races were grown in regions to which they were native, by scientists familiar with their background, origin and uses. Among the data collected were tassel morphological data. This included branch number and in some publications the numbers of both primary and secondary branches. These data have been analyzed here in relation to data on elevation of adaptation, plant size and vigor and other traits. Evidence has been sought for heterosis of branch number among races of hybrid origin and for any evolutionary trends in branch numbers.

2.2 Branch Number of Indigenous Races of Maize

Total branch numbers were available for 215 races listed in the 10 publications in the “Races of Maize” series. In many countries there were data provided for both primary and secondary branches. I have summarized total branch number data in Appendix Table 2-1 for 215 races. This often includes races grown in more than one century, occasionally in quite different ecosystems. Branch numbers ranged from a low value of 2.8 to a high value of 58.2 with an overall average of 27.0 ± 10.7 branches. The data have been plotted in Figure 2-1 together with an approximately normal distribution curve created following

spreadsheet procedures of Brewbaker (2003). The data closely fit the normal curve, with cumulative chi-square value of 9.47 ($P>0.50$)

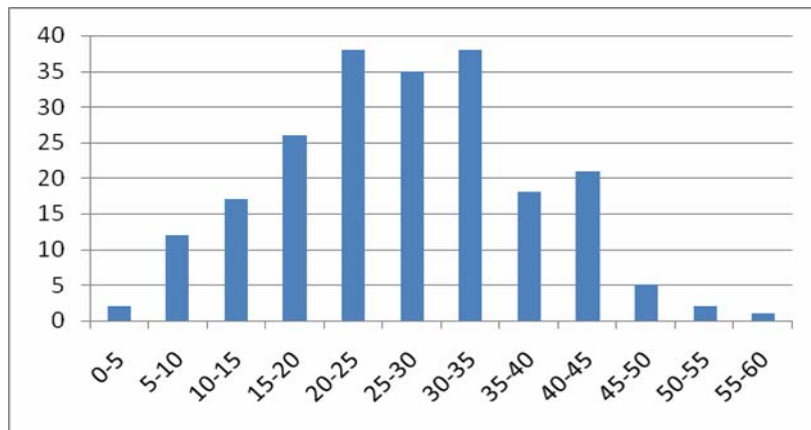


Figure 2-1 Tassel branch number variation among the races of corn

Major races are those that are grown over more extensive regions, usually representing more significant crop varieties. A summary of branch numbers among races grown in multiple countries is presented. The 31 races averaged 28.8 branches (range 4.4 to 45.2) with an overall standard deviation of 3.61. This resulted in a coefficient of variation of 12.6%, a value that can be considered small and representing low genotype by environment variation. Among the most widely grown races in this table are Cuzco (17.1 ± 0.9), Cateto (26.4 ± 3.7) and Tuxpeno (28.6 ± 5.6). The data showed a slight tendency for positive correlation for standard deviation with branch number.

2.3 Branch Number and Elevation of Adaptation

The elevations of adaptation are presented by the authors of the Races in Maize series of publication. These ranged from sea level to 3500 meters, the latter in high Andean mountains. Branch numbers were notably higher among races from lower elevations. Data

for branch number and elevation were highly negatively correlated with a value of $r = -0.465$. Only 14 out of the 215 races had average numbers less than 10 branches, and these were almost entirely from the high elevations. Examples include the very primitive Palomero Toluqueño from highland Mexico (3.6 branches), Cacahuacintle from southern Mexico and Guatemala (5.6 branches) and Confite Puñeno of Peru (6.0 branches).

Branch numbers for 19 ancient indigenous races are summarized in Table 2-1. The values averaged 16.9 and range from 3.6 to 32.1, with standard deviation of 8.42. It is evident from this table also that highland races (including the three mentioned above) dominated the group with low branch numbers. One of the most significant races in the evolution of maize-based civilizations (Brewbaker, 1979) was Nal-Tel, which has a high tassel branch number (22.8) similar to the average seen in Figure 2-1.

Table 2-1 Summary Branch numbers for 19 ancient indigenous races

Ancient Races	Branch Number
Palomero Toluqueno	3.6
Cacahuacintle	5.6
Confite Puneno	6
Sub-race Elotes Occidentales	8.8
Harinoso de Ocho	10
Confite Puntiaquedo	11.8
Kculli	12.6
Chapalote	13
Confite Morocho	14.9
Oloton	16.8
Maiz Dulce	18.1
Caingang	22.7
Lenha	22.8
Nal-Tel	22.8
Moroti Precoce	25.4
Moroti Guapi	26.5
Moroti	30.2
Entrelacado	32.1
Average	16.87
Std	8.42

Husk numbers were shown (Brewbaker and Kim, 1979) to range widely among the races of maize, averaging 11.1 and ranging from 5 to 22. Variation among 128 races was closely correlated with elevation of adaptation of the race, with $r = -0.732$.

2.4 Branch Numbers and Agronomic Traits

The Races of Maize series of publications included detailed data of many types on agronomic traits. Among those of interest were traits reflecting plant vigor (height, leaf

number), days to flowering and ear characters. A correlation matrix of ten of these values is presented in Table 2-3. The data were derived from 166 races.

Table 2-2 Correlation coefficients among major morphological traits in 166 maize races

	Branch	Elev. In m.	Maturity DTS	Leaf No.	PH	EL	Row	ED	KD	Husk No.
Branch										
Elev. In m.	-0.465									
Maturity DTS*	-0.193	0.541								
Leaf No.	0.473	-0.417	-0.193							
PH*	0.587	-0.445	-0.306	0.784						
EL*	0.289	-0.255	-0.121	0.426	0.634					
Row	0.182	-0.191	-0.213	0.113	0.121	-0.062				
ED*	-0.011	0.136	0.304	0.072	0.162	0.252	0.107			
KD*	-0.225	0.475	0.468	-0.233	-0.13	0.057	-0.215	0.659		
Husk No.	0.674	-0.745	-0.517	0.625	0.646	0.309	0.297	-0.096	-0.426	

* DTS is day to silk; PH is plant height; EL is ear length; ED is ear diameter; KD is kernel depth.

Tassel branch numbers correlated highly (Table 2-3) with elevation ($r = -0.465$), as did husk numbers ($r = -0.745$). It can be argued for husk numbers that insect pests are more common in the lower elevation, but increased tassel branching would not seem to relate to pest damage. Instead it is clear that branch numbers were higher on plants with high leaf number ($r = 0.475$), with greater plant height ($r = 0.587$) and higher husk number ($r = 0.674$). Relevant correlations are those of plant height to husk number and leaf number to husk number. In general it appears that more robust varieties with larger plants having more leaves and husks also have larger tassels with more branching. Interestingly there was a positive correlation of 0.289 between ear length and tassel branch number, somewhat similar to the correlation of leaf number and ear length. Branch numbers show no other significant correlation with maturity, ear row number, ear diameter of kernel

depth (Table 2-2). It is evident that most ear traits are inherited independently from genes affecting branching of the male inflorescence.

2.5 Heterosis for Branch Number among Races of Hybrid Origin

Authors of the race publications have listed putative parents for races of suspected or known hybrid origin. The data for 32 of these races have been summarized in Table 2-4, with racial parentage and original country of the data. Branch number for the 32 races averaged 26.5 and ranged from 9.2 to 47.2. When data for the hybrid races were compared with those of the parental averages, heterosis values could be calculated. These averaged 8.3% but ranged widely. Positive heterosis values included 19 races with 14 exceeding 10% heterosis. Negative values included 13 races with 8 exceeding 10%. It can be concluded that there is a general trend toward positive heterosis for tassel branch number.

It can be assumed that hybrid races were selected by farmers for higher yield that in turn reflected larger plants with more leaves and husks. The correlations shown above for branch number and plant height, leaf number and husk number probably account indirectly for the positive heterosis trend observed in Table 2-3 for hybrid vs. their parent races.

Table 2-3 Heterosis for branch number among races of hybrid origin

Race	Land	Hybrid Parents	Race	P1	P 2	PAVG	Heterosis	
			Branches					
Amagaceno	Col	Chococeno x Montana	44.3	41.5	50.1	45.8	-3.28%	
Ancashino	Per	Huayleno x Arequipeno	22.0	18.2	31.9	25.1	-12.18%	
Arequipeno	Per	Chaparrano x Cuzco	31.9	40.0	17.5	28.8	10.96%	
Bolita	Mex	Tabloncillo x Zapalote Chico	17.4	8.8	18.9	13.9	25.63%	
Cabuya	Col	Clavo x Sabanero	43.9	41.2	36.2	38.7	13.44%	
Celaya	Mex	Tabloncillo x Tuxpeno	21.1	8.8	34.3	21.6	-2.09%	
Chalqueno	Mex	Tuxpeno x Conico Norteno	10.7	34.3	17.5	25.9	-58.69%	
Chancayano	Per	Alazan x Pardo	28.3	35.6	21.0	28.3	0.00%	
Chandelle	Ven	Pollo x Puya	34.2	19.3	33.1	26.2	30.53%	
Chirimito	Ven	Pira & Nal-Tel	46.7	36.2	22.8	29.5	58.31%	
Comiteco	Mex	Tehua x Oloton	21.3	27.7	16.8	22.3	-4.27%	
Comun	Col	Amagaceno x Costeno	44.5	44.3	34.2	39.3	13.38%	
Conico Norteno	Mex	Conico x Celaya	17.5	5.5	21.1	13.3	31.58%	
Curagua Grande	Chi	Curagua x Cristallino. Chileno	34.0	43.2	29.8	36.5	-6.89%	
Cuzco Cristalino Amarillo	Per	Huancavelicano x Uchuquilla	18.9	14.8	16.2	15.5	21.82%	
Cuzco-Huilacaparú	Bol	Huilcaparu x Cuzco	16.6	22.9	17.5	20.2	-17.99%	
Granada	Per	Kculli x Huayleno	24.7	12.6	18.2	15.4	60.39%	
Guaribero	Ven	Chirimito x Araguato	43.6	46.7	33.5	40.1	8.73%	
Huancavelicano	Per	Confite Morocho x Kculli	14.8	14.9	12.6	13.8	7.64%	
Huevito	Ven	Canilla x Andaqui	32.7	28.0	42.1	35.1	-6.70%	
Jala	Mex	Comiteco x Tabloncillo	17.9	21.3	8.8	15.1	18.94%	
Montana	Col	Pira Naranja x Sabanero	50.1	58.2	36.2	47.2	6.14%	
Pagaladroga	Per	Mochero x Confite Puntiaquedo	32.8	38.8	11.8	25.3	29.64%	
Pepitilla	Mex	Vandeno x Palomero?	21.8	20.8	3.6	12.2	78.69%	
Puya	Col	Clavo x Tuxpeno	33.1	41.2	34.3	37.8	-12.32%	
Puya Grande	Col	Puya x Tuxpeno	30.2	33.1	34.3	33.7	-10.39%	
SanGeronimoHuancavelicano	Per	Huancavelicano x Paro	18.8	14.8	17.0	15.9	18.24%	
Tabloncillo	Mex	Harinoso x Reventador	8.8	10.0	8.4	9.2	-4.35%	
Tuxpeno	Mex	Olotillo x Tepecintle	34.3	30.3	24.7	27.5	24.73%	
Vandeno	Mex	Tuxpeno x Zapalote Grande	20.8	34.3	23.9	29.1	-28.52%	
Yucatan	Col	Andaqui x Comun	43.6	42.1	44.5	43.3	0.69%	
Zapalote Chico	Mex	Nal-Tel x Tepecintle	18.9	22.8	24.7	23.8	-20.42%	
Zapalote Grande	Mex	Zapalote Chico x Tehua	23.9	18.9	27.7	23.3	2.58%	
						AVG	26.6	8.30%

2.6 Branch Numbers of Modern Inbred lines

It was of interest to compare the tassel branch numbers of historic races of maize with the numbers of branches in modern corn inbreds. A total of 73 inbreds were used in this investigation and were planted at the Waimanalo Research Station in 2008 and 2009. Each inbred was planted in a 20-plant plot thinned to a single plant per hill. Ten plants were randomly selected for counts of branch numbers when tassels were fully emerged. Branch numbers included all of the primary and secondary branches and excluded the central spike.

The data for 73 inbred lines are summarized in Appendix Table 2-2. Branch numbers ranged from a low value of 2.5 to a high of 36.5 with an overall average of 14.4 ± 2.6 branches. The data have been plotted in Figure 2-2, and closely follow the normal distribution curve. Among the 73 inbred lines were 7 with fewer than 5 branches, including A619, DB544, GT601, Hi31 (a conversion of B68), Hi60 (a conversion of Mo17) and W182. All of these except Georgia's GT601 are modern temperate inbreds of Corn-Belt adaptability. Only three inbred lines had more than 30 branches; these were Hi28, CIMA21 and CM103.

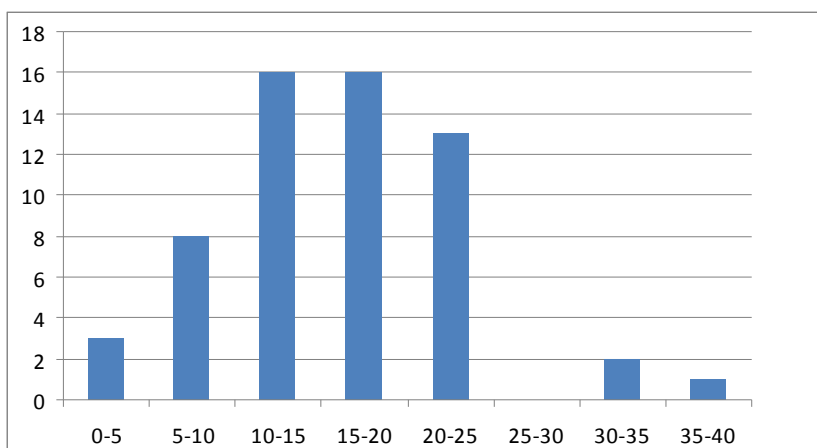


Figure 2-2 The distribution of branch number for 73 inbred lines

Comparing overall means, the modern inbreds averaged only 14.4 ± 2.6 branches while the indigenous races averaged 27.0 ± 10.7 branches. Not only do modern inbred lines have smaller tassels compared to the races, but they show much less variation around these means. It is evident that this is the result both of inbreeding per se and long-term selection by corn breeders. This presumably reflects the fact that smaller tassels can save more energy and increase grain yield.

