MORPHO-AGRONOMIC VARIABILITY AND EXPRESSION OF *Sh2* AND *Bt2* GENES IN SOME ADVANCED CORN LINES UNDER TROPICAL AND DRYLAND ENVIRONMENT

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MORPHO-AGRONOMIC VARIABILITY AND EXPRESSION OF *Sh2* AND *Bt2* GENES IN SOME ADVANCED CORN LINES UNDER TROPICAL AND DRYLAND ENVIRONMENT

ABSTRACT

Twelve new composite corn lines were characterized and their genotypic and phenotypic variability was studied. Experiments were conducted at two different test locations with tropical and dryland climates (Malaysia and Iran). Initially, all twelve lines were grown for morph-agronomic evaluation in the tropical environment. Ultimately, based on their morphological and physiochemical performance, six outstanding lines were selected for the evaluation of genotypic and phenotypic variability in yield and yield components in the dryland environment. Analysis of variance for all traits except tasseling date showed significant differences among the corn advance lines at the first test location. The highest genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were observed for yield per plant (30.48%, 38.70%) and ear number per plant (20.01%, 29.35%) respectively. Maximum heritability was obtained for 1000 kernel weight (91.35%), followed by plant height (74.78%) and ear weight (74.31%). Maximum genetic advance was also observed for yield per plant (16116.36), followed by 1000 kernel weight (3342.31) and ear weight (1815.61). At the second test location, the analysis of variance showed significant difference in all traits among the six composite corn lines, except silking date and ear number per plant, which showed no significant difference. The highest GCV and PCV were observed for yield per plant, with 20.56% and 27.89%, respectively. Maximum heritability was obtained for ear length (92.58%), followed by 1000 kernel weight (88.01%) and kernel number per ear (75.27%). Maximum genetic advance was also observed for kernel number per ear (1990.12), followed by yield per plant (491.85)

and plant height (344.80). Furthermore, the mean squares from the combined variance analysis of the two test locations showed highly significant variation in genotype, location and interaction between location and genotype for all characteristics studied. Genotypic variation was significant for silking date (MS = 11.89), plant height (MS = 334.69), ear weight (MS = 1654.90), kernel number per ear (MS = 1528.81) and 1000 kernel weight (MS = 2458.67). However, location variation was significant for all characteristics. In fact, both location and genotype interacted significantly with all characteristics except tasseling date and ear length, which showed non-significant variation. In general, based on the DMRT results UM 1 exhibited the highest range of yield and other effective yield components across the two test locations. Furthermore, quantitative real-time PCR was used to measure the RNA transcription levels of various genes including the *Sh2* and *Bt2* genes and *18s* as a housekeeping gene in the leaf and endosperm tissue of six composite corn lines selected at the two test locations. The *Sh2* and *Bt2* genes presented upregulated expression levels in leaf and endosperm tissue at two different test locations. The correlation coefficient between the expression levels of *Sh2* and *Bt2* and the morphological traits showed that the expression level of the *Bt2* gene in leaf tissue was not significantly associated with any traits, whereas the expression of this gene in endosperm tissue was positively and significantly associated with ear number per plant, ear weight, ear length, kernel number per ear, 1000 kernel weight and yield. Similarly, the expression level of the *Sh2* gene in leaf tissue showed no significant correlation with any traits, whereas the expression of this gene in endosperm tissue demonstrated positive and significant association with ear number per plant, ear weight, ear length, 1000 kernel weight and yield.

Keywords: Morpho-agronomic Variability, *Sh2* and *Bt2* genes, Gene Expression, RTPCR

VARIASI CIRI AGRONOMIK DAN EKSPRESI GEN *Sh2* DAN *Bt2* DALAM KOMPOSIT JAGUNG TERBAIK DI KAWASAN TROPIKA DAN KERING

ABSTRAK

Dua belas jagung komposit baru telah dicirikan dan variabiliti genotip dan fenotip dua belas komposit ini dikaji. Eksperimen telah dilakukan di dua lokasi ujian yang berbeza, di kawasan iklim tropika dan kering (Malaysia & Iran). Pada mulanya semua dua belas komposit ini ditanam untuk penilaian ciri agronomik di persekitaran tropika dan akhirnya berdasarkan prestasi morfologi dan fisiokimia, enam komposit yang terbaik telah dipilih untuk menilai variabiliti genotip dan fenotip bagi hasil dan komponen hasil di persekitaran kering. Analisis varians untuk semua ciri kecuali tarikh 'tasseling' telah menunjukkan perbezaan yang ketara di antara jagung komposit di lokasi ujian pertama. Koefisien varian genotip (GCV) dan Koefisien varian fenotip (PCV) tertinggi telah diperhatikan masing-masing untuk hasil setiap tumbuhan (30.48%, 38.70%) dan bilangan telinga setiap tumbuhan (20.01%, 29.35%). Warisan maksimum telah diperolehi dalam berat 1000 kernel (91.35%), diikuti oleh tinggi tumbuhan (74.78%), dan berat telinga (74.31%). Kemajuan genetik maksimum juga telah diperhatikan untuk hasil setiap tumbuhan (16116.36), diikuti oleh berat 1000 kernel (3342.31) dan berat telinga (1815.61). Di lokasi ujian kedua, analisis varians telah menunjukkan perbezaan ketara di antara enam jagung komposit terpilih untuk semua ciri kecuali tarikh 'silking' dan bilangan telinga setiap tumbuhan yang tidak menunjukkan perbezaan ketara. GCV dan PCV tertinggi telah diperhatikan untuk hasil setiap tumbuhan, dengan masing-masing 20.56% dan 27.89%. Warisan maksimum telah diperolehi dalam panjang telinga (92.58%), diikuti oleh berat 1000 kernel (88.01%) dan bilangan kernel setiap telinga (75.27%). Kemajuan genetik maksimum juga telah diperhatikan untuk bilangan kernel setiap telinga (1990.12), diikuti oleh hasil setiap

tumbuhan (491.85) dan tinggi tumbuhan (344.80). Selain itu, rata-rata kuadrat daripada analisis varians gabungan di dua lokasi ujian telah menunjukkan variasi yang sangat ketara dalam genotip, lokasi dan interaksi di antara lokasi dan genotip untuk semua ciri yang dikaji. Variasi genotip adalah penting untuk tarikh 'silking' (11.89), tinggi tumbuhan (334.69), berat telinga (1654.90), bilangan kernel setiap telinga (1528.81) dan berat 1000 kernel (2458.67). Walau bagaimanapun, variasi lokasi pula adalah penting untuk semua ciri. Kedua-dua lokasi dan genotip berinteraksi dengan ketara untuk semua ciri kecuali tarikh 'tasseling', dan panjang telinga yang menunjukkan variasi tidak ketara. Selain itu, kuantitatif PCR masa nyata telah digunakan untuk mengukur tahap transkripsi RNA pelbagai gen termasuk: *Sh2* dan *Bt2* gen, juga *18s* sebagai gen 'housekeeping' dalam tisu daun dan endosperm enam jagung komposit terpilih di dua lokasi ujian. Gen *Sh2* dan *Bt2* telah menunjukkan ekspresi tahap 'upregulation' dalam tisu daun dan endosperm a di lokasi ujian pertama. Selain itu, gen *Sh2* dan *Bt2* telah menunjukkan ekspresi tahap 'upregulation' dalam tisu daun dan endosperm a di lokasi ujian kedua. Koefisien korelasi di antara tahap ekspresi gen *Sh2* dan *Bt2* dengan ciri morfologi menunjukkan bahawa tahap ekspresi gen *Bt2* dalam tisu daun tidak ketara berkaitan dengan mana-mana ciri, manakala ekspresi gen ini dalam tisu endosperm a ketara berkaitan dan menunjukkan korelasi positif dengan bilangan telinga setiap tumbuhan, berat telinga, panjang telinga, bilangan kernel setiap telinga, berat dan hasil 1000 kernel. Tahap ekspresi gen *Sh2* dalam tisu daun tidak menunjukkan korelasi yang ketara dengan mana-mana ciri manakala ekspresi gen ini dalam tisu endosperm ketara berkaitan dan menunjukkan korelasi positif dengan bilangan telinga setiap tumbuhan, berat telinga, panjang telinga, berat dan hasil 1000 kernel.

Kata kunci: Ciri agronomik Variasi, Gen *Sh2* dan *Bt2*, Ekspresi gen, RTPCR

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LIST OF SYMBOLS AND ABBREVIATIONS

µl	:	Microliter
PCR	:	Polymerase chain reaction
RT-PCR	:	Reverse transcription Polymerase chain reaction
DMRT	:	Dancan's multiple range test
KW	:	Kernel weight
wt	:	Wieght
pl	:	plant

CHAPTER 1: INTRODUCTION

1.1 General introduction

The cereals, including maize, account for 70% of food production worldwide, and in addition, maize is an economically important crop in the United States. Maize is also the best-studied and most tractable genetic system among the cereals, making it the premier model system for studying this important group of crops, as well as other monocots. Although cereals are of economic importance and a greater understanding of their genes will have great impact, much interesting biology can also be learned from these species. For example, the recent diversification of the grasses makes them an ideal collective system for dissecting genetic control of morphological and genomic diversity (Bennetzen et al., 2001). Maize (meɪz/ *mayz;* *Zea mays* subsp. *mays*), known in some English-speaking countries as corn, is a large grain plant domesticated by indigenous people in Mesoamerica in prehistoric times. Most historians believe maize was domesticated in the Tehuacan Valley of Mexico (Gibson & Benson, 2002a). Scientists believe people living in central Mexico developed corn at least 7000 years ago. It was started from a wild grass called teosinte. Teosinte looked very different from our corn today. The kernels were small and were not placed close together like kernels on the husked ear of modern corn. Also known as maize Indians throughout North and South America, eventually depended upon this crop for much of their food. From Mexico maize spread north into the Southwestern United States and south down the coast to Peru. About 1000 years ago, as Indian people migrated north to the eastern woodlands of present day North America, they brought corn with them. Maize is the most widely grown grain crop throughout the Americas, with 332 million metric tons grown annually in the United States alone. Approximately 40% of the crop is used for corn ethanol (Pollack, 2011). Genetically modified maize made up 85% of

the maize planted in the United States in 2009. Prior to their domestication, maize plants only grew small, one-inch long corn cobs, and only one per plant. Many centuries of artificial selection by the indigenous people of the Americas resulted in the development of maize plants capable of growing several cobs per plant that were usually several inches long each (Spielvogel, 2006). The maize (*Zea mays*) kernel represents one of the most important sources of human nutrition either directly, in the form of tortillas, chips, flakes, vegetable oil, or sweet corn, or indirectly, as energy-rich fodder for farm animals. All three parts of the kernel contribute to its nutritional value. The endosperm contains mainly starch and protein, the embryo is rich in fatty acids, and the surrounding pericarp provides fiber. These reserve substances are accumulated during the filling or maturation phase, which is preceded by the early or developmental phase and followed by the dehydration or desiccation phase. The knowledge of the molecular mechanisms governing the development, filling, and maturation of the maize kernel is in sharp contrast to its agronomic and economic importance. Despite considerable progress in recent years, the number of genes with a clearly identified function in kernel development remains extremely limited and is a long way short of the 1,000 genes estimated to be involved in seed development in Arabidopsis (McElver et al., 2001).

A genetic variant that accumulates more sugar and less starch in the ear is consumed as a vegetable is called sweet corn (*Zea mays* L. var. saccharata). Sugar-rich varieties called sweet corn are usually grown for human consumption as kernels, while field corn varieties are used for animal feed, various corn-based human food uses and as chemical feedstock. Sweet corn is harvested in the "milk stage", after pollination but before starch has formed, between late summer and early to mid-autumn. Field maize is left in the field very late in the autumn to thoroughly dry the grain, and may, in fact, sometimes not be harvested until winter or even early spring (Fernandez, 2011). Standard sweet

corn is differs from field corn by a mutation which causes the kernels to accumulate about two times more sugar more than field corn and significantly less starch. The primary difference is gene expression that determines endosperm carbohydrate content as well as many other genes that affect maize growth (Erdal et al., 2011; Najeeb et al., 2011; Znidarcic, 2012). Yield gains due to the genetic improvement have been smaller in sweet corn than in field corn. Sweet corn breeders have often focused on improving quality and ear appearance, rather than on enhancing yield (Tracy, 1993). Moreover, all commercial sweet corn hybrids are based on one or more defective endosperm mutants, and production of high quality seed is more difficult for sweet corn than for most types of corn (Tracy, 1994). Additionally, the narrow genetic base of sweet corn may limit genetic gains for yield. The polygenic nature of this trait has directed the breeding efforts towards the development of new sweet corn hybrids with improved eating quality and favorable ear and kernel traits (Hansen et al., 1977; Has, 1999; Has & Cabulea, 1998; Kaukis & Davis, 1986; Tracy, 1994). Recently all the corn now grown in the United States is of hybrid varieties. Seed is obtained by crossing inbred lines which are obtained by self-pollination through several generations. This results in reduced vigor and yield but increased uniformity in the inbreds. A number of maize (*Zea mays* L.) endosperm mutants that affect the quantity and quality of carbohydrates in the endosperm, as well as kernel development and morphology, have been identified and extensively studied (Shannon, 1984). Endosperm genes that have been used in commercial sweet corn cultivars are Amylose-extender1, Brittle1, Brittle2, Dull1, Shrunken2, Sugary1, Sugary enhancer1 and Waxy1. Plant ADP-glucose pyrophosphorylase (AGPase) catalyzes the production of ADP-glucose (ADPGlc) and inorganic pyrophosphate (PPi) from Glc-1-P and ATP, thus generating the nucleotide sugar. The *Sh2* and brittle endosperm2 (*Bt2*) genes encode the large and small subunit of ADP-glucose pyrophosphorylase respectively (Bhave et al., 1990; Tsai & Nelson,