2.7 Branch Number and Agronomic Traits

In the past 30 years, Dr. Brewbaker and colleagues assembled many data on the agronomic traits of the inbred lines maintained by Hawaii Foundation Seeds (www.ctahr.hawaii.edu/hfs). These data are summarized in publications by Kim et al. (1988) and by Brewbaker et al. (1989). In comparing these data with those above for branch number of 73 inbred lines, we found 43 corresponding inbreds that could be used to compute correlation analyses among the agronomic traits and branch numbers. Our primary interest was to assess the correlation of branch numbers with these agronomic traits, and to compare the results with previous correlation analysis among the races.

In the analysis of modern inbred lines the branch numbers correlated highly with husk numbers ($r = 0.629$). This had also been observed in the analysis of races, where $r = 0.674$. This suggests an intrinsic genetic mechanism linking tassel branch number and husk number. Each represents a form of secondary meristematic activity--one affecting the central spike of a tassel and the other affecting the central branch ("shank") of an ear. It is common to find low husk numbers in modern corn hybrids, often the result of selection to

facilitate harvest and threshing. This is particularly notable for sweet corns. High husk numbers are associated with reduced ear-insect damage in the tropics (Brewbaker and Kim, 1979).

The correlation coefficient between tassel branch numbers of inbreds and numbers of leaves was relatively high, $r = 0.491$. This was also consistent with the results from the racial data where $r = .473$. In contrast the tassel branch number of races was highly correlated with plant height ($r = 0.587$) but this correlation did not exist for inbred lines ($r = 0.109$). It can be argued that inbred lines were selected by breeders to favor small tassels irrespective of plant size, presumably to reduce energy loss in the tassel and pollen production.

CHAPTER 3

IDENTIFICATION OF THE BRANCHED-TASSEL GENE

3.1 Introduction

The purpose of this study was to identify the genetic control of a highly tassel branched character encountered among near isogenic lines of the inbred Hi27 (Brewbaker 2012) and known as “branched tassel” or “*Brta*”. I was interested in the character and quality of this gene, as well as its linkage relationships. Tassel branching was recorded for all available NILs in the Hi27 series, and generation mean analysis methods were used to investigate gene action of *Brta*.

Near isogenic lines (NILs) are important genetic stocks for investigating the function and regulation of genes. They are also useful for isolating genes (Kojima et al. 1998). Genes of interest are backcross-introgressed into a stable genetic background, which is often an excellent inbred line adapted to local environmental conditions. Near isogenic lines have been used to evaluate and map the major genes and QTLs for specific traits in plants. Over 100 NILs have been based on Hi27, an elite inbred flint corn derived from Colombia (Brewbaker 2012). These materials have become a valuable resource in studying maize morphological traits and mapping the functional genes in the maize genome.

3.2 Materials and Methods

3.2.1 Generation mean analysis

Generation mean analysis utilizes observed means and variances of various generations. The generations should be derived from a cross between two parents that are homozygous for differences in a trait of interest. Effects of gene action which can be revealed in this analysis are additivity, dominance and three types of non-allelic interactions (“epistasis”). The theoretical foundation of generation mean analysis will be summarized here from a classic textbook on biometrical genetics by Mather and Jinks (1977).

When two alleles are involved (disomic inheritance), there will be three genotypes, AA, Aa, and aa in a segregating locus. There are two parameters required in order to measure the differences in phenotypic expression of these three genotypes. The mid-point between two homozygotes AA and aa is defined as m , mid- parent. A parameter a is defined to measure the departure of each homozygote AA and aa from the mid-parent, while other parameter d measures the departure of heterozygote Aa from m . Thus, parameters a and d represent additive and dominance effects, respectively.

In figure 3-1, the genotype AA has an expression, $m+a$, while aa equals $m-a$ and Aa $m+d$. When dominance is absent, d will be zero and consequently the heterozygote's expression will equal m . In the case of complete dominance, d equals a . In the rare event that Aa falls outside the range between AA and aa, then it will display over-dominance.

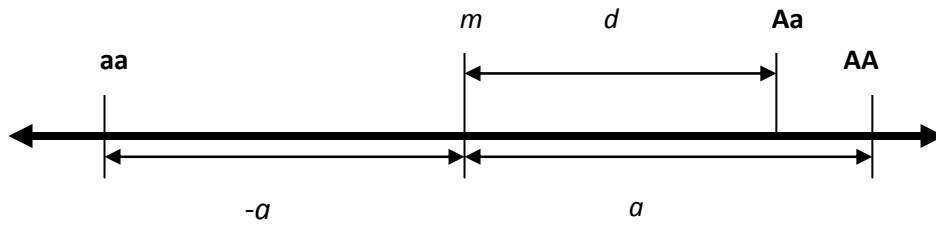


Figure 3-1 The a and d increments of the gene difference A-a. Deviations are measured from the mid-parent, m , midway between the two homozygous AA and aa. Aa may lie on either side of m and the sign of d will vary accordingly (Mather and Jinks, 1977).

Individual genes that contribute to gene effects normally cannot be distinguished. Considering two homozygous lines which differ at two loci, A-a and B-b, with no interaction or linkage between them. There will be two possible combination of genes in two lines. If one of them is AABB, then the other will be aabb. If the effect of these genes are simply additive, the first will depart from mid-point by a_a+a_b and the second by $-(a_a+a_b)$. If the lines are AAbb and aaBB, they will depart from mid-point by a_a-a_b and $-a_a+a_b$, respectively. When k loci are involved, $[a]$ symbolized their pooled additive effects. Similarly, when two homozygous lines are crossed, the phenotypic expression of heterozygotes will have pooled dominance effects represented by $[d]$. In each case, individual gene effects can be either positive or negative, and thus tend to balance out each others' effects.

Genotypes at the "A" locus will appear in the ratio of $1/4AA$, $2/4Aa$, $1/4aa$ when an F_2 is produced. Therefore, this gene will contribute $1/4a_a+2/4d_a-1/4a_a=1/2d_a$ to the departure of average expression in F_2 from the mid-parent. When extended to k genes, the F_2 mean

becomes $1/2[d]$ and the mean phenotype of the F_2 will be $F_2 = m + 1/2[d]$. In the same way, we can generate $B_1 = m + 1/2[a] + 1/2[d]$ and $B_2 = m - 1/2[a] + 1/2[d]$. Components of means for generations that can be derived from two homozygous parents, P_1 and P_2 , are summarized in Table 3-1.

Table 3-1 Components of means for different generations of a GMA on the six-parameter models (Mather and Jinks, 1977). Note that 3 parameter model involves only m , $[a]$ and $[d]$.

Generation	Six-parameter model					
	M	[a]	[d]	[aa]	[ad]	[dd]
P_1	1	1	0	1	0	0
P_2	1	-1	0	1	0	0
F_1	1	0	1	0	0	1
F_2	1	0	0.5	0	0	0.25
B_1	1	0.5	0.5	0.25	0.25	0.25
B_2	1	-0.5	0.5	-0.25	-0.25	0.25

3.2.2 Parent inbreds for generation mean analysis

Two near isogenic lines (NILs) of $Hi27 (fl\ v4)^{Hi27}$ and $(v4\ fl)^{Hi27}$ were created by backcrossing MGC stock 63-2370-5/2367-2 ($lg\ gl2\ fl\ v4$) with $Hi27$ to make the conversions from 1969. After six generations of backcrossing, selfing, fl and $v4$ NILs were selected out respectively according to their own morphological traits, followed by one of the final result revealed that both of them are $(fl\ v4)$ double mutants due to their close proximity on the chromosome 2 ($fl1$ is on the coordination 75.7, while $v4$ is on 88.0 by Map Genetic 2008 2). In our observation, the line selected by fl mutant have an unusually large tassel branch number, while the line selected by $v4$ have a normal number like that of other NILs of $Hi27$. Therefore, in our experiment, the branched tassel NILs $(fl\ v4)^{Hi27}$ and non-branched tassel NILs $(v4\ fl)^{Hi27}$ or wild type $Hi27$ were used to

produce F1, F2 and two backcross populations (BC1 and BC2). Inbred (fl v4)^{Hi27} was selected by the trait of floury endosperm. The gene controlling the floury endosperm was proposed dominant and of dosage effect by the previous study. Inbred (v4 fl)^{Hi27} was selected by the trait of virescent seedling about 2-3 weeks after planting. In our stocks, both inbreds had the double mutant phenotypes mentioned above, even after numerous generations, were assumed to be highly homozygous.

Field evaluations for six generations (P1, P2, F1, F2, B1, B2) were carried out in spring of 2008 at Waimanalo Agricultural Research Station of the University of Hawai'i. The experiment was designed in randomized complete blocks with two replications. Plots consisted two or four 4.5-meter rows for non-segregating populations (P1, P2, F1) and four and six rows for the two backcrosses and F2 populations, respectively. Row and hill spacing were 0.75 and 0.25 m, respectively. Two untreated seeds were planted per hill and resulting plants were thinned to one per hill at around 3 weeks after planting. Tassel counting was directly conducted in the field when the tassel completely showed up on the top of plant at the stage of sexual mature of plants.(about 50-60 days after planting). For the non-segregating populations, ten or twenty sample plants in each row were randomly selected to count. The branch number included all primary and secondary branches excluding the central spike. For the segregating populations (F2, B1 and B2), the branch numbers of all plants were recorded excepting only stunted or diseased individuals, because the segregation ratio was as important as the mean and standard deviation in the subsequent analysis.

3.2.3 Statistical Method

Generation mean analysis was conducted under the conventional assumptions of no epistasis or linkage. Additive and dominance genetic variances ($\sigma_A^2 + \sigma_D^2$) and narrow-sense heritability (nH) were estimated following the method of Warner (1952), in which $\sigma_A^2 = 2\sigma_{F2}^2 - (\sigma_{B1}^2 + \sigma_{B2}^2)$, $\sigma_D^2 = \sigma_{F2}^2 - (\sigma_A^2 + \sigma_E^2)$ and $nH = \sigma_A^2 / \sigma_{F2}^2$

Data from all samples for each generation were calculated by averaging variance of each replication in same generation. Variance of mean for a generation was obtained by its variance divided by number of plants in the generation. Standard error of a generation was a square root of variance of mean for the generation.

Gene effects based on a six parameters were estimated using the nonweighted generation means analysis described by Gamble (1962) and are defined as follows:

$$\text{Mean [m]} = F2$$

$$\text{Additive [a]} = B1 - B2$$

$$\text{Dominance [d]} = -0.5P1 - 0.5P2 + F1 - 4F2 + 2B1 + 2B2$$

$$\text{Additive} \times \text{Additive [aa]} = -4F2 + 2B1 + 2B2$$

$$\text{Additive} \times \text{Dominance [ad]} = -0.5 P1 + 0.5P2 + B1 - B2$$

$$\text{Dominance} \times \text{Dominance [dd]} = P1 + P2 + 2F1 + 4F2 - 4B1 - 4B2$$

The minimum number of genes (N) controlling the trait of tassel branch number was estimated following Mather and Jinks (1982) as:

$$N = (P2 - P1)^2 / 8 \times [\sigma^2 F2 - (\sigma^2 F1 + \sigma^2 P1 + \sigma^2 P2) / 3]$$

These effective factor formulas assume that the segregating genes for the trait of interest are all located in one parent, not linked, have equal effects, with no genotype \times environment effects, and no epistatic or dominance effects (Wright, 1968).

3.3 Results

3.3.1 Hypothesis for Monogenic genetics

The NIL of Hi27, (fl v4)^{Hi27} was observed in 2007 to have an unusually compact and erect tassel. Seed from these selfed virescent plants were floured and were planted in 2008. When the tassel branch numbers were counted, they averaged 23 or about twice that of Hi27. This stock traced back to a chromosome 2 stock from the Maize Genetic Cooperation Center (gl2 lg v4 b fl1) crossed with Hi27 in 1969 by Brewbaker (2012). The fifth backcross to Hi27 was selfed in 95-974 from which the (fl v4) double mutant (lacking gl2 and lg) was observed in plot 98-2653 (the sixth backcross).

The highly branched tassel was designated Brta by Brewbaker and Huang (2009) after showing it to be monogenic. At the same time, another NIL (v4 fl)^{Hi27} selected by virescent seedling trait has 12.0 branches like the wild type Hi27, though it possessed the same double mutant with (fl v4)^{Hi27}.

In addition, in a trial planted 11/16/2007 under severe winter stress (low light, heavy rain, yield reduced 75%), several genes on the same chromosome with fl and v4 (chromosome 2) were studied. The seasonal effect was so obvious that the plants were very

weak and the branch numbers of all genotypes were dramatically decreased. However, we still observed a significant difference between (fl v4)^{Hi27} and (v4 fl)^{Hi27} in branch number. Additionally, there are other genes, including lg, gl2, sk, gs2, on chromosome 2 with fl and v4. By the Map Genetic 2008 2, gs2 is on 50.0, characterizing green stripe on leaf; gls2 is on 30.5, characterizing glossy and shiny bright green leaf. The two mutants have the normal tassel branch number as Hi27. sk1, silkless ears1 mutant, is on 57.0, showing an interesting result in this trial. A double mutant (sk1 fl) was employed to study the branch number. There is a significant difference between the homozygous (sk1 fl) inbred and heterozygous (sk1 fl)/(+ fl) in branch number. While (sk1 fl) homozygous averaged 0.4 branches, the (sk1 fl)/(+ fl) had an value of 1.80.