1966). One class of the many genes in maize has been described that greatly alters starch content of the seed. Mutation at either of two unlinked loci, shrunken-2 (*Sh2*) or britrle-2 (*Bt2*), gives rise to a severely shrunken and brittle types of sweet corn seed. The *Bt2* gene, which codes for the small subunit of ADP-glucose pyrophosphorylase (AGPase), is a key enzyme in starch synthesis. The *Bt2* gene generates two transcripts, referred to as *Bt2a* and *Bt2b* (Rosti & Denyer, 2007). The classical transcript (*Bt2a*), coding for a cytoplasmic isoform, was almost exclusively expressed in the developing endosperm, whereas *Bt2b*, an alternative transcript coding for a plastidial isoform, was expressed in almost all tissues. The classical studies of Tsai and Nelson (1966) revealed that both loci affect the starch synthetic enzyme ADP-glucose pyrophosphorylase. This enzyme catalyzes the reversible synthesis of ADP-glucose and pyrophosphate from ATP and glucose 1 phosphate (Tsai & Nelson, 1966). It may represent one of the main regulatory steps in the biosynthesis of starch in plants (Preiss, 1982) and glycogen in bacteria (Preiss, 1984). Hannah and Nelson (1975, 1976) suggested that both loci were structural genes for endosperm ADP-glucose pyrophosphorylase. Starch composes approximately 70% of the dry weight of the maize kernel and is, therefore, the major component of the seed weight (Hannah & Nelson, 1975; Hannah & Nelson, 1976). However, Hannah (1980) in another set of experiments reported that, two differentially heat-labile forms of ADP-glucose pyrophosphorylase were detected (Hannah et al., 1980). All *Sh2* and *Bt2* mutants analyzed greatly reduced or abolished the major heat-labile form of the enzyme but only moderately reduced the minor heat-stable form of the enzyme. These results raised the possibility that *Sh2* and/or *Bt2* might act post-transcriptionally or post-translationally. Furthermore, in the *Sh2* revertants obtained by excision of the transposable element Ds, the enzyme levels were approximately equal to wild type (Tuschall & Hannah, 1982). Neither of these two results was consistent with the post-transcriptional role for *Sh2* or *Bt2* in controlling the enzyme action, and the

results strongly supported the idea of the two genes being structural genes for the enzyme. However, these somewhat complicated and possibly conflicting observations concerning the nature of genetic control of ADP-glucose pyrophosphorylase by the *Sh2* and *Bt2* genes necessitated more direct molecular studies.

Maize yields have improved drastically over the last 40 years. Yield increases over that time period have been shown to be largely due to hybrids with greater crowding stress tolerance combined with increased plant populations (Duvick, 2005). Maize production practices to increase yield need to be better understood, including the relation of grain yield and yield components. Agriculture is an information-intensive industry from an essential point of view. Many factors such as sowing date, soil type, fertilizer, location, hybrid, season duration, etc. influence yield and yield components of a grain crop and they are well needed by agricultural experts (Matsumoto, 1998). Exploring the agricultural technologies of traits related to the control of crop grain yield reductions has a poor record of application (Fischer, 2011). As population rises, less land will be devoted to agriculture, meaning increased production will have to come from increased yields. Path coefficient analysis is often used as a tool to better understand yield components. Obtaining ideal plant populations in maize is necessary in order to maximize grain yields. Optimal plant population also varies with hybrids, environments and yield goals, thus all of these factors must be considered when determining appropriate plant populations and maximizing yield. Plant population alters yield components as well, and understanding yield components will help to better understand the effect of plant population on grain yield (Milander, 2015). Maize (*Zea mays* L.) yield is a function of the number harvested kernels per unit land area and the individual kernel weight (KW). Kernel weight and its development show a wide variability due to genotype, environment, crop management, and all possible interactions. Commercial maize hybrids differ markedly in the patterns (rate and

duration of kernel growth) behind differences in final KW (Borras & Gamban, 2010; Shekoofa et al., 2012, 2013).

Maize (*Zea mays* L.) possesses enormous genetic variability which coupled with its biological diversity make it a model crop for study of genetic principles which are of practical significance to plant breeders (Singh & Chaudhary, 1985). It does not survive in its wild form probably because of the highly cross pollinated nature (Menkir & Ingelbrecht, 2007). The ultimate goal of various breeding methods in maize is the production of improved genotypes. Hence short cut but efficient methods are needed for isolation and identification of superior genotypes which can be used in hybrid breeding programs (Arshad et al., 2003). For selection of inbred lines tolerant to inbreeding depression and being superior in genetic potential, early generation testing is desirable (Barata & Carena, 2006). In a breeding program for hybrid production, too many lines are produced making their handling and evaluation pretty hard and time-consuming. In order to solve this problem, early generations of selfed progeny are out crossed to a common tester for yield and general performance. Lines with poor combing abilities are discarded and only good performance lines are further selfed and selected in subsequent cycles of selection (Kempthorne, 1957). Considering the different climate conditions of maize cultivated regions of the world, the production of improved hybrids with good performance for a single climate is of great value. The same genotypes in different climates may not show the same performance. Rahman et al. (2010) tested 24 maize S2 lines using line × tester analysis for some traits related to earlier flowering and ear height and revealed highly significant differences among the testcrosses for measured parameters. They recommended these testcrosses to be included in further breeding programs for developing maize germplasm with earlier flowering and desirable plant and ear height attributes (Ram & Singh, 2003).

The yield of sweet corn (*Zea mays* L.) under tropical environment is very low although it is enriched with several vitamins, minerals and good amount of free sugar. On the other hand field corn, which yield is much higher compare to sweet corn but with low nutritive value and very low free sugar. To overcome the low productivity and low nutritive values in corns, an attempt was taken to develop high yield potential corn variety with superior nutritive value and free sugar at Institute of Biological Sciences, University of Malaya, Malaysia. A series of crossing were made between field and sweet corn in 2009-2010. Generations of several crosses (F1 to F6) raised and finally, twelve genotypes were identified as outstanding. In the present investigation it was tried to characterize the morph-agronomic characters of these advance lines. It was also tried to evaluate the presence of *Sh2* and *Bt2* genes and their expressions in these lines. However, the improvement of nutritive value was not considered here since it is already done by other group of researchers. The research in this study helps us to better understand maize grain yield and its components along with effects of factors such as environment, plant population, and hybrid on these components.

1.2 Hypothesis

a. The advance UM corn lines may show morpho-agronomic variability in tropical and dryland environments.

b. The *Sh2* and *Bt2* genes may be present in the advance UM corn lines and ensure the relative transcript levels of *Sh2* and *Bt2* genes are upregulated in leaf and endosperm tissue.

c. The expression levels of the *Sh2* and *Bt2* genes in leaf and endosperm tissue may be correlated with morphological variability in selected advance UM corn lines.

1.3 Objectives of study:

The objectives of this study are summarized as:

1. To evaluate the morpho-agronomic traits of selected advance corn lines in tropical and dryland environments.
2. To detect the presence and expression levels of the *Sh2* and *Bt2* genes in the leaves and endosperm of corn lines grown in tropical and dryland environments by PCR and RT-PCR.
3. To analyse the relationship between the morpho-agronomic variability of corn lines and expression levels of the *Sh2* and *Bt2* genes in the leaves and endosperm of corn plants.