When I finally put all these genes in a line, it was concluded that there might be a factor that influenced branch number and located closely to fl gene, probably on the sk side. This hypothesis also can explain why the (v4 fl) mutant had normal tassel branch number because the long distance of v4 away from the sk side. So according to the above data, a hypothesis can be made that there is a gene located closely to floury-1 gene on chromosome 2, and it functions to increase the branch number.

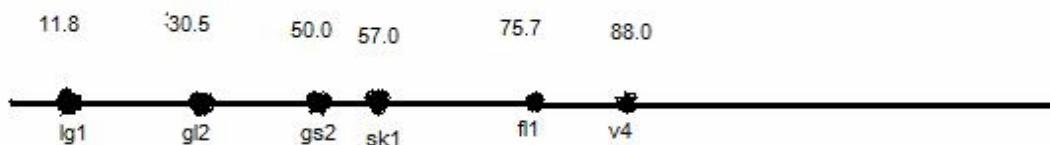


Figure 3-2 Arrangement for the mapped genes on chromosome 2

3.3.2 Population means

Average branch numbers were calculated in a generation mean analysis planted in February 2008, with Parent 1 being Hi27 and Parent2 being (fl v4)^Hi27 NILs (branched). In this trial, one row of (v4 fl)^Hi27 was planted instead of Hi27 due to their non-significant difference in the trait of interest as demonstrated in previous experiments. For the same reason, the other parent populations characterized by branched tassel consisted of two genotypes of (fl v4) and (fl v4)y NILs. Also, F1 populations included two hybrids of (fl v4)/(+ +) and (fl v4)y/(+ +)y. For the segregating populations B1, B2 and F2, the seeds were classified as normal and floury and planted on the separated plots, respectively.

Table 3-2 The tassel branch numbers for two parents, Hi27 and (fl v4) mutant, F1, F2 and backcross (BC1, BC2) generations.

Generations	Count	Mean	Variance	SE	CV
P1	40	11	3.95	0.31	18.10%
P2	40	20	6.2	0.39	12.40%
F1	60	15.7	8.4	0.37	18.50%
F2	80	15.8	12.3	0.39	22.20%
BC1	80	13.4	16.9	0.46	30.60%
BC2	80	16.9	10.1	0.35	18.80%

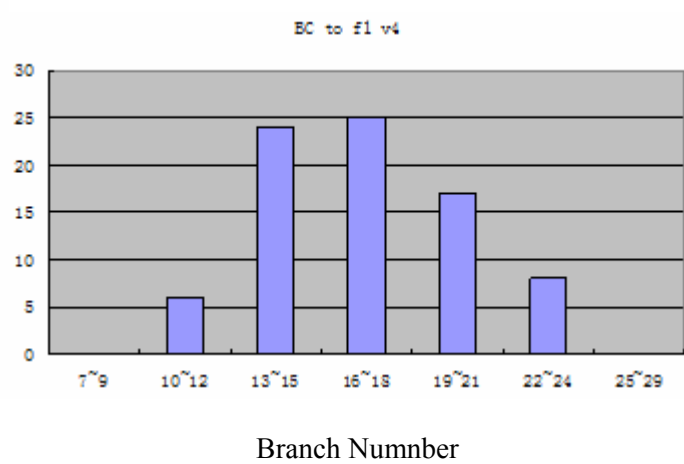
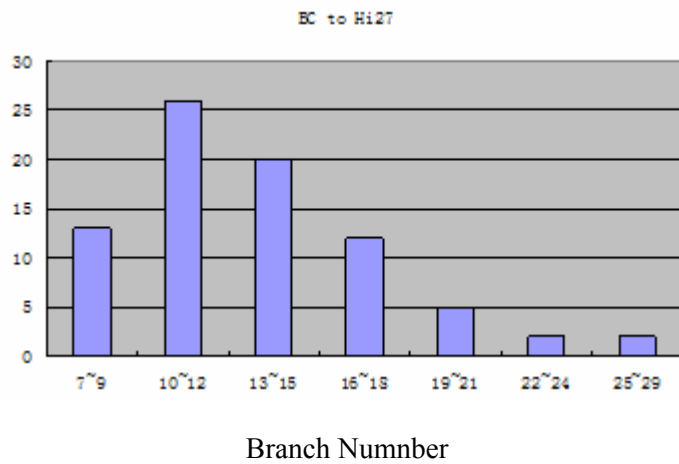
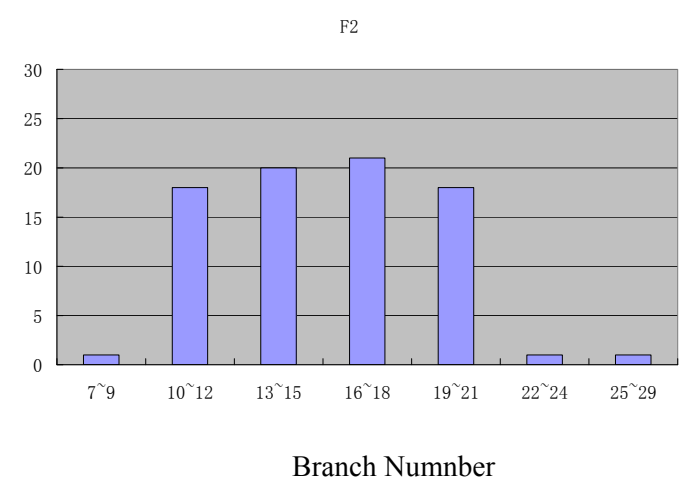
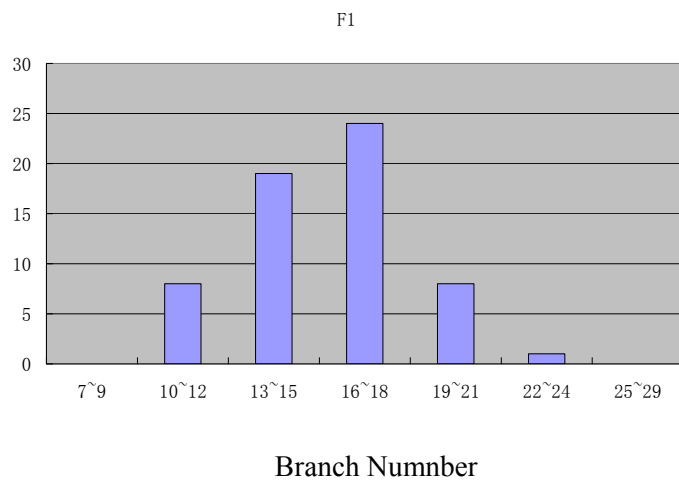
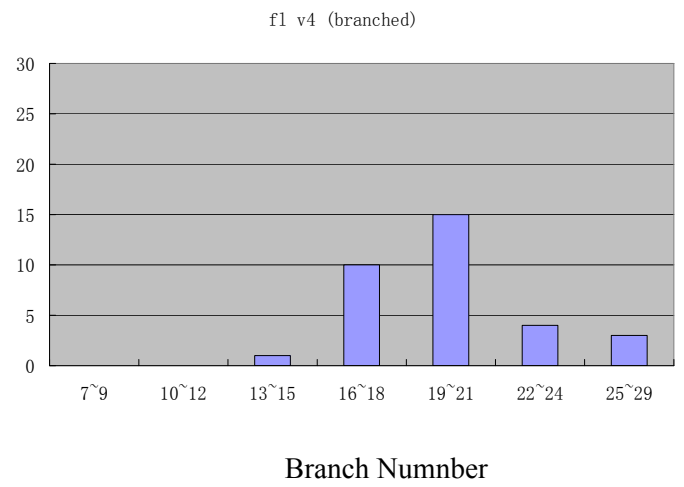
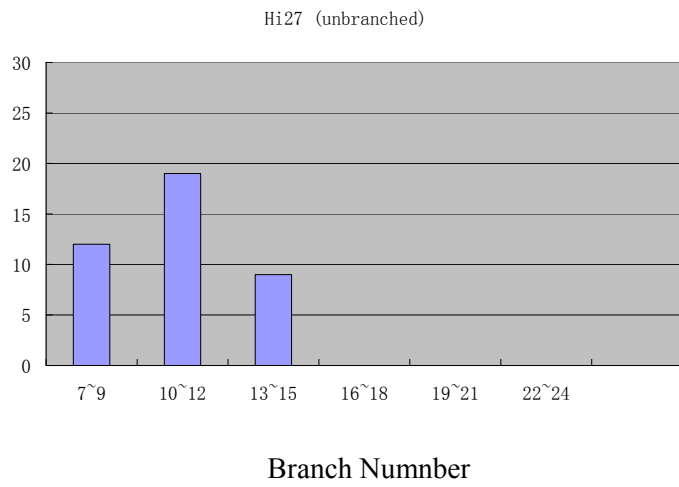


Figure 3-3 Frequency Distribution of tassel branch number in six generations of $Hi27 \times (f1 v4)^{Hi27}$.

In this experiment, P1 averaged 11.0 for tassel branch, while P2 averaged 20.0. This result was approximately compliance with our previous estimation that the (fl v4)^{Hi27} mutant was two times that of the wild type Hi27 for branch number. The average of F1 was on the mid-point of two parents, indicating a large additive effect. All the non-segregating population (P1, P2, F1) showed acceptable uniformity with a CV less than 20%.

The mean of F2 was nearly identical to the F1 but the variance was bigger due to segregation. BC1 (13.4) and BC2 (16.9) approximately located on the midpoint between P1 and F1, and between P2 and F1, respectively. It can confirm the large additive component. Frequency distributions of the six generations for branch number were graphed in 3-count intervals in Figure 3-2. From the distribution graphs, the modes of two parents are located on range 10~12 and range 19~21, respectively. The vast majority of F1 and F2 progenies were just between the values that represent their parents (between 10~12 and 19~21). It indicated no evidence of heterosis for my trait of interest. Actually, the parents of this GMA analysis were NILs of Hi27, and they differed only by a very short chromosome segment, so heterosis was not expected. Backcross distributions were a little skewed toward the few-branched P1 parent.

F2, B1 and B2 were the segregating generations in the GMA population for the studied trait. In each of the segregating generations, I classified seed into two groups by the kernel characters (flint vs floury), because the Brta gene appeared to be closely linked with the

floury-1 gene. Tassel branch numbers were collected and presented under this classification in table 3-3.

Table 3-3 Tassel branch numbers for Hi27 × (fl v4)[^]Hi27 GMA populations

Generation	Genotype	Counting No.	Mean	Stdev	CV
P1	HI27 or (v4 fl)	40	11	1.99	18.10%
P2	(fl v4) or (fl v4)y	40	20	2.49	12.40%
F1	HI27 ×(v4 fl) [^] Hi27	60	15.7	2.9	18.50%
F2	F1 self (+)	40	14.8	3.84	25.90%
	F1 self (fl)	40	16.7	2.9	17.40%
BC1	F1 ×Hi27 (+)	40	11.2	3.24	29.00%
	F1 ×Hi27 (fl)	40	15.5	3.73	24.10%
BC2	F1×(fl v4) (+)	40	15.7	2.86	18.20%
	F1×(fl v4) (fl)	40	18	3.09	17.20%

BC1 and BC2 generated very obvious monogenic effect for tassel branch number within their own generation respectively. The non-floury seed of BC1, which should not carry Brta in genome, averaged on 11.2. It is very close to the number of P1 (11.0). The floury seed of BC1, which should be the genotype of Brta/+, grew out the average of 15.5 for tassel branches. It is very close to the average of F1 (15.7). In the same way, BC2 should demonstrate two genotypes that were Brta/+ and Brta/Brta, which should have the comparable average with my F1 (15.7) and P2 (20.0) for the tassel branch, respectively. In this case, it seems to conform to the expectation.

F2 generation should present 1:2:1 segregation for monogenic character, especially for the gene with dosage effect. The overall average of F2 15.8 was in accordance with that

model. However, due to the limit I used the floury trait as the marker to group and the complexity in detailed level for the endosperm character, it is impossible to exactly classify the Brta character by employing the fl gene as markers in F2 generation.

3.3.3 Generation mean analysis

In order to further confirm and extend the conclusions I achieved above, the classic generation mean analysis was employed, although it generally is used on the GMA population formed by two parents that are genetically distant.

Generation mean analysis provided estimates of six parameters (gene effects) for the trait of branch number. (Table 3-4) Mean effects (m) were calculated simply as the mean of the F2 progeny. Additive (a) effect, derived by comparing B1 and B2 progenies (Gamble, 1962), was very significant in this study. In addition, dominance also showed significant effect, but relatively less so than additivity.

Table 3-4 Estimates of gene effects for branch number in the GMA combination

Parameters	Gene effects		
$m = \text{mean effect} =$	15.80	\pm 0.21	**
$a = \text{additive effect} =$	-3.50	\pm 0.30	**
$d = \text{dominance eff.} =$	-2.40	\pm 1.07	*
$aa = \text{add} \times \text{add} =$	-2.60	\pm 1.03	*
$ad = \text{add} \times \text{dom} =$	1.00	\pm 0.34	*
$dd = \text{dom} \times \text{dom} =$	4.40	\pm 1.57	*

Additionally, the minimum numbers of effective factors or gene loci governing the trait of interest was estimated as 1.64 by the Castle-Wright formula. It was approximately consistent with my conclusion that monogenic effect was identified.