CHAPTER 2: LITERATURE REVIEW

2.1 Corn and its importance

Beginning about 2500 BC, the crop spread through much of the Americas. The region developed a trade network based on surplus and varieties of maize crops. After European contact with the Americas in the late 15th and early 16th centuries, explorers and traders carried maize back to Europe and introduced it to other countries. Maize spread to the rest of the world because of its ability to grow in diverse climates. An influential 2002 study by Matsuoka et al. has demonstrated that, rather than the multiple independent domestications model; all maize arose from a single domestication in southern Mexico about 9,000 years ago (Matsuoka et al., 2002). The study also demonstrated that the oldest surviving maize types are those of the Mexican highlands. Later, maize spread from this region over the Americas along two major paths. This is consistent with a model based on the archaeological record suggesting that maize diversified in the highlands of Mexico before spreading to the lowlands. Corn as we know it today would not exist if it weren't for the humans that cultivated and developed it. It is a human invention, a plant that does not exist naturally in the wild. It can only survive if planted and protected by humans. Sugar-rich varieties called sweet corn are usually grown for human consumption as kernels, while field corn varieties are used for animal feed, various corn-based human food uses (including grinding into cornmeal or masa, pressing into corn oil, and fermentation and distillation into alcoholic beverages), and as chemical feedstocks. Corn is often classified as dent corn, flint corn, flour corn, popcorn, sweet corn, waxy corn, and pod corn. The remainder of this discussion will be concerned only with dent corn, which is the major type cultivated in the United States. The great variability of the corn plant led to the selection of numerous widely adapted varieties which hardly resembled one another. The plant may have ranged from no more than a couple of feet tall to over

20 feet. It was not like the uniform sized plant that most people know today. The principal role of the corn plant during the 19th century was closely tied to the development of the Midwest. In the movement westward, corn found its major home in the woodland clearings and grasslands of Ohio, Indiana, Illinois, Iowa, and adjacent states. These were places where it had not been grown widely in prehistoric times (Gibson & Benson, 2002b).

2.2 The genetics of maize plant

Through the study of genetics, we know today that corn's wild ancestor is a grass called teosinte. Teosinte doesn't look much like maize, especially when you compare its kernels to those of corn. But at the DNA level, the two are surprisingly alike. They have the same number of chromosomes and a remarkably similar arrangement of genes (SCAUO Time). Maize is a diploid with 20 chromosomes (n=10). The combined length of the chromosomes is 1500 cM. Some of the maize chromosomes have what are known as "chromosomal knobs": highly repetitive heterochromatic domains that stain darkly. Individual knobs are polymorphic among strains of both maize and teosinte. In fact, teosinte cans cross-breed with modern maize varieties to form maize-teosinte hybrids that can go on to reproduce naturally. Scientists study teosinte-maize hybrids and their offspring through the process of genetic archaeology. This process helps geneticists understand what is happening at the DNA level to make teosinte and maize so different. In the 1930s, Beadle studied teosinte-maize hybrids and showed that their chromosomes are highly compatible. Later, he produced large numbers of teosinte-corn hybrids and observed the characteristics of their offspring. By applying basic laws of genetic inheritance, Beadle calculated that only about 5 genes were responsible for the most-notable differences between teosinte and a primitive strain of maize. Using more-modern techniques, another group of scientists analyzed the DNA from teosinte-maize

offspring. They too noticed that about 5 regions of the genome (which could be single genes or groups of genes) seemed to be controlling the most-significant differences between teosinte and maize (Beadle, 1930).

2.3 The morphology of maize plant

Maize typically produces branches at only two or three of the nodes along the main stem. Axillary buds are present at some of the other nodes but they are arrested early in development. Each of the branches that are produced is composed of nodes and short internodes, averaging about 1 cm in length. Unlike teosinte, the number of internodes in the lateral branch is greater than the number in the main stalk above the point of attachment of the branch. The architecture of the lateral branches of the maize plant is strikingly different from that of teosinte. Much of the interest and controversy surrounding the domestication of maize arises from the fact that these two plants have such strikingly different morphologies. For maize line W22, a branch attached at the fifth node below the main tassel will be composed of about 12 internodes and have 12 husk leaves. The leaves (husks) along the lateral branch are composed largely of sheath with only a small (if any) blade attached to it. The husks are arranged in a spiral phyllotaxy along the branch, rather than the alternate phyllotaxy for leaves on the main stem of the plant. Secondary branches are normally absent (aborted). Finally, the lateral branch is terminated by a female inflorescence or ear, which is tightly enclosed within the spirally arranged husks because of the failure of the internodes of the branch to fully elongate. The divergent morphologies of maize and teosinte lateral branches reveal the complex set of evolutionary changes involved in the origin of maize plant architecture. These include: (1) arresting the full elongation of the internodes, (2) arresting the growth of the leaf blade, (3) arresting the outgrowth of some axillary buds, (4) suppressing the outgrowth of secondary branches, (5) increasing the number of leaves

(husks) formed on the branch, (6) changing leaf (husk) phyllotaxy from alternate to spiral, and (7) replacing the tassel (male inflorescence) at the tip of the lateral branch with an ear (female inflorescence). Noticeably, most of these changes involve arresting the growth of particular organs. This suggests that a common developmental process (repression of organ growth) expressed at distinct points in space or time of organogenesis produced this suite of correlated changes. The cob (rachis) of the maize ear, like that of its teosinte counterpart, is composed of invaginated internodes or cupules. Maize cupules are arranged polystichously (in four or more ranks) around the circumference of the ear with usually 100 or more cupules in a single ear. Unlike teosinte, the cupules of maize are shallow, often collapsed, and they do not envelop the kernels. Maize cupules may be indurate, but the outer glumes are softer than the highly indurated glumes of the teosinte ear. In contrast to teosinte, there are two spikelets associated with each cupule, one pedicellate and the other sessile. Thus, an ear with four ranks of cupules will have eight rows of kernels. The female spikelets of maize also differ from those of teosinte in that they are oriented perpendicular and not parallel to the axis of the ear. Finally, maize ears lack abscission layers as found in teosinte, so the ear remains intact at maturity (Iltis, 2000; Weatherwax, 1935).

2.4 Different types of corn plant

Corn variation may be artificially defined according to kernel type as follows: dent, flint, flour, sweet, pop and pod corn. Except for pod corn, these divisions are based on the quality, quantity and pattern of endosperm composition in the kernel and are not indicative of natural relationships. Endosperm composition may be changed by a single gene difference, as in the case of floury (*fl*) versus flint (*FI*), sugary (*su*) versus starchy (*Su*), waxy (*wx*) versus non-waxy (*Wx*), and other single recessive gene modifiers that have been used in breeding special-purpose types of corn. The quantity or volume of

endosperm conditioning the size of the kernel (e.g., the difference between dent and flint corns or flint corn and popcorn) is polygenic and, in the latter example, is of some taxonomic significance. The pod corn trait is monogenic and more of an ornamental type. The major gene involved (*Tu*) produces long glumes enclosing each kernel individually, such as occurs in many other grasses (Brown et al., 1986).