3.4 Discussion

Mickelson and his colleagues use B73×Mo17 RILs population as materials to detect three quantitative trait loci (QTL) controlling tassel branch number on chromosome 2 (2002). Berke and Rocheford (1999) have the similar study by studying Illinois High Oil (IHO) as materials and found a QTL on chromosome 2 which functioned to affect tassel branch number.

Gene effects have varied expression magnitude under different background and environment. In the Hi27 near isogenic lines population and the tropical environment in Hawaii, the Brta loci showed a very significant effect and present an obvious dosage effect. It is very close to the floury gene and virescent gene on chromosome 2. The determining for the exact location will need further research on the molecular level.

CHAPTER 4

DIALLEL ANALYSIS OF TASSEL BRANCH NUMBER IN MAIZE

4.1 Introduction

This study was conducted to determine general (GCA) and specific (SCA) combining ability effects for tassel branch number in maize through diallel analysis. Eight elite maize inbred lines representing diverse heterotic groups with tropical and temperate backgrounds were crossed in a diallel. The 28 hybrids and 8 inbred parents were evaluated in trials planted at Waimanalo in March 2009, May 2009, December 2009 and September 2011. An earlier set of data from a planting in December 2008 was discarded due to bad germination and weak plants during a severe winter season.

4.2 Materials

4.2.1 Maize inbred lines

Eight tropically adapted maize inbred lines originating from different research institutions were employed in this study (Table 4-1). These inbreds were elite and widely used in hybrids and they ranged greatly in origin (tropical, temperate) and type (dent, flint). All 8 inbreds had been converted to resistance to Maize Mosaic Virus in Hawaii (Brewbaker, 1997; Brewbaker and Josue, 2008).

Table 4-1 Maize inbred lines for diallel analysis

Inbred	Source	Origin	Seed type	Breeder
Hi53	ICAL210	Cuban Flint 5832#	Tropical flint	Arboleda
Hi57	Ki9	Suwan 1(S)C4(=KU1409)	Tropical flint	Sujin/Sutat
Hi60	Mo17	XI187-2×C103	Corn belt dent	Zuber
Hi61	N3y	White dents (=SR52F)	Southern dent	Nelson
Hi62	Pi17	Tropical×Temp	Tropical flint	Logroño
Hi65	Tx601	Yellow Tuxpan	Tropical dent	Bockholt
Hi67	Tzi18	SeteLagoas TZSR×7729	Tropical flint	Kim
Hi26	Hi26	CM202(=CI21E)	Southern dent	Brewbaker

4.2.2. Diallel hybrids

The eight inbreds were crossed in a diallel manner excluding reciprocals (Griffing 1956, Method 2) by Dr. A. Josue during the fall of 2003 and spring of 2004 at Waimanalo Research Station. Each inbred was used as either a male or female and the F1 seeds were bulked at harvest. Additional crosses were made in 2008 to supplement seed supplies. In the evaluation trials the hybrids and parents were blocked separately and randomized within blocks. Hybrid H1035 (Hi26 x Hi63) was used as the hybrid and inbred borders.

Entries were grown in two row plots 5 m long with rows spaced 0.75 m apart. Two seeds were planted per hill using a hand jab planter at 0.25 m spacing. They were thinned to one plant per hill(53,333 plants per hectare) at around the 5 to 8 leaf stage. Diallel entries were grown and evaluated in a series of plantings that began in May and July of 2009, February of 2010, followed by November 2011.

4.3. Methods

4.3.1 Waimanalo environmental conditions

At the Waimanalo Research Station the mean monthly temperature recordings during the growing period from 2008 to 2010 ranged from 21.6 to 26.5 °C, the lowest of which occurred in January and highest in August and September (Figure 2-1). The trends in temperature followed the temperature records obtained from 1989 to 2009 at Waimanalo.

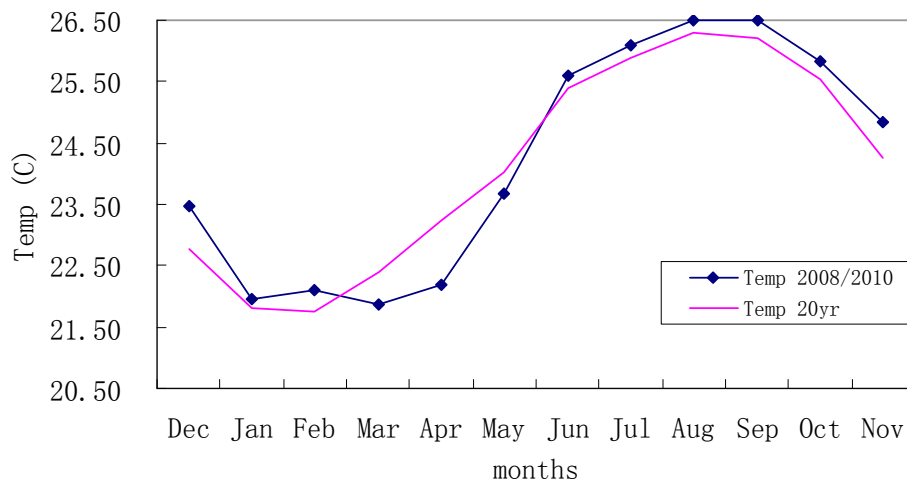


Figure 4-1 Average temperature from 2008-2010 and 20 years period (1989-2009) at Waimanalo, Hawaii.

4.3.2 Traits measured

The primary trait considered in this study was the branch number of the maize male inflorescence. Tassel branch numbers included both the primary and secondary branches. Counting was conducted in the field at the stage that the tassels were completely emerged. The inbred parents generally matured later than their hybrid counterparts, so the date of measurements was about one week earlier for hybrids than for inbreds. For each entry ten

representative plants were selected as samples to count and record in 2009/2010 trials, while five plants were counted in 2011 trial.

4.3.3 Diallel analysis

A diallel series of crosses provides plant breeder and geneticist with a unique look at the performance of the parental lines in different cross combinations and insight into gene action controlling expression of the traits under study. A full diallel cross is comprised of a series all possible combinations of single crosses among “n” parents, the number of subsequent F1 crosses represented by n^2 . A partial or half-diallel would combine “n” parents in half of the possible combinations to result in $[n(n-1)/2]$ crosses. Choice of a half-diallel is traditional when there is no implied evidence of maternal inheritance.

There are two well-established models for diallel analysis devised by Griffing (1956) and by Gardner and Eberhart (1966) that are used to estimate genetic effects from a diallel. Basic assumptions of these models include diploid inheritance, two alleles per locus, and no epistasis. These assumptions limit extrapolation from the results and have invited criticism of this type of analysis (Baker, 1978).

Diallel analysis with both approaches provides estimations of genetic effects by partitioning them into general and specific combining abilities. The general combining ability, GCA, is the effect that the parent has on all of its progeny with different parents. GCA is the expression of the additive genetic effects, those that can be most easily influenced by selection and where the plant breeder can make rapid gain

through selection. Specific combining ability, SCA, represents the departure from additive effects and is represented by a parent's superior or inferior performance in a cross with another specific parent when compared to expectations based on parental average performance. SCA includes non-additive effects like dominance, epistasis, or multiplicative gene action (Sprague and Tatum, 1942)

The diallel analysis of this set of data followed the example of Problem 8b in "Quantitative Genetics on a Spreadsheet" (Brewbaker, 2003), in which data were taken from replicated block trials. In my analysis the data are sample data within rows of the hybrids and inbreds, and replication-based error is supplanted with sampling error for analysis. The analysis generally was based on four sets of data with varied samples for the 36 entries. All data were taken in corn research fields at Waimanalo areas in which replication effects for small trials of this type (36 rows) were rarely noted for yield over 30 years of study of Dr. Brewbaker and his students. The data were subjected to both fixed model (GCA and SCA effects) and random model (heritabilities) following the textbook examples.

Among the four sets of data, the set planted on March of 2009 includes two replications. In the subsequent analysis, the two blocks of data were combined into one set in order to have a clear contrast with the other three sets from different planting months. T tests were made between the two replications of data before combining and no significant difference was found, which is consistent with our long term observation as mentioned

above. The other three sets of data are from the planting on May 2009, December 2009 and September 2011, respectively.

4.4 Results

4.4.1 Mean performance and analysis of variance

Inbreds had an average branch number of 14.2 over the five sets of data (Table 4-2). They ranged from 3.4 for inbred Hi60 (temperate, Missouri) to 24.4 for Hi67 (tropical, Nigeria). The branch numbers were essentially identical in the four plantings, ranging from 14.0 in the 9/2011 planting to 14.6 in 5/2009.

Table 4-2 Mean branch numbers of inbreds from four planting dates

Entry	Pedigree	Branch Number				Mean
		3/2009	5/2009	12/2009	9/2011	
Hi53	ICAL210	14.9	17.4	16.0	16.2	16.1
Hi57	Ki9	14.1	19.2	15.3	16.0	16.2
Hi60	Mo17	3.2	2.7	2.1	5.6	3.4
Hi61	N3y	20.1	13.3	16.7	15.6	16.4
Hi62	Pi17	12.8	10.2	12.7	9.8	11.4
Hi65	Tx601	10.6	17.2	15.3	17.6	15.2
Hi67	TZi18	26.8	24.0	27.7	19.0	24.4
Hi26	Hi26	10.4	12.4	8.3	12.4	10.9
Inbred Means		14.1	14.6	14.3	14.0	14.2

Hybrids had an average branch number of 18.0 over the four planting dates (Table 4-3). The lowest number occurred in the 9/2011 planting (17.4), followed by the 12/2009 (17.6) and 5/2009 (17.8) plantings.

Table 4-3 Mean branch number of hybrids from three planting dates

Entry	Pedigree	Branch Number					MPH (%)
		3/2009	5/2009	12/2009	9/2011	Mean	
Hi53×Hi57	ICAL210×Ki9	23.5	25.4	21.5	23.8	23.6	46.00%
Hi53×Hi60	ICAL210×Mo17	16.3	16.3	11.1	15.6	14.8	51.80%
Hi53×Hi61	ICAL210×N3y	28.9	23.1	27.7	20.6	25.1	54.20%
Hi53×Hi62	ICAL210×Pi17	17.9	17.4	18.3	18.6	18.1	31.40%
Hi53×Hi65	ICAL210×Tx601	22.6	23.9	21.3	22.6	22.6	44.50%
Hi53×Hi67	ICAL210×Tzi18	29.8	26.0	30.2	29.8	29.0	43.10%
Hi53×Hi26	ICAL210×Hi26	22.6	18.2	21.9	13.8	19.1	41.70%
Hi57×Hi60	Ki9×Mo17	16.1	15.0	10.7	15.8	14.4	47.30%
Hi57×Hi61	Ki9×N3y	19.4	19.4	19.4	24.8	20.8	27.40%
Hi57×Hi62	Ki9×Pi17	21.6	17.8	15.2	18.4	18.3	32.70%
Hi57×Hi65	Ki9×Tx601	20.3	24.3	19.9	23.0	21.9	39.70%
Hi57×Hi67	Ki9×Tzi18	24.8	25.8	23.8	22.2	24.2	19.20%
Hi57×Hi26	Ki9×Hi26	19.8	19.1	19.6	18.2	19.2	42.00%
Hi60×Hi61	Mo17×N3y	10.9	11.4	10.4	9.4	10.5	6.20%
Hi60×Hi62	Mo17×Pi17	11.5	8.0	10.0	12.2	10.4	41.10%
Hi60×Hi65	Mo17×Tx601	11.7	11.0	9.1	12.6	11.1	19.50%
Hi60×Hi67	Mo17×Tzi18	17.9	13.9	14.4	13.8	15.0	8.10%
Hi60×Hi26	Mo17×Hi26	13.2	10.9	9.7	12.0	11.4	60.40%
Hi61×Hi62	N3y×Pi17	15.4	13.4	17.3	11.8	14.5	4.20%
Hi61×Hi65	N3y×Tx601	19.8	22.2	17.4	16.6	19.0	20.30%
Hi61×Hi67	N3y×Tzi18	24.6	21.5	23.8	19.4	22.3	9.40%
Hi61×Hi26	N3y×Hi26	19.4	14.8	18.5	18.4	17.8	30.20%
Hi62×Hi65	Pi17×Tx601	16.1	16.3	15.9	12.4	15.2	14.40%
Hi62×Hi67	Pi17×Tzi18	21.1	19.7	18.7	17.4	19.2	7.60%
Hi62×Hi26	Pi17×Hi26	17.5	14.3	13.2	13.4	14.6	31.30%
Hi65×Hi67	Tx601×Tzi18	22.3	20.9	21.9	19.4	21.1	6.80%
Hi65×Hi26	Tx601×Hi26	17.3	15.7	14.0	15.6	15.7	20.20%
Hi67×Hi26	Tzi18×Hi26	15.5	12.8	17.7	16.8	15.7	-10.90%
Hybrid Means		19.2	17.8	17.6	17.4	18.0	28.20%

*MPH =mid parent heterosis (%)

Analysis of variance for branch number (Table 4-4) revealed significant differences among planting dates ($P < 0.05$). It was clear that the 9/2011 planting had the lowest branch number for all entries. The greatly reduced temperature and terrible weather of winter in

Waimanalo can account for these performances. Variation among the inbreds and hybrids were significant ($P < 0.01$). The comparison between parents group and hybrids group was also significant ($P < 0.01$), which is consistent to the result that heterosis was significant. The interaction effects of dates and entries were not significant.