The U.S. Corn Belt dents originated from the hybridization of the Southern Dent or late-flowering maize race called Gourd seed, and the early-flowering Northern Flints. Dent corn is characterized by the presence of corneous, horny endosperm at the sides and back of the kernels, while the central core is a soft, floury endosperm extending to the crown of the endosperm where, upon drying, it collapses to produce a distinct indentation. Degree of denting varies with the genetic background. Nearly all varieties grown in the U.S. are yellow, with only a few white endosperm types grown. Dent corn is used primarily as animal food, but also serves as a raw material for industry and as a staple food. Upwards of 93 percent of dent corn produced (including the corn equivalent of by-product feeds from corn processing) is used as animal feeds. However, it is still an important human food and industrial material, entering into many specialized products via the dry- or wet-milling industry in the U.S. Yellow dent corn sells at market price as it enters the normal feed grain or milling channels. However, white dent often receives a premium price in the dry milling industry, where it is utilized for certain human food products because of its whiter starch. The flint corns mostly have a thick, hard, vitreous (glassy) or corneous endosperm layer surrounding a small, soft granular center. The relative amounts of soft and corneous starch, however, vary in different varieties. Generally, the kernels are smooth and rounded, and the ears long and slender with a comparatively small number of rows or kernels. In temperate zones, flint corn often matures earlier, germinates better, and has more spring vigor, more tillers and fewer prop roots than dent strains. Very little flint corn is produced and utilized in the U.S.

today, although it was undoubtedly grown extensively up through colonial times. Generally, yields are lower than our Corn Belt dents, in part because of relatively little breeding work done. Flints are more extensively grown in Argentina and other areas of South America, Latin America and southern Europe where they are used for feed and food. The flour Corn is one of the oldest types of corn, tracing back to the ancient Aztecs and Incas. American Indians ground the soft kernels for flour. Floury maize types have soft starch throughout, with practically no hard, vitreous endosperm and thus are opaque in kernel phenotype. Kernels tend to shrink uniformly upon drying, so usually have little or no denting. When dry, they are easy to grind, but may mold on the mature ear in wet areas. In the U.S., flour corn has limited production and is restricted to the drier sections. It is grown widely in the Andean region of South America. Popcorns are perhaps the most primitive of the surviving races of maize. This corn type is characterized by a very hard, corneous endosperm containing only a small portion of soft starch. Popcorns are essentially small-kerneled flint types. The kernels may be either pointed (rice-like) or round (pearl-like). Some of the more recently developed popcorns have thick pericarps (seed coats), while some primitive semi-popcorns, such as the Argentine popcorns, have thin pericarps. Popcorn is a relatively minor crop compared to dent corn. It is used primarily for human consumption as freshly popped corn or as the basis of popcorn confections. Isolated planting is not necessary, since there are no major xenia effects on popping expansion and many types of popcorn are cross-sterile with field corn. Most popcorn acreage is grown under contract. Although conditions for growing popcorn are the same as for dent corn, special harvesting, drying and storage practices are necessary to maintain popping quality. Pod corn (tunicate maize) is more of an ornamental type. The major gene involved (*Tu*) produces long glumes enclosing each kernel individually, such as occurs in many other grasses. The ear is also enclosed in husks, as with other types of corn. Homozygous pod corn usually

is highly self-sterile, and the ordinary type of pod corn is heterozygous. Pod corn may be dent, sweet, waxy, pop, flint or floury in endosperm characteristics. It is merely a curiosity and is not grown commercially (Brown et al., 1986). The sugar-rich varieties of corn called sweet corn. The most common form of sweet corn, commonly referred to as the standard sugary (*su*) corn, is thought to have originated from a mutation in the Peruvian race Chullpi. Most certainly it was grown and used by Native American Indians in pro-Colombian times. In sweet corn, the sugary gene prevents or retards the normal conversion of sugar into starch during endosperm development, and the kernel accumulates a water-soluble polysaccharide called 'phytoglycogen'. The higher content of water-soluble polysaccharide adds a texture quality factor in addition to sweetness. Sweet corn is eaten in the immature milk stage and is gaining importance as a popular vegetable in urban markets. Now-a-days the standard sugary corns are being modified with other endosperm genes and gene combinations that control sweetness to develop new cultivars. At least 13 endosperm mutants, in combination with sugary, have been studied for improving sweet corn. Except for sugary, the genes used in breeding act differently to produce the taste and texture deemed desirable for sweet corn. Therefore, the growers must consider genetic type of the varieties when making selections for planting. The genetic type is not readily identifiable by cultivar name alone (Tracy, 2000).

2.5 Composite line development from sweet corn and field corn

Heterosis, or hybrid vigor, is the better performance of a hybrid relative to the parents, and is the outcome of the genetic and phenotypic variation. Most traits of economic importance are qualitative and controlled by several to many major genes (Ali et al., 2012). The simple act of crossing different strains resulted in higher yields and stronger plants. Hybrid maize generates high yields, increased value and reduced

production costs. The plants are bigger, stronger and more vigorous. This hybrid vigor, or heterosis, occurs when crossing two genetically unrelated inbred parents to create a hybrid. To produce hybrid seed, two inbreds are planted together and the tassels removed from one before any pollen is shed. One of the first things the plant scientists noticed when they began crossing different pure lines were that hybrid plants were usually more vigorous than their parents. Generally heterosis can be divided into two broad categories, true heterosis and pseudo heterosis. In case of true heterosis, there is an increase in general vigor, yield and adaptation. In case of pseudo heterosis, the F1 hybrid exhibits increase in vegetative growth only. It refers to the superiority of F1 over the standard commercial check variety. So, it is also called economic heterosis or superiority over checks (Sharief et al., 2009). Recently it has been divulged that the utilization of heterosis is extremely effective for the genetic improvement of different traits and that the concepts of combining ability are the fundamental tools for enhancing productivity of different crops in the form of F1 hybrids (Flint-Garcia et al., 2009). Heterosis occurred in the F1 hybrids for all traits of interest, but their values varied among crosses and characters. However, Genotypes harboring desirable attributes and stable performance are vital, both as cultivars and as source of desirable germplasm for further improvement. Maize production can be boost up by providing some relevant and basic information about the pattern and genetic variability to the breeding community. Combining ability studies provide information on the genetic mechanisms controlling the inheritance of quantitative traits and enable the breeders to select suitable parents for further improvement or use in hybrid breeding for commercial purposes. In biometrical genetics two types of combining abilities are considered i.e. general combining ability (GCA) and specific combining ability (SCA). General combining ability refers to the average performance of the genotype in a series of hybrid combinations and is a measure of additive gene action whereas; specific combining ability is the performance

of a parent in a specific cross in relation to general combining ability (Sharief et al., 2009). The design has been widely used in maize breeding by several workers and continues to be applied in quantitative genetic studies in maize due to its significance (Sharma et al., 2004). Early generation testing is extremely important for efficient evaluation and production of inbred lines of maize. The use of testers in a maize recurrent selection program has been well documented (Rademacher et al., 1999).