Table 4-4 ANOVA for tassel branch number across planting dates

Source of Variance	Df	SS	MS	F	F0.05	F0.01
Times	3	41.75	13.92	3.07*	2.69	3.97
Entry	35	3747.92	107.08	23.62**	1.54	1.83
Parents	7	1009.62	144.23	31.81**	2.10	2.81
Hybrids	27	2384.66	88.32	19.48**	1.59	1.92
P vs H	1	353.63	353.63	77.99**	3.93	6.88
T × E	105	476.08	4.53	0.11	1.25	1.37
Sampling Error	1129	44836.76	39.71			
Total	1272	49102.51				

Mean F1 performance ranged from 10.4 for Hi60×Hi62 to 29.0 for Hi53×Hi67. Hybrids with comparably low branch numbers were Hi60×Hi61 and Hi60×Hi65, possessing 10.5 and 11.1 branches, respectively. Hybrid Hi60×Hi61 was the lowest for branch number in the 3/2009 (10.9) and 9/2011 (9.4) plantings. For the 12/2009 planting, Hi60×Hi65 (9.1) was the lowest, followed by Hi60×Hi26 (9.7), and Hi60×Hi62 (10.0). Hi60×Hi62 (8.0) from 5/2009 planting possessed the lowest number in our record. On the other hand, the higher branch number hybrid lines besides Hi53×Hi67 (29.0) were Hi53×Hi61 (25.1), Hi57×Hi67 (24.2), Hi61×Hi67 (22.3), Hi53×Hi57 (23.6). In all planting dates hybrid Hi53×Hi67 had the highest branch number (30.2) in 3/2009 trial.

4.4.2 Heterosis for branch number

Hybrids consistently exceeded inbreds in tassel branch number (comparing Tables 4-2 and 4-3). Mid-parent heterosis (MPH, %) was calculated by dividing the difference between hybrid branch number and average of its parents by the mid-parent value. These MPH values averaged 28.2% showing significant heterosis for the branch number. The MPH values ranged from -10.9% for Hi67×Hi26 to 60.4% for Hi60×Hi26 (Table 4-3). Hybrid Hi53×Hi60, Hi53×Hi61, Hi57×Hi60 had comparably high MPH values of 51.8%, 54.2%, 47.3%, respectively.

MPH values were then calculated separately for the 8 parental inbreds (Table 4-5). As noted the values averaged 31.0% overall, but they ranged significantly from 13.4% for Hi67 to 48.0% for Hi53. The data suggest differences in genes governing branching among the eight parents. However the data within parents generally ranged widely and the eight values were poorly correlated ($R^2 = 22.3\%$) with inbred average branch numbers, inferring no simple GCA variations.

Table 4-5 Mean MPH values for the 7 hybrids involving each of the 8 inbred parents

	Hi53	Hi57	Hi60	Hi61	Hi62	Hi65	Hi67	Hi26
	46.00%	47.30%	6.20%	4.20%	14.40%	6.80%	-10.90%	41.70%
	51.80%	27.40%	41.10%	20.30%	7.60%	20.20%	43.10%	42.00%
	54.20%	32.70%	19.50%	9.40%	31.30%	44.50%	19.20%	60.40%
	31.40%	39.70%	8.10%	30.20%	31.40%	39.70%	8.10%	30.20%
	44.50%	19.20%	60.40%	54.20%	32.70%	19.50%	9.40%	31.30%
	43.10%	42.00%	51.80%	27.40%	41.10%	20.30%	7.60%	20.20%
	41.70%	46.00%	47.30%	6.20%	4.20%	14.40%	6.80%	-10.90%
Mean	48.00%	39.40%	37.40%	20.90%	24.90%	28.50%	13.40%	35.80%

Branch numbers for the 8 inbreds were compared with their corresponding hybrid array means and all were statistically significant (Table 4-6). The hybrid array means across planting dates were highest for Hi53 (21.7), followed by Hi67 (20.9) and the lowest for temperate inbred Hi60 (12.5). The hybrid array means were consistently lowest for Hi60, which were 13.9, 12.4, 10.8 and 13.1 for the four planting dates. The hybrid of Hi53 were highest for the array means in 3/2009 (23.1), 5/2009 (21.5) and 12/2009 (21.7) plantings, while Hi57 had the highest in the 9/2011 (20.9) trial. Correlation analysis was conducted between inbred line means and their hybrid array means. A highly significant average of 0.88 occurred across dates (Table 4-6). The correlation coefficient (r) was highest in the 5/2009 planting (r=0.90, P<0.01), followed by 12/2009 (r=0.86, P<0.01) and 9/2011 (r=0.85, P<0.01).

Table 4-6 Correlations between inbred means and hybrid array means for branch number within the three planting dates.

	Inbred Mean					Array Mean				
	3/2009	5/2009	12/2009	9/2011	mean	3/2009	5/2009	12/2009	9/2011	mean
Hi53	14.9	17.4	16.0	16.2	16.1	23.1	21.5	21.7	20.7	21.7
Hi57	14.1	19.2	15.3	16.0	16.2	20.8	21.0	18.6	20.9	20.3
Hi60	3.2	2.7	2.1	5.6	3.4	13.9	12.4	10.8	13.1	12.5
Hi61	20.1	13.3	16.7	15.6	16.4	19.8	18.0	19.2	17.3	18.6
Hi62	12.8	10.2	12.7	9.8	11.4	17.3	15.3	15.5	14.9	15.7
Hi65	10.6	17.2	15.3	17.6	15.2	18.6	19.2	17.1	17.5	18.1
Hi67	26.8	24.0	27.7	19.0	24.4	22.3	20.1	21.5	19.7	20.9
Hi26	10.4	12.4	8.3	12.4	10.9	17.9	15.1	16.4	15.3	16.2
Mean	14.1	14.6	14.3	14.0	14.2	19.2	17.8	17.6	17.4	18.0
Correlation Coefficients						0.78	0.90	0.86	0.85	0.88

4.4.3 Diallel analysis for tassel branch number

Diallel analysis was conducted to estimate general combining ability (GCA) and specific combining ability (SCA) effects for maize tassel branch number. General combining ability effect is a measure of additive gene effects, while specific combining ability is a measure of non-additive gene effects. Diallel analysis was based on Griffing's (1956) Method 2, Model 1 (Fixed effects Model) analysis, which included the parents without the reciprocal crosses and spreadsheet methods of analysis were adopted from Problem 8b of "Quantitative Genetics on a Spreadsheet" (Brewbaker, 1994).

The analysis of GCA and SCA on the 28 F1 hybrids for tassel branch number was conducted across the four Waimanalo planting dates (Table 4-8). For the combined analysis of GCA effects, Hi67 had the highest GCA (4.1), followed by Hi53 (3.0). The lowest GCA was -6.0 of Hi60. For the analysis of SCA effects, Hi67×Hi26 was observed to have the lowest SCA (-3.6), while Hi53×Hi67 the highest (4.73). Hybrids with higher SCA effects also included Hi53×Hi61 (4.1), Hi57×Hi65 (2.48), Hi53×Hi65 (2.21).

Table 4-7 Tassel branch numbers of inbreds and their hybrid combinations across Waimanalo planting dates.

	Hi53	Hi57	Hi60	Hi61	Hi62	Hi65	Hi67	Hi26	Array mean
Hi53	16.1	23.6	14.8	25.1	18.1	22.6	29.0	19.1	21.7
Hi57		16.2	14.4	20.8	18.3	21.9	24.2	19.2	20.3
Hi60			3.4	10.5	10.4	11.1	15.0	11.4	12.5
Hi61				16.4	14.5	19.0	22.3	17.8	18.6
Hi62					11.4	15.2	19.2	14.6	15.7
Hi65						15.2	21.1	15.7	18.1
Hi67							24.4	15.7	20.9
Hi26								10.9	16.2

Table 4-8 GCA effects (on diagonal) and SCA effects (below diagonal) for tassel branch number across Waimanalo planting dates.

	Hi53	Hi57	Hi60	Hi61	Hi62	Hi65	Hi67	Hi26
Hi53	2.99							
Hi57	1.40	1.99						
Hi60	0.66	1.24	-6.00					
Hi61	4.10	0.77	-1.46	0.82				
Hi62	0.06	1.25	1.41	-1.35	-2.16			
Hi65	2.21	2.48	-0.30	0.78	-0.07	0.23		
Hi67	4.73	0.92	-0.23	0.26	0.15	-0.36	4.07	
Hi26	0.89	1.95	2.21	1.71	1.52	0.18	-3.60	-1.94

Analysis of variance for GCA and SCA effect was also conducted using the combined data from different dates. Both GCA and SCA effects were highly significant ($P < 0.01$). The relative importance of GCA and SCA effects was assessed using the mean square ratio of GCA to SCA. In this case, the value of the ratio is 15.7 showing greater contribution of additive gene effects.

Table 4-9 Analysis of variance for combining ability effects for tassel branch number across Waimanalo planting dates.

Source	df	SS	MS	F (fixed)	F 0.05	F 0.01
GCA	7.00	746.07	106.58	94.03**	2.10	2.81
SCA	28.00	190.12	6.79	5.99**	1.58	1.91
ERROR	105.00	119.02	1.13			
Ratio of GCA:SCA 15.70						

Heritability of tassel branch number was also calculated by use of the data in this trial. The narrow sense and broad sense heritability were 74.6% and 95.8%, respectively. It indicated that the additive effects played a primary role in the heredity of tassel branch number. In addition, the dominant effect and other genetic effects also make some influence on the performance of tassel branch number, consistent with the fore mentioned result that specific combining ability effect was significant.

4.5 Discussion

The inbred materials used in this study represent different heterotic groupings originating from different geographical locations. In Hawaii, these inbreds were selected during several generations of inbreeding and backcrossing to incorporate resistance to maize mosaic virus, rusts, blights and other disease. (Brewbaker, 1997). Inbred branch numbers maximized for Hi67 (27.0), an inbred derived from the tropical flint Tzi18. The lowest inbred number (3.3) was Hi60, derived from the corn belt dent Mo17. The tropical varieties generally were more close to the ancestor of corn which may need to have a

bigger tassel to propagate in wild condition. The temperate counterparts were bred to adapt to the high density planting and mechanical harvest, in which small tassels were enough to provide the pollen and favorable for increasing yield.

. Mid-parent heterosis was positive and large (average 28%) for essentially all hybrids. Inbred tassel branch numbers were highly correlated with their corresponding hybrid array means ($r = 0.733$). Highest hybrid array means were observed for Hi53 and Hi67. Variations among planting dates were also highly significant for tassel branch number related to large seasonal differences in light and temperature across the Waimanalo growing planting dates.

General combining ability (GCA) and specific combining ability (SCA) effects were determined for tassel branch number across the three Waimanalo planting dates. Information on the genetic control was based on GCA and SCA mean squares, measures of additive and non-additive effects, respectively. Based on the magnitudes of GCA effects, inbreds Hi53 and Hi67 consistently increased tassel branch number, while inbred Hi60 (Mo17) always reduced the number in all different temperature and light condition.

High ratios of GCA to SCA mean squares were observed for tassel branch number indicating that genetic control is largely due to the additive type of gene action. The ratio of GCA to SCA mean squares for tassel branch number was 15.7 for the combined analysis. It confirms the major role of GCA effects which is consistent with the previous studies by other people. Schuetz and Mock (1978) used GMA and an analysis of tassel branch numbers and their implications in breeding for small tassels. Five of these set of six

single-crosses showed significant additive effects, while non-additive effects were significant in three of the six. They proposed that additive, dominance and epistatic gene actions all influenced the inheritance of tassel branch number, but additive gene action was the most important.

With the prevalence of GCA and additive gene effects for tassel branch number, this trait can be altered with breeding methods such as those used in population improvement. Hybrid breeding methods that help exploit non-additive effects could also be used, considering the small but significant SCA effects for tassel branch number we observe. In my related studies of year-round plantings (Chapter 6) the seasonal effects at Waimanalo on branch number can be very great, and genotype \times environment interactions may also become a significant factor in breeding for tassel branch number. The breeding and evaluation in multiple planting dates through the year for this study may be required.

CHAPTER 5

TASSEL BRANCH NUMBERS IN RECOMBINANT INBRED LINES

5.1 Introduction

Recombinant inbred lines (RILs) are produced from the hybrid of two parent inbreds by inbreeding with single-seed descent or by dihaploidization. These inbreds represent recombinations of parental genes in relatively homozygous lines that can be repeatedly evaluated. The RILs provide a relatively clear view of segregating QTLs (quantitative trait loci) affecting the character being studied, to the extent that the recombinations are not affected by linkage drag, epistasis, and mean:variance correlations. A large series of RILs was created in Hawaii by Dr. Hyeun Gui Moon (1995) to evaluate quantitative trait variations among tropical and temperate maize inbreds. His data included tassel-branch numbers from two of the sets of RILs also used in this thesis study, Sets B and G.

Where segregations among RILs appear to involve few loci, statistical methods that relate the data to calculated probability curves can aid interpretation (Moon et al., 1995, 1999). These methods include the direct comparison of segregations with predicted curves by use of chi-square or least squares analysis (Brewbaker, 2003, Ex. 10c). Moon (1995) interpreted his data on Set B and G RILs as best fitting a digenic model. The model assumed high variances for genotypes similar to those he observed for his relatively small sets of data on the parental inbreds. Moon's model errs in that it implicates an F₂ segregation of two loci with heterozygotes and homozygotes. The model is more correctly

suggested to be the 1:4:6:4:1 ratio of homozygotes that occur from a four-QTL model with each alleles (e.g., A vs. a) having similar quantitative effect and acting without epistasis or complications due to linkage. A similar three-gene model would create a 1:3:3:1 ratio of homozygous inbreds. The genetic interpretation of RIL segregations is often assisted by coordination with data on molecular markers, thus allowing more precise mapping of QTLs, as in the use of RFLP markers by Moon et al. (1995), Ming et al. (1997) and Mickelson et al. (2002).