2.6 Multigene engineering of sucrose biosynthesis in plant

Carbon metabolism in plants has been studied for many years and, with the advance of molecular genetic techniques, much progress has been made in understanding how the enzymes involved influence particular pathways. Most of this work has been performed in leaves of the model plant Arabidopsis thaliana, or in seeds and tubers of starch storing crops such as maize, rice and potato. Despite their agronomic importance, less work has been performed on sucrose storing plants. Plants are sessile autotrophic organisms that utilize light and water in the process of photosynthesis to produce ATP (adenosine tri-phosphate) and NADP (nicotinamide adenine dinucleotide). These molecules provide energy to fix absorbed carbon dioxide (CO_2) into triose phosphates (TPs), such as glyceraldehyde-3-phosphate (G3P), via the reductive pentose phosphate cycle (otherwise known as the Calvin Cycle). Fixed carbon has many fates, one of which is the production of the disaccharide sucrose (β-D fructofuranulosyl-(2→1)-α-D-glucopyranoside). Photosynthate is transported out of the chloroplasts in the form of a variety of sugars by a number of transporters depending on the environmental conditions. During the day triose phosphates (TPs) are manufactured in the Calvin Cycle and can be exported by a triose phosphate/phosphate translocator (TPT) out of the chloroplast (Fliege et al., 1978; Flugge, 1999). Within the plastid TPs can also be used to synthesize starch, which acts as a transient carbon store that is degraded to soluble

sugars during the dark period (Kaiser & Heber, 1984; Weber et al., 2000). At night the starch is mobilized to glucose and maltose which are then exported into the cytosol by the pGlcT (Weber et al., 2000) and MEX1 (Niittyla et al., 2004) transporters respectively.

Triose phosphates are generated by the Calvin Cycle within the chloroplasts, from where they are transported into the cytosol to be converted into sucrose. This sugar is then moved out of the cell into the apoplast via sucrose transporters (SUTs) or to other cells through plasmodesmata. Modelling of the pathway using known kinetic parameters of the different enzymes has indicated that two enzymes, namely fructose 1,6-bisphosphatase (FBPase) and sucrose phosphate synthase (SPS), exhibit a high degree of control over sucrose levels (Grof & Campbell, 2001). The extent to which FBPase and SPS contribute to control the synthesis pathway changes depending on environmental conditions. Limiting conditions such as low CO_2, radiation flux or photosynthetic rates result in FBPase having a greater level of control by shifting focus to providing sufficient metabolites for efficient Calvin Cycle turnover. Under non-limiting conditions, when photosynthetic rates are higher, SPS seems to exert more control and thus have a greater influence on maximum flux (Huber & Huber, 1996; Stitt, 1989; Stitt & Quick, 1989). Two isoforms of FBPase exist localised in the chloroplast (cpFBPase) and cytosol (cyFBPase), both of which convert fructose-1,6-bisphsophate (FBP) to fructose-6- phosphate (F6P) (Serrato et al., 2009). cpFBPase helps with Calvin Cycle turnover and provides substrate for starch synthesis (Fridlyand et al., 1999; Serrato et al., 2009). cyFBPase is more involved in sucrose synthesis by catalysing the first irreversible reaction of the conversion TPs into sucrose (Daie, 1993; Serrato et al., 2009). Both SPS and SuSy are able to synthesize sucrose, with SPS playing a more prominent role in leaf sucrose synthesis. In Arabidopsis, SuSy appears to have little control over leaf sucrose concentrations (Barratt et al., 2009). Leaf SPS

activity on the other hand has been determined to have a positive correlation with leaf sucrose content of tomato, sugarcane and Arabidopsis (Grof et al., 1998; Murchie et al., 1999; Signora et al., 1998; Worrell et al., 1991). Anti-sense SPS Arabidopsis leaves had lower sugar levels and no significant reduction in starch (Strand et al., 2000), suggesting that SPS is under the control of other sensing and regulatory mechanisms. The extent of SPS control over changes in carbon metabolism is supported by over-expression studies that were performed using tomato plants. Expression of the maize SPS gene, linked to the rubisco small subunit (rbcs) promoter resulted in a six fold increase in leaf SPS activity in addition to increased photosynthesis and sucrose synthesis (Worrell et al., 1991). Enzyme activities are greatly influenced by post-translational modification, especially phosphorylation by a number of protein kinases, including the sucrose non-fermentable related kinase (SnRK1) and a camodulin-like domain protein kinase (CDPK), as well as interactions with 14-3-3 regulatory proteins (Gibson & Benson, 2002a; Hrabak et al., 2003; Huang & Huber, 2001; Huber & Huber, 1996; Lunn & MacRae, 2003; Pagnussat et al., 2002; Toroser et al., 1998). In its inactive form, a phosphorylated SPS is dephosphorylated by a 2A protein phosphatase to become active. The active form has a much higher affinity to its substrate and is less likely to be inhibited by Pi (inorganic phosphate) (Huber & Huber, 1996; Stitt et al., 1988; Trevanion et al., 2004). The enzyme sucrose-6-phosphate phosphatase (SPP) catalyses the final step in sucrose synthesis by dephosphorylating sucrose-6-phosphate (S6P) (Lunn et al., 2000). It has been hypothesised that SPS and SPP form a complex in which sucrose is produced directly from UDP Glucose (UDPGlc) and F6P (Lunn & MacRae, 2003). Furthermore changes in sucrose levels only occurred after repression of 90% of SPP activity, indicating less control of this enzyme over leaf carbon metabolism than SPS (Chen et al., 2005). In many, but not all, plants sucrose is the most important form of soluble carbon.

The genes *Sh2* and *Bt2* govern ADPG-pyrophosphorylases that block starch synthesis and create extremely sweet phenotypes (Brewbaker, 2010). The promoter of *Sh2* gene is a strong tissue specific promoter to the endosperm of monocot seeds like maize, rice and wheat (Andersen et al., 1991; Bhave et al., 1990; Chen et al., 2003; Smidansky et al., 2002; Smidansky et al., 2007). The *Sh2* encodes the large subunit of ADP glucose pyrophosphorylase which is involved in starch biosynthesis (Hannah, 1997; Lloyd et al., 2005; Preiss, 1997; Zeeman et al., 2010). Although promoters from ADP-glucose pyrophosphorylase genes from other species have been shown to be either constitutive, with especially high expression in sink tissues (Cognata et al., 1995). As it was the only promoter successfully isolated, it was decided to continue with the assessment of its activity in sugarcane callus.

2.7 Modifications of maize kernel by the segregation of major modifier genes

Corn may be altered by genetic means to produce modifications in carbohydrate, protein, oil and other properties. As a result of modifications of ordinary dent types, new corn specialties have been created. Among them are augmented sugary kernel, waxy-maize, amylomaize, and high-lysine or modified-protein corn (Brown et al., 1986). In augmented sugary kernel type of sweet corns, the sugars are modified (increased) by the action of other genes (modifiers), either partially or completely. Major modifier genes of kernel sweetness are shrunken-2 (*Sh2*), brittle-2 (*Bt2*), sugary enhancer (*se*), brittle (*bt*) and shrunken-4 (*sh4*). The shrunken 2 (*Sh2*) sweet corn, also called super sweet, has two main advantages over the other types: 1) it is at least two to three times sweeter, and 2) the conversion of sugar to starch is negligible, thus this corn type will remain sweet up to 10 days after harvest if cooled properly followed by refrigeration (Tracy, 1994). Attempts to breed with *Sh2* gene and with the gene sugary enhancer (*se*) that delays kernel dry-down largely failed as a result of severe kernel rots.