5.2 Methods and Materials

Three sets of recombinant inbred lines that had been produced by Dr. H. G. Moon (Moon, 1995) were evaluated for tassel branch number variations. These populations were known as Set B, Set D and Set G and had the following parentage:

Set B: B73 (Iowa) x Nar330 (Colombia)

Set D: B73 (Iowa) x TZi4 (Nigeria)

Set G: Hi31 (Iowa) x Ki14 (Thailand)

Some of these inbreds have been further improved for performance in Hawaii (Brewbaker and Josue 2003) and are maintained by Hawaii Foundation Seeds (www.ctahr.hawaii.edu/hfs) as Hi47 (=B73), Hi68 (TZi4), Hi60 (Mo17) and Hi58 (Ki14). Inbred Nar330 is also known in the literature as Nariño 330, given by a breeder in Thailand to reflect its Colombian origin.

Sets B and D were planted in May and June 2011 in unreplicated 5m plots with the parental inbreds as checks in two plots. Five or six tassel-branch data were taken in these RILs and only from representative, healthy plants. Set G was planted with the parents in September 2012, and data taken on eight representative plants per RIL. These three trials represent the best season for corn growth at Waimanalo (Jong et al., 1982), with high homogeneity characterizing environment and the experimental area; i.e., essential freedom from weeds, diseases, pests. This homogeneity was evident in low CVs for the three sets of data, and most of the RILS were high in vigor and yield. One disease was present in these trials but not severe in the seasons these data were taken; this was MCMV (maize chlorotic mottle virus).

5.3 Results

5.3.1 Set B

Set B was derived from parent inbreds B73 and Nar330, also studied by Moon (1995). The parents averaged 5.5 and 18.6 tassel branches, a large difference (13.1 branches) that is typical of comparisons of temperate inbreds (B73 from Iowa) and tropical inbreds (Nar330 is from Colombia). There were 89 RILS that provided adequate tassel-branch data (six data per RIL), and these averaged 11.86 branches, approximately midway between the parents. The average standard deviation for the 89 values was 1.57, providing a CV of 13.3% for these data and indicating relatively low uncontrolled variation in the fields. The minimum RIL had 5.5 branches, comparable to the B73 parent. The maximum RIL had 24.7 branches and significantly exceeded that of the Nar330 parent. The RIL data

as percentages of the total 89 values are graphed in Figure 5.1, showing a near-normal curve peaking at 12 branches (essentially midpoint of the parents). However, ten of the 89 inbreds showed transgressive segregation, exceeding the high-branch parent to provide a curve skewed from normality. Analysis of the means and variances of the 89 inbreds revealed a correlation of 0.626 that is considered high enough to account for this skewness. Statistical tests for normality as discussed by Brewbaker (2003) require accurate assessment of variance of parental means, and these data were too limited to validate such a test, but deviation from normality (skewness) appeared to be significant. Moon's data for 94 Set D RILs were graphed in Moon et al. (1995; Figure 3f), and appear very similarly skewed to those from the present study in Figure 5.1. A major difference was that Moon's parental means were different than here, B73 at a high 9.1 tassel branches (vs. 5.1) and Nar330 somewhat low 16.4 (vs. 18.6). Averages for the RILs were similar (13.7 vs. 11.9). Interpreting his data from a twice-replicated trial with only one "typical" datum per plot, Moon (1995) concluded that the segregation could adequately be explained by his "digenic" model of QTLs acting without dominance, producing a highly significant F test ($F=103^{**}$). Moon's model applied to RILs is effectively of four identical QTLs acting without dominance or interaction, producing an expected RIL ratio of 1:4:6:4:1 with 0, 2, 4, 6, and 8 "dominant" alleles among the 16 genotypes. Multiple QTLs were inferred by Mickelson et al. (2002) to account for variation among 180 RILs from B73 x Mo17. In their study B73 averaged 7.1, Mo17 averaged 10.1, and RILs averaged 8.9 with very wide range of 3.8 to 26.8. Nine of the 180 RILs showed significant transgressive segregation. Six QTLs were tentatively mapped by use of molecular markers, of which one on

Chromosome 2 had a significant effect. B73 has an unusually erect tassel (Mickelson et al., 2002) with branch angle much less than that of Nar330. Variations of this trait were observed but showed no evident correlation with branch number, as noted also by Moon (1995).

5.3.2 Set D

Set D was derived from parent inbreds B73 and TZi4. They averaged 5.1 and 19.4 tassel branches in this trial, again reflecting a large difference in branch number (14.3) typical of temperate (B73) and tropical inbreds (TZi4 was bred in Nigeria by CTAHR graduate Dr. S. K. Kim). There were 73 RILs that provided tassel-branch data (5 plants per RIL), and these averaged 13.36 branches, slightly exceeding the midpoint between the parents. The average standard deviation for the 73 values was a high 4.71. This leads to a calculated CV of 35% for these data that suggests relatively high error variance possibly related to the type of QTLs involved or to field variability. The minimum RIL had 4.8 branches, comparable to the B73 parent. The maximum RIL had 25.2 branches, significantly exceeding that of the Nar330 parent. The RIL data are also graphed in Figure 5-1, showing a near-normal curve peaking at 14 branches (essentially midpoint of the parents). As in Set B, ten of the 73 inbreds of Set D exceeded the high-branch parent to provide a curve similarly skewed from normality. Analysis of the means and variances of the 73 inbreds revealed a correlation of 0.431 that perhaps accounts for this skewness. As in Set B it can be concluded for Set D RILs that several or many gene loci contribute to the genetic variation in tassel branch numbers.

5.3.3 Set G

Set G was derived from parent inbreds B68 and Ki14. The 90 RILs and their parents were planted in Sept. 2012 and 8 data were taken per RIL. The parents averaged 4.9 and 20.3 tassel branches in this trial, again reflecting the large difference in branch number (15.4) typical also of Sets B and D. There were 90 RILS that provided tassel-branch data, and these averaged 11.13 branches, slightly below the midpoint (12.1) between the parents. The average standard deviation for the 90 values was a low 1.53, leading to a calculated CV of 13.7% for these data. The minimum RIL had 4.25 branches, little less than that of the Hi31 parent. The maximum RIL had only 17.88, significantly fewer than that of the Ki14 parent. The RIL data are graphed in Figure 5-1, and show the closest fit to normality of the three sets of RILs. Notably there was no transgressive segregation of RILs exceeding branch numbers of the tropical parent, and there was no significant correlation ($r = .210$) of means and variances for these data. Dr. Moon recorded tassel branch data for 110 RILs of similar origin as used in the present study, together with a large number of other agronomic traits (Moon, 1995; Chapter 8.2). His parent Hi31 averaged 5.63 branches, Ki14 averaged only 9.83, and the 110 RILs averaged 7.97 (range 3.0 to 12.7). A graph of Moon's data (Moon, 1995; Figure 8.7) is similar to that for the data taken in 2012 (Figure 5.1), again only slightly skewed to the higher numbers. Ki14 was advanced through several cycles of recurrent selection and renamed Hi58, and it is not clear why the present inbred has a much higher branch number than when studied by Moon. When Moon's data were compared with his "digenic model" (i.e. 4 genes for RILs), the F value (38.9**) indicated a satisfactory fit. The data were significantly discrepant from his

“monogenic” model. Ming et al. (1997) studied the segregation of QTLs affecting resistance to maize mosaic virus (MMV) in Set G with the use of RFLP markers, establishing that a major controlling locus was on Chromosome 3. Moon (1995) also plotted segregations of many other traits in this extremely heterogeneous set of RILs, including tassel branch angle (Ki14 has a very lax tassel, Hi31 very upright). No obvious correlations were reported in these studies with tassel branch number.

5.4 Graphing and Discussion of RIL data

The three sets of RILs had very similar parentage with respect to tassel branching, with the temperate parents averaging about 5 branches and the tropical ones about 19 (Table 5-1). The averages of the RILs closely approximated the midpoints of these parents. Range values (Table 5-1) were similar for Sets B and D, with significant transgressive segregation for high branch number. Set G showed no transgressive segregation. The relatively high correlations of means and variances for Sets B ($r = .626$) and D ($r = .431$) are suggested to account for the transgressive segregations. The mean:variance correlation for Set G was a non-significant $r = .210$. Breeding inbreds with fewer branches from any of these three populations would be difficult due to their rarity. In contrast Mickelson et al. (2002) observed considerable transgressive segregation in North Carolina toward lower numbers in their study of RILs from B73 (10.1 branches) and Mo17 (7.5 branches). It is to be noted that these temperate inbreds grow much larger in temperate climates, flowering about 3 weeks later than in the short-day tropics (Jong et al., 1982) and obviously having

more tassel branches. In Hawaii’s winters both of these inbreds are badly dwarfed and often have less than 3 tassel branches.

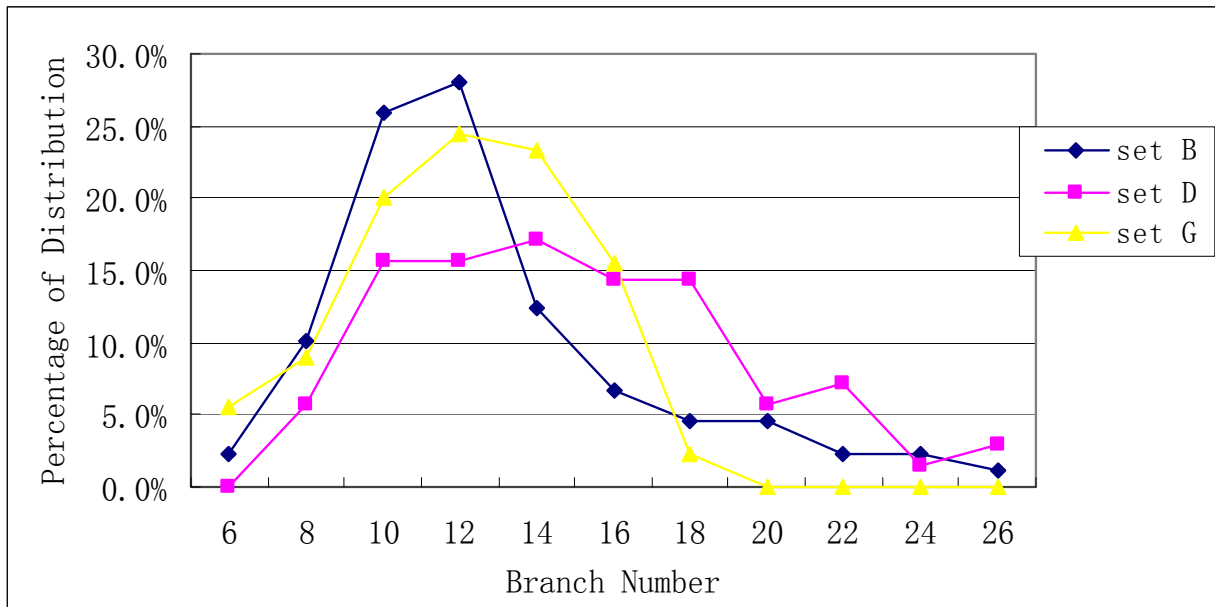
Table 5-1 Summary of tassel-branch data for RIL Sets B, D, and G. Data are numbers of tassel branches.

SET	Parents	P1	P2	MidPt	Avg. RIL	RIL Range	No. RILs
B	B73 x Nar330	5.1	18.6	11.8	11.9	5.5-24.7	89
D	B73 x TZi4	5.1	19.4	12.2	13.4	4.8-25.2	73
G	Hi31 x Ki14	4.9	20.3	12.6	11.1	4.3-17.9	90

The RIL data have been graphed in Figure 5-1 for the three sets, using the values converted to percents of the totals to allow direct comparison. While they are similar in many ways, Set G is the most normalized and approximates the 4-gene expected ratio of 1:4:6:4:1 (with approximate branch number values of 5:9:13:16:19). This is in effect Moon’s so-called “digenic model” for which his Set G data fit very well ($F=38.9^{**}$), i.e., a four QTL segregation among inbred lines. Heterosis for branch numbers is very great as reported in this thesis for diallels, but heterosis is not a factor influencing RIL data. It is concluded that these three sets of RILs are approximated well by a four-gene model of QTL segregation, where each locus acts similarly (e.g., alleles like “a” contribute 1.25 branches and alleles like “A” contribute 20/6 or 3.3 branches for variation from 5 to 20 tassels among parental inbreds. Transgressive segregation is best explained by the correlations of means and variances commonly observed for tassel-branch data and found to be significant here for Sets B and D. In their early studies of the heritability of tassel

branching, Sheutz and Mock (1978) concluded that their GMA data from six sets of hybrids could best be approximated by a model of five quantitative loci acting additively with some nonadditivity.

Figure 5-1 Tassel branch number distributions of three sets of RIL data expressed as percentage of the total number of inbred lines (89 for Set B, 73 for Set D, 90 for Set G)



CHAPTER 6

GENOTYPE BY ENVIRONMENT INTERACTION OF TASSL BRANCHING IN DIFFERENT SEASONS

6.1 Introduction

Tassel branch numbers have been observed by many authors to vary with the environment, in a way similar to most quantitative traits in maize. These variations are obvious in our studies in Hawaii that have been confined to growth at the Waimanalo Research Station, latitude 20N. Seasonal variations at this station are very great with respect to the growth and yield of maize, resulting in 100% differences in yield from winter to summer (Jong et al., 1982). Similarly, the sizes of maize plants and their tassels and ears can vary at least by a doubling from the short days of winter, with high cloud cover and low light intensity, to the long days of summer. It is less clear whether these environmental variations are represented also by differences in reactions of different genotypes, i.e., in G x E (genotype by environment) interaction. Jong et al. (1982) reported major GxE variations with respect to grain yield at Waimanalo, with temperate hybrids responding much greater to the short winter days and low light than did the tropical hybrids. Bechoux et al. (2000) reported that tassel branch numbers and spikelet pairs of two inbreds grown in greenhouse pots were differentially affected by chilling, unaffected by changes in light quality and flooding, and only slightly changed under drought and mineral deficiency.