However, breeders in Australia and Thailand have succeeded in marketing *Sh2* hybrids under farm conditions that lack fusarium pressure, unlike Hawaii's year-round growers. Sweet corn production in Thailand is dominated about equally by hybrids *Sh2* and *bt1* (Brewbaker et al., 2006). The *Sh2* varieties assume tremendous market potentiality. However, problem of germination is always associated with such varieties due to lack of starch in the seed endosperm. Germination is particularly problematic when sown as kharif crop in Indian conditions. Thus such varieties should preferably be grown during rabi season to meet their demand in local market. Such corn may also be grown to catch international seed market of *Sh2* corn. However, it is recommended to use such corn in combination with *su* corn, so that the problem of germination may be attained with little compromise with sweetness. In these partial modifications, the sugary (*su*) kernels are modified by the segregation of major modifier genes such that about 25 percent of the kernels are double-mutant endosperm types possessing the enhanced benefits of the modifier. The addition of the sugary enhancer (*se*) gene along with one of the major modifier genes like *Sh2* will further modify some of the sugary kernels to about 44 percent double-mutant endosperm types rather than 25 percent. Besides, there are other genes with minor modifying effects of kernel sweetness: amyloseextender (*ae*), dull (*du*), floury (*fl)*, floury-2 (*fl2*) opaque (*O*), opaque-2 (*O2*), sugary-2 (*su2*) and waxy (*wx*). Many of these modifiers are known to be present in sweet corn backgrounds either in the segregating or homozygous state (Tracy, 1993).

Waxy corn was introduced to the U.S. from China in 1908. Although China was the original source, waxy (*wx*) mutations have since been found in American dent strains. Its name derives from the waxy appearance of the endosperm exposed in a cleanly cut cross-section. Common corn starch is approximately 73 percent amylopectin and 27 percent amylose, whereas waxy starch is composed entirely of amylopectin, which is the branched molecular form. Amylomaize (High-amylose corn) is the generic name for

corn that has amylose content higher than 50 percent. The endosperm mutant amylose extender (*ae*) found by R. P. Bear, increases the amylose content of the endosperm to about 60 percent in many dent backgrounds (Vineyard et al., 1958). Modifying factors alter the amylose contents as well as desirable agronomic characteristics of the grain. The amylose-extender gene expression is characterized by a tarnished, translucent, sometimes semi -full kernel appearance. High-amylose grain is grown exclusively for wet milling. The two types produced commercially are Class V (amylose content, 50-60 percent) and Class VII (amylose content, 70-80 percent). High-lysine corn is the generic name for corn having an improved amino acid balance, thus a better protein quality for feeding and food use compared to ordinary dent types. Mertz in 1964 discovered that the single recessive gene, opaque-2 (*O2*), reduced zein in the endosperm and increased the percent of lysine to improve nutritional quality. Other genes with similar gross effects on protein quality exist in corn, but attempts to improve corn protein quality have been primarily based on use of the opaque-2 gene and modified opaque-2 germplasm (Mertz et al., 1964).

The so-called ornamental corn or "Indian" corns commonly show segregation for alleles of several genetic factors that control the production of anthocyanins and related pigments in the aleurone, pericarp and plant tissues of corn. The kernels may be segregating for various color expressions; and variation of color may even be expressed within a kernel, depending upon the genetic factors involved and their interaction during development of the kernel. Ornamental corns may be dent, sweet, pop, flint or floury endosperm types. Apart from genetic studies, they are a curiosity and are only grown for ornamental and decorative purposes (Brown et al., 1986).

2.8 Corn yield and yield influencing characters

Maize (*Zea mays* L.) is one of the most important cereal crops in the worldwide. Maize is used like a human food, livestock feed, for producing alcohol and non-alcohol drinks, built material, like a fuel and like medical and ornamental plant (Bekric & Radosavljevic, 2008). Since, the grain yield in maize is quantitative in nature and polygenically controlled, effective yield improvement and simultaneous improvement in yield components are imperative (Bello & Olaoye, 2009). Yield can be considered to be the result of the interaction of genotype, management, and environmental factors (Fageria et al., 2006). Selection on the basis of grain yield character alone is usually not very effective and efficient. However, selection based on its component characters could be more efficient and reliable (Alvi et al., 2003). Yield is composed of physical components that directly correlate to the amount of grain produced by the crop. Yield components are interrelated, have compensatory effects, and develop sequentially at different stages. First order yield components of maize consist of the number of ears (or ears per plant), kernels per ear, and kernel weight. First order yield components are sometimes referred to as primary components and have a direct effect on final yield as well as indirect effects through later developing yield components (Fageria et al., 2006). Yield components that can be considered second order or secondary are those that indirectly effect yield through their effect on first order components. These components consist of rows per ear, ear length, kernels per rows, and ear circumference. Previous studies (Agrama, 1996; Mohammadi et al., 2003) of maize yield components have used ears plant $^{-1}$ as a primary component rather than ears m $^{-2}$. When looking at yield on an area basis rather than a per plant basis, ears m^{-2} gives a more accurate measure of yield per unit area than ears plant^{-1}, which is of greatest importance to farmers. Path coefficient analysis has indicated that the number of ears plant^{-2} had a larger effect on grain yield than any of the other yield components (Agrama, 1996) as also found for

other crops such as wheat (*Triticum aestivum L.*) (Dhungana et al., 2007) and barley (*Hordeum vulgare L.*) (Dofing & Knight, 1994). Abendroth et al. (2011) stated that higher yield per acre is generally produced with relatively high plant populations combined with an adequate number of kernels ear^{-1} rather than a low plant population with a large kernels number ear^{-1}. This leads one to believe that ears m^{-2} has a larger correlation with yield in maize than the other components; however this correlation is highly dependent on time of stress. Early-season growing conditions influence the number of ears m^{-2} (or ears plant^{-1}). Mid-season growing conditions affect kernels ear^{-1}. Potential kernels ear^{-1} are a direct result of number of rows and kernels per row. Number of rows is determined at V7 stage (according to the leaf collar) method shortly after the ear is initiated (Stevens et al., 1986), Number of rows is strongly related to the genetics of the hybrid and is only effected by serious environmental stresses (Abendroth et al., 2011; Begna et al., 1997; Svecnjak et al., 2006). Potential number of kernels ear $^{-1}$ is determined from V7 through V15 or V16 (Uribelarrea et al., 2002). Pollen shed is a critical factor and ultimately determines whether or not potential ovules will be fertilized (Abendroth et al., 2011). Silking is the stage of the crop in which it is most sensitive to water and heat stress which can reduce kernel number (Westgate et al., 2004). At silking, the total number of kernels ear $^{-1}$ is determined; however kernel abortion during early grain fill can reduce kernel number. The correlations between traits is also great importance for success in selections to be conducted in breeding programs, and it is the most widely used one among numerous methods that can be used (Yagdi & Sozen, 2009). Multivariate methods have three main purposes: summarizing information, eliminating “noise” from the data sets and revealing the structure of the data sets (Crossa et al., 1990; Gauch Jr, 1992). Multivariate methods can also be used for determining grain yield stability and identifying genotypic groups possessing

desirable traits (Lin et al., 1986). Cluster analysis can identify differences among genotypes for the breeder via classification of genotypes (Sabaghnia et al., 2012).