It has been common in Waimanalo to observe major decreases in tassel branch numbers during Hawaii's winter season, which is characterized by short days, lower temperature, higher precipitation and more wind. The objectives of these studies were to quantify these environmental effects on tassel branch number and seek evidence for G×E interactions.

6.2 Materials and Methods

6.2.1 Plant materials

Eight different genotypes (inbreds and hybrids) were selected as the entries in this set of trials. These genotypes have quite distinctive genetic backgrounds. CML223 is a yellow flint inbred from Zimbabwe; GT601 is a yellow dent inbred derived from the population GT-MAS:GK in Georgia; Hi47 is the converted Iowa inbred B73; Hi1035 is a hybrid of temperate dent Hi26 and tropical flint Hi63; Hi27 is a tropical flint inbred derived from CM104, a Colombian line of Cuban Flint origin. The other three genotypes, d^{Hi27}, (fl v4)^{Hi27} and ra2^{Hi27} are near isogenic lines of Hi27, with mutants introgressed by at least six backcrosses. These genotypes broadly cover temperate and tropical variations in maize. Preliminary investigations had shown a large range in tassel branch numbers (~5 to 45) among these genotypes, while high uniformity existed within each. Therefore, these materials appeared to be adequately representative of germplasms to study the environmental effects and G×E of interested traits.

6.2.2 Methods

Entries were grown in two row plots 5 m long spaced 0.75 m apart. Two seed were planted per hill using a hand jab planter at 0.25 m spacing and thinned to one plant (53,333 plants per hectare) at around the 5-8 leaf stage. Three seasons were chosen for trials with two replications each in December of 2008 (12/2008), February of 2009 (02/2009) and April of 2009 (04/2009), respectively. The plant growth stages ranged from December of 2008 to June of 2009, including the more severe winter conditions (low temperature, short-day time, etc.) and the normal summer condition in Hawaii. Mean monthly temperatures were recorded during the growing period from Dec. 2008 to June 2009 (Figure 6.1). The lowest period occurred from January to April (around 22°C), and then the temperature began to quickly increase up to 25.6°C in June.

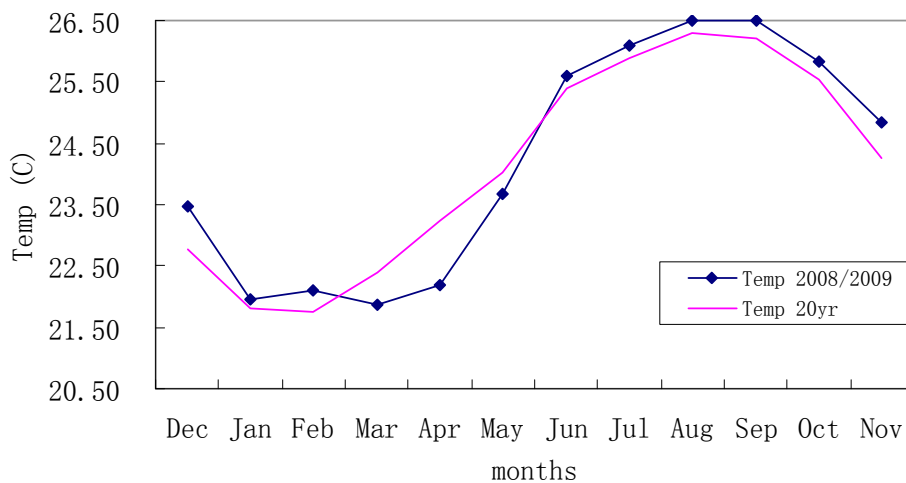


Figure 6-1 Average temperature from Dec. 2008 to Nov. 2009 and the comparable 20-year period (1989-2009) at Waimanalo Hawaii.

Additional environmental factors included the shorter daylength, reduced light and increased wind velocities of winter that contribute to the stressful conditions for corn

growth. These conditions are especially characteristic of windward areas of the islands of Hawaii (Brewbaker, 2003).

The traits considered in this trial were the number of lateral tassel branches, excluding the central spike in counting. Data were taken in the field when the tassel was completely emerged, generally 45-55 days after planting). Ten plants were randomly selected as the samples in each plot.

6.3 Results

Tassel branch numbers (TBN) differed greatly for the eight entries under the different environmental conditions of the three seasons of planting. These data are summarized in Table 6-1. Tassel branch numbers showed a tendency to increase as daylength and temperatures rose and weather became better (increased incident light, reduced precipitation, mild wind). Average branch numbers of the eight genotypes ranged significantly from 5.1 (Hi47) to 40.2 (ra2).

Table 6-1 Average tassel branch numbers for three distinctive environments in Waimanalo

Genotypes	12/2008		02/2009		04/2009		Mean
	Rep 1	Rep 2	Rep 1	Rep 2	Rep 1	Rep 2	
CML223	13.2	13.6	18.0	17.6	18.2	18	16.4
GT601	8.9	7.1	9.0	4.6	7.3	5.3	7.0
H1035	13.1	12.3	14.3	15.0	17.1	17.1	14.8
HI27	11.0	11.1	11.0	11.3	14.3	12.5	11.9
d1^Hi27	6.0	5.8	5.1	7.2	5.9	1.7	5.3
(fl v4)^Hi27	14.1	15.8	17.9	20.4	24.8	25.4	19.7
ra2^Hi27	44.4	43.0	42	41.5	31.3	39.0	40.2
Hi47	5.6	5.0	4.5	4.2	5.4	5.6	5.1
Season Mean	14.3		15.2		15.6		

Analyses of variance (Table 6-2) were conducted on the data summarized in Table 6-1 to study environment effects and the interaction of genotype and environment. The three seasons were significantly different ($P=0.01$) indicating environmental factors made a considerable effect for branch number between the three planting times. Replicates within each season shown no significant difference. Genotypes were highly significant as expected. Interaction of genotypes and environment in this study was significant in the analysis, indicating a differential response of some genotypes to the environmental factors.

Table 6-2 Analysis of Variance of pooled data for tassel branch number

Sources	df	SS	MS	F (fixed)	F0.05	F0.01
Total	407	47530.7				
Seasons	2	11.83	5.92	39.46**	9.55	30.82
Rep in season	3	0.45	0.15	0.00		
Genotypes	7	5558.59	794.08	270.16**	2.49	3.64
G×E	14	236.86	16.92	5.76**	2.20	3.07
Exp. Error	21	61.73	2.94	0.03	1.59	1.91
Sampling error	360	41661.24	115.73			

CV=19.53%

The sampling error for the 408 data was represented by a CV of 19.53% (Table 6-2). Exceptional variation uniquely characterized the mutant, d1^{Hi27}, which had high CVs (ranging from 28% to 54% for the three seasons). The mutant d1^{Hi27} is a dwarf that clearly was shaded by neighboring plots of normal plants, notably in the short-day winter season when the sun's angle at noon in Hawaii is about 43°. This environmental factor may

have increased the variance and unfairly affected the interpretation of G×E. Therefore a second calculation was made excluding the data for the d1^{Hi27} dwarf (Table 6-3).

Table 6-3 Analysis of Variance of pooled data for tassel branch number excluding d^{Hi27}

Source	d.f.	SS	MS	F (fixed)	F0.05	F0.01
Total	356	42516.97				
Seasons	2	19.01	9.50	12.93*	9.55	30.82
Rep in season	3	2.21	0.74	0.00		
Genotypes	6	4903.73	817.29	300.03**	2.66	4.01
G×E	12	223.01	18.58	6.82**	2.34	3.37
Exp.error	18	49.03	2.72	0.02	1.64	1.99
Sampling error	315	37319.99	118.48			

CV=16.56%

This calculation resulted in a reduced CV from 19.53% to 16.56%, and a reduction in the significance of Seasons from P=0.01 to P=0.05. There was however no essential difference in terms of the conclusions regarding significance of environmental effects and interaction of G by E.

Interaction of Genotype by Environment was showed by plotting the relation of the means of genotypes in different planting times (Figure 6-2). For the eight genotypes studied, I could classify them into three groups by their rough trending. The Group I including GT 601, Hi47 and d1^{Hi27}, represented insensitive varieties due to their little response to the changed environments. Group II comprised of Hi27, H1035, CML223, (fl v4)^{Hi27}, which showed a tendency of increasing branches from stressful winter to

favorable spring and summer. Group III is $ra2^{Hi27}$ in my study, showing a decline tendency for the branch number.

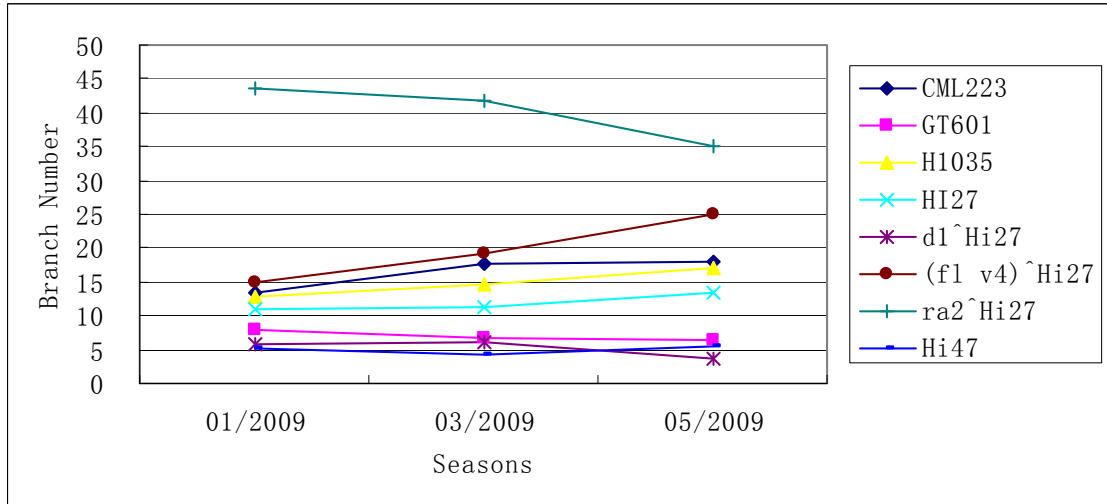


Figure 6-2 Plot for the TBN mean and planting times for different genotypes

6.4 Discussion

The number of tassel branches was influenced by both internal genotype and external environment. The interaction effect represents the response between biological character and nature. For interaction effect, the striking point is the three near-isogenic lines (NILs) of $Hi27$ and the wild type were spread into three groups I classified. Especially in the comparison between $(fl\ v4)^{Hi27}$ and $ra2^{Hi27}$, the former one showed a strong uptrend while the latter one performed an obvious declining tendency. Among the NILs, they are just one gene different one another, but the only different gene determined their varied response to the environmental changing in terms of branch number. In previous study, I observed that $Brta\ (fl\ v4)$ and $ra2$ have their own separate pathways in the process of affecting male inflorescence development. Mutant $ra2$ is a recessive gene, which

makes long branches instead of switching to make short branches (spikelet pairs) (McSteen, 2001). Brta is a co-dominant gene according to the previous study. The mechanism it branched the tassel is not clear yet, but it is obvious that Brta doesn't share the same mechanism with ra2 due to the different environmental response. It is probable that the two genes function in different stages of inflorescence development, so they generate diverging results when under the same environmental conditions.

Appendix Table 2-1 Tassel Branch Number for 215 Indigenous Races

Race	Primary branches	Secondary branches	Percent of Secondary (%)	Total
Races in west indies				
Cuban Flint	19.9	5.3	20.9	25.1
Haitian Yellow	21.7	8.9	29.1	30.6
Costal Tropical Flint	26.4	10.1	27.7	36.5
Maiz Chandelle	22.5	10.6	32.0	33.0
Early Caribbean	17.4	5.0	22.1	22.4
St. Croix	16.0	4.6	22.3	20.6
Tuson	22.5	6.5	22.3	28.9
			MEAN	28.2
Races in Venezuela				
Chirimito	34.0	12.7	27.2	46.7
Araguito	25.4	8.1	24.2	33.5
Pollo	14.9	4.4	22.8	19.3
Canilla Venezolano	26.2	7.7	22.7	33.9
Pira	27.5	8.7	24.0	36.2
Cariaco	29.4	7.6	20.5	37.0
Guaribero	31.0	12.6	28.9	43.6
Sabanero	25.8	10.4	28.7	36.2
Huevito	24.5	8.2	25.1	32.7
Puya	25.8	7.3	22.1	33.1
Tuson	26.4	8.2	23.7	34.6
Cuba Yellow Flint	25.7	6.0	18.9	31.7
Chandelle	26.2	8.0	23.4	34.2
Costeno	26.6	7.6	22.2	34.2
Puya Grande	24.6	5.6	18.5	30.2
Tuxpeno	26.3	8.0	23.3	34.3
Comun				
Negrito	27.8	8.3	23.0	36.1
Cacao	32.4	13.0	28.6	45.4
			MEAN	35.2
Races in Mexico				
A.Ancient Indigenous				
Palomero Toluqueno	3.5	0.1	2.8	3.6
Arrocillo Amarillo				
Chapalote	10.9	2.1	16.0	13.0
Nal-Tel	16.0	6.8	30.0	22.8
B.Pre-Columbian Exotic				

Cacahuacintle	5.2	0.4	8.0	5.6
Harinoso de Ocho	8.8	1.2	12.0	10.0
Sub-race Elotes Occidentales	8.0	0.8	9.5	8.8
Oloton	12.9	3.9	23.5	16.8
Maiz Dulce	15.3	2.8	15.4	18.1
C.Prehistoric Mestizos				
Conico	5.1	0.4	7.0	5.5
Reventador	7.9	0.5	5.9	8.4
Tabloncillo	7.8	1.0	11.5	8.8
sub-race Perla	11.5	1.7	13.0	13.2
Tehua	21.9	5.8	21.1	27.7
Tepecintle	19.4	5.3	21.5	24.7
Comiteco	17.4	3.9	18.3	21.3
Jala	15.7	2.2	12.3	17.9
Zapalote Chico	15.9	3.0	16.0	18.9
Zapalote Grande	20.9	3.0	12.6	23.9
Pepitilla	19.4	2.4	10.9	21.8
Olotillo	20.0	10.3	34.0	30.3
Tuxpeno	18.2	4.7	20.5	22.9
Vandeno	17.0	3.8	18.2	20.8
D.Modern Incipient				
Chalqueno	9.6	1.1	10.1	10.7
Celaya	17.6	3.5	16.8	21.1
Conico Norteno	14.3	3.2	18.3	17.5
Bolita	14.1	3.3	19.0	17.4
			mean	16.6
Races in Bolivia				
Confite Puneno	2.6	0.2	6.1	2.8
Altiplano	12.0	3.2	20.9	15.2
Patillo	8.4	0.5	5.4	8.9
Kcello	7.8	3.5	31.0	11.3
Kulli	10.4	1.6	13.5	12.0
Huilcaparu	15.7	7.2	31.3	22.9
Chake-Sara	14.0	3.8	21.2	17.8
Aysuma	8.4	1.5	15.4	9.9
Patillo Grande	13.4	2.8	17.3	16.2
Checchin	10.8	1.9	14.7	12.7
Cuzco-Huilacaparú	12.8	3.8	22.7	16.6
Paru	14.7	3.2	17.9	17.9
Chuspillu	19.6	6.2	24.0	25.8

Cuzco Boliviano	11.7	4.4	27.1	16.1
Pisankalla	8.5	0.8	8.6	9.3
Uchuquilla	12.3	3.9	24.2	16.2
Karapampa	3.8	2.0	34.0	5.8
Argentino	22.7	7.8	25.5	30.5
Ninuelo	11.3	3.3	22.8	14.6
Camba	29.7	12.0	28.8	41.7
Morado	26.2	9.9	27.4	36.1
Perola	23.9	8.0	25.0	31.9
Yunqueno	27.0	8.0	22.8	35.0
Pojoso Chico	26.4	9.5	26.5	35.9
Cholito	26.4	8.7	24.7	35.1
Cubano Dentado	20.2	5.4	20.9	25.6
Cateto	23.0	6.9	23.0	29.9
Pororo	26.7	5.0	15.8	31.7
Coroico Blanco	33.6	9.8	22.6	43.4
Coroico Amarillo	30.8	9.4	23.3	40.2
Coroico	28.1	10.0	26.2	38.1
Enano	23.5	4.3	15.3	27.8
			mean	22.9
Races in Colombia				
A.Primitive				
Pollo	19.6	15.6	44.3	35.2
Pira	31.0	19.6	38.7	50.6
B.Races Probably Introduced				
Pira Naranja	37.6	20.6	35.4	58.2
Clavo	26.1	15.1	36.7	41.2
Guirua	25.5	18.1	41.5	43.6
Cariaco	30.2	15.9	34.4	46.1
Andaqui	24.9	17.2	40.8	42.1
Imbricado	26.8	14.4	35.0	41.2
Sabanero	18.6	11.4	38.0	30.0
C.Colombian Hybrid Races				
Cabuya	24.7	19.2	43.8	43.9
Montana	29.3	20.8	41.5	50.1
Capio	20.7	21.3	50.7	42.0
Amagaceno	28.2	16.1	36.4	44.3
Comun	27.7	16.8	37.8	44.5
Yucatan	27.7	15.9	36.4	43.6
Cacao	25.6	15.4	37.6	41.0
Costeno	25.4	11.5	31.2	36.9

Negrilo	20.8	7.3	26.0	28.1
Puya	24.0	9.8	29.0	33.8
Puya Grande	27.3	17.3	38.7	44.6
Chococeno	27.7	13.8	33.3	41.5
			mean	42.0
Races in Cuba				
White Pop	21.6	4.6	17.5	26.2
Yellow Pop	23.7	11.3	32.4	35.0
White Dent	17.0	3.1	15.4	20.1
Canilla	20.2	7.8	28.0	28.0
Tuson	27.5	13.8	33.3	41.3
Criollo	22.6	8.6	27.6	31.2
Argentino	25.7	8.9	25.8	34.6
				30.9
Races in Chile				
Harinoso Tarapaqueno	19.9	9.3	31.8	29.2
Choclero	24.8	7.3	22.8	32.1
Camelia	23.8	8.2	25.6	32.0
Curagua	36.3	6.9	16.0	43.2
Curagua Grande	27.9	6.1	17.9	34.0
Cristalino Chileno	24.2	5.6	18.7	29.8
Dentado Comercial	27.4	6.1	18.1	33.5
Aeaucano	18.6	4.3	18.9	22.9
Cristlino Norteno	16.9	3.8	18.2	20.7
Dulce(Evergreen)	18.9	3.4	15.2	22.3
Dulce(Golden Bantam)	17.0	3.5	16.9	20.5
			mean	29.1
Races in Ecuador				
Canguil	10.5	1.4	11.8	11.9
Sub-raza Grueso	12.6	0.9	6.9	13.5
Sabanero Ecuatoriano	20.1	8.4	29.5	28.5
Cuzco Ecuatoriano	12.2	4.4	26.5	16.6
Mishca	10.1	4.9	32.7	15.0
Patillo Ecuatoriano	10.9	2.4	17.9	13.3
Racimo de Uva	10.7	2.7	20.1	13.4
Kcello Ecuatoriano	17.3	7.6	30.6	24.9
Chillo	20.3	9.6	32.1	29.9
Chulpi Ecuatoriano	24.5	8.7	26.2	33.2
Morochon	19.6	8.7	30.7	28.3
Huandango	16.9	7.8	31.6	24.7
Monatana Ecuatoriano	18.8	4.4	19.0	23.2

Blanco Harinoso Dentado	21.6	10.0	31.6	31.6
Conico Dentado	22.7	1.1	4.6	23.8
Uchima	25.0	9.3	27.2	34.3
Clavito	22.5	7.6	25.2	30.1
Pojoso Chico Ecuatoriano	34.2	12.1	26.1	46.3
Tusilla	27.7	10.9	28.2	38.6
gallina	32.7	9.3	22.2	42.0
Candela	32.0	12.9	28.8	44.9
Chococeno	36.4	11.0	23.1	47.4
				28.0
Races in Peru				
Primitive Races				
Confite Morocho	10.7	4.2	28.2	14.9
Confite Puntigudo	9.0	2.8	23.7	11.8
Kculli	10.3	2.3	18.3	12.6
Confite Puneno	5.9	0.1	1.7	6.0
Enano				
Anciently Derived Races				
Huayleno	14.9	3.3	18.1	18.2
Chullpi	18.0	4.5	20.0	22.5
Granada	15.2	9.5	38.5	24.7
Paro	11.5	5.5	32.4	17.0
Morocho	9.9	2.0	16.8	11.9
Huancavelicano	11.8	3.0	20.3	14.8
Mochero	23.6	15.2	39.2	38.8
Pagaladroga	21.5	11.3	34.5	32.8
Chaparreno	25.8	14.2	35.5	40.0
Rabo de Zorro	16.0	12.6	44.1	28.6
Piricinco	25.0	11.0	30.6	36.0
Ancashino	15.7	6.3	28.6	22.0
Shajatu	18.8	12.7	40.3	31.5
Alazan	23.6	12.0	33.7	35.6
Sabanero	16.3	8.8	35.1	25.1
Uchuquilla	17.5	9.0	34.0	26.5
Cuzco Cristalion Amarillo	12.6	6.3	33.3	18.9
Cuzco	12.9	4.6	26.3	17.5
Pisccorunto	7.8		0.0	7.8
Lately Derived Races				
Arequipeno	18.4	13.5	42.3	31.9
Huachano	19.2	11.6	37.7	30.8
Chancayano	18.8	9.5	33.6	28.3

San Geronimo Huancavelicano	15.0	3.8	20.2	18.8
Perla	20.8	8.0	27.8	28.8
Rienda	20.5	19.3	48.5	39.8
Maranon	18.6	9.3	33.3	27.9
Chimlos	23.7	10.4	30.5	34.1
Cuzco Gigante	12.3	4.7	27.6	17.0
Introduced Races				
Pardo	12.9	8.1	38.6	21.0
Aleman	20.4	8.0	28.2	28.4
Chuncho	24.9	10.9	30.4	35.8
Arizona	16.5	5.3	24.3	21.8
Incipient And Imperfectly Defined Races				
Jora	21.1	8.6	29.0	29.7
Coruca	23.8	19.2	44.7	43.0
Morocho Cajabambina	15.8	5.6	26.2	21.4
Morado Canteno	12.8	5.6	30.4	18.4
Sarco	13.6	4.4	24.4	18.0
Perlilla				
			mean	24.6
Races in Brazil and adjacent areas				
1.Indigenous				
Moroti	24.4	5.8	19.2	30.2
Moroti Precoce	19.8	5.6	22.0	25.4
Moroti Guapi	20.9	5.6	21.1	26.5
Caingan	18.7	4.0	17.6	22.7
Lenha	20.2	2.6	11.4	22.8
Entrelacado	26.3	5.8	18.1	32.1
2.Ancient Commercial				
Cristal Sulino	20.3	4.2	17.1	24.5
Cristal	22.3	5.0	18.3	27.3
Cristal Semi-Dentado	22.9	4.6	16.7	27.5
Canario de Ocho	15.0	3.9	20.6	18.9
Cateto Sulino Precoce	21.5	4.5	17.3	26.0
Cateto Sulino	18.7	3.9	17.3	22.6
Cateto Sulino Escuro	20.0	4.5	18.4	24.5
Cateto Sulino Grosso	18.5	3.2	14.7	21.7
Cateto	24.0	5.3	18.1	29.3
<i>Cateto Assis Brasil</i>	22.0	4.2	16.0	26.2
<i>Cateto Grande</i>	16.0	5.1	24.2	21.1
Cateto Nortista	23.8	6.0	20.1	29.8
Cateto Nortista Precoce	25.2	7.3	22.5	32.5

3.Recent Commercial				
Dente Riograndense				
Dente Riograndense Rugoso	19.4	4.1	17.4	23.5
Dente Riograndense Liso	21.4	4.1	16.1	25.5
Dente Paulista	20.6	4.5	17.9	25.1
Dente Branco				
Dente Branco Riograndense	19.2	4.3	18.3	23.5
Dente Branco Paulista	23.6	4.7	16.6	28.3
Semi-Dentado				
Semi-Dentado Riograndense	20.5	4.1	16.7	24.6
Semi-Dentado Paulista	25.1	5.1	16.9	30.2
Cravo Riograndense	20.8	3.0	12.6	23.8
Cravo Paulista	19.4	3.8	16.4	23.2
4.Exotic Commercial				
Hickory King	15.7	4.2	21.1	19.9
Tuson	14.0	3.6	20.5	17.6
			mean	25.2

Appendix Table 2-2 Tassel Branch Number for 73 Inbred Lines

INBRED	ORIGIN	BRANCH NUMBER
A619 bm2		4.7
CI64	USDA	11.5
CI66	USDA	15.7
CIMA21	CIMMYT	36.5
CM103	India	30.4
CML223	CIMMYT	15.2
CML295	CIMMYT	15.6
DB544	Korea	2.5
Fla2AT116	Florida	17.9
GT601	Georgia	4.4
Hi25	Hawaii	10.5
Hi26	Hawaii	8.4
Hi27	Hawaii	12.2
Hi28	Hawaii	32.0
Hi29	Hawaii	12.8
Hi30	Hawaii	19.5
Hi31	Hawaii	4.8
Hi33	Hawaii	10.5
Hi34	Hawaii	17.6
Hi35	Hawaii	22.4
Hi36	Hawaii	11.3
Hi37	Hawaii	10.1
Hi38	Hawaii	9.3
Hi39	Hawaii	12.7
Hi41	Hawaii	12.9
Hi42	Hawaii	22.6
Hi43	Hawaii	16.7
Hi44	Hawaii	6.0
Hi45	Hawaii	11.9
Hi46	Iowa/Hi	12.4
Hi47	Iowa/Hi	6.7
Hi48	India/Hi	17.1
Hi50	Florida/Hi	16.8
Hi51	Florida/Hi	21.9
Hi52	Florida/Hi	14.5
Hi53	Colombia/Hi	18.0

Hi54	Colombia/Hi	19.3
Hi55	Colombia/Hi	17.0
Hi56	Kenya/Hi	22.0
Hi57	Thailand/Hi	20.9
Hi58	Thailand/Hi	18.1
Hi60	Missouri/Hi	4.7
Hi61	Zimbabwe/Hi	17.1
Hi62	Philippines/Hi	8.8
Hi63	Philippines/Hi	11.9
Hi64	Philippines/Hi	14.2
Hi65	Texas/Hi	20.3
Hi67	Nigeria/Hi	22.0
Hi68	Nigeria/Hi	15.2
IAN1		11.2
IAN13		9.2
IAN14		10.4
IAN2		10.0
IAN5		12.4
IAN8		12.9
KP58K	Indiana	18.9
KS23-6		18.8
Mp68:616	Mississippi	20.1
N3		14.5
Nar330	Thailand	19.3
Ph DMRS5	Thailand	17.7
Ph102-28	Philippines	22.7
R18		21.6
SG18	Indiana	20.1
TLR		3.3
Tx5855	Texas	16.0
TZi14	Nigeria	16.3
TZi17	Nigeria	25.0
TZi3	Nigeria	6.4
TZi31	Nigeria	6.5
TZi35	Nigeria	16.6
W182 bm2		4.9
W64A bm2		7.2

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