2.9 Genetic Variability and Association Analysis of correlation and heritability

Genetic variation is a prerequisite for any crop improvement. Knowledge of genetic variation and relationships between accessions or genotypes is important to understand the genetic variability available and its potential use in breeding programs (Beyene et al., 2005; Thormann et al., 1994). An insight into the magnitude of variability is of utmost importance as it provides the basis for effective selection (Singh, 2005). The composition of the phenotype (the observable properties of an organism), is simply expressed as the outcome of three major sources of variation: the genotype, the environment which includes all factors external to the plant that affect development and growth, and interactions of all kinds (Lee, 2006). Falconer (1989), Banziger and Cooper (2001) described genetic variance as a measure of the extent of genetic differences among the germplasm units (individuals or families) evaluated (Bennetzen et al., 2001; Falconer, 1989). The partitioning of variance into its components allows the breeders to estimate the relative importance of the various determinants of the phenotype, in particular the role of heredity versus environment. Genetic gains from phenotypic selection have been assessed for many plant species and environments, and the progress has been varied (Duvick, 1986; Volenec et al., 2002). Despite instances of spectacular success, phenotypic selection has revealed little about the fundamental basis of progress achieved by plant breeding (Lee, 2006). The most important factor influencing selection gains is the amount of available genetic variation for general adaptation and traits necessary for improved production under specific constraints (Blum, 1988; Ceccarelli, 1989; Vasal et al., 1997). In agreement with this report, others also indicated that selection cannot create variability but can act on heritable variability already existing in

the population (Hallauer & Filho, 1988; Singh & Chaudhary, 1985). Genetic variation can be created by domestication, germpasm collection, plant introduction, hybridization (intervarietal, distant, somatic), mutation, polyploidy, somaclonal variation and genetic engineering (Singh, 2005). It is considered best to start selection on high performing and agronomically desirable germplasm exhibiting large variation for stress tolerant traits (Vasal et al., 1997). Different mating designs are used in the estimation of genetic variability and other components of variance. On the contrary, there is a method without mating design for estimation of genetic variances in a population that tests the unselected inbred lines themselves (Hallauer & Filho, 1988). They pointed out that although no mating is used, variability among inbred lines can be used as an estimation of genetic variability of a reference population. The total variance of a given character is its phenotypic variance (σ_p^2) and environmental variance (σ_e^2) which is that part of the phenotypic variance attributed to environmental conditions (Douglas S. Falconer et al., 1996). The total genetic variance (σ_g^2) also known as variance of genotypic value, is the part of phenotypic value which can be attributed to genotypic differences among the phenotypes (Dudley & Moll, 1969). Total genetic variance is further portioned into additive genetic variance (σ_A^2), dominance genetic variance (σ_D^2) and epistatic genetic variance (σ_1^2). The additive genetic variance, which is the variance of breeding values, is the important component. It determines the observable genetic properties of the population and the response of the population to selection. A primary goal of any plant breeding program is to develop and identify high yielding transgressive segregants. Kisha et al. (1997) indicated that populations with greater genetic variance are expected to produce higher yielding transgressive segregants than populations having lower genetic variance (Kisha et al., 1997). Genetic improvement of a crop is pivoted on the strength of genetic diversity within the crop species. Adequate variability provides options from which selections are made for improvement and possible hybridization.

Genotypic correlations had been used as an effective tool to determine the relationships among agronomic traits in genetically diverse population for enhanced progress in crop improvement. Binodh (2008) reported that information on character association in crops is important for effective and rapid selection in crop improvement (Binodh et al., 2008). Estimation of genotypic and phenotypic correlation among characters is useful for the formulation of a breeding program. Correlation measures the degree of association, genetic or non-genetic, between two or more characters and is measured by a correlation coefficient (Hallauer & Filho, 1988). The cause of correlation in crop plants can be genetic or environmental (Falconer, 1989; Hallauer & Filho, 1988). Two types of correlations, phenotypic and genetic, are commonly discussed in plant breeding. Phenotypic correlation (r_P) involves both genetic and environmental effects. It can be directly observed from measurements of the two characters in a number of individuals in a population (Hallauer & Filho, 1988). Genetic correlation is the association of breeding values (i.e., additive genetic variance) of the two characters (Falconer, 1989). Both measure the extent to which degree the same genes or closely linked genes cause co-variation in two different characters (Hallauer & Miranda Filho, 1988). Genetic correlations inherently have large errors because of difficulties to avoid the directional effects of confounding factors on additive correlation estimates (Falconer, 1989). Betran et al. (2003d) observed negative phenotypic correlations between grain yield and anthesis date. Pixley and Bjarnason (2002) reported positive and significant correlation between ear height and grain yield for Quality Protein Maize (QPM) cultivars. Genetic correlations for grain yield measured in stress and non-stress environments have been sufficiently low to make direct selection under stress, and more effectively in maize, than indirect selection under optimal conditions (Banziger & Lafitte, 1997; Brun & Dudley, 1989; Byrne et al., 1995). Moreover, the genetic correlation for grain yield between stress and optimal environments seems to decrease as stress intensity increases

(Banziger & Lafitte, 1997; Cooper et al., 1997; Fukai & Cooper, 1995). This suggests that selection in optimal environments could not be effective in identifying superior genotypes for stress environments. Correlation analysis between grain yield and secondary traits must be interpreted with care, because results are often confounded by genetic differences among genotypes for other traits or by the presence of outliers (Blum, 1988; Bolanos & Edmeades, 1996). Monneveux et al. (2005) studied the correlation of grain yield with ears per plant, grains per ear, grain weight, anthesis-silking interval, and plant and ear heights and reported a significant correlation of grain yield with grain weight under optimum N, and with anthesis-silking interval and grains per ear under low N conditions. High grain yields were associated with short anthesis-silking interval, increased number of ears per plant, and delayed leaf senescence (Banziger & Lafitte, 1997). They reported that absolute values for genetic correlations with grain yield were the highest for ears per plant, followed by leaf senescence, and anthesis-silking interval. In the diallel cross progeny of 10 QPM inbred lines, Hadji (2004) observed positive and significant correlations between grain yield and ear height, plant height, ears per plant, ear length, ear diameter, kernels per row and kernel weight. The same trend was observed for genotypic correlation of grain yield with these traits but with larger magnitude than the corresponding phenotypic correlation. Where Genotype x Environment interaction between stressed and unstressed environment is high, genetic correlations between the two environments are usually low (Itoh & Yamada, 1990). Banziger et al. (1997) observed positive genetic correlation between grain yields under low and high N. It is likely that a selection environment more similar to the target environment would result in larger selection gain (Banziger & Lafitte, 1997). Success of breeders in changing the characteristics of a population depends on the degree of correspondence between phenotypic and genotypic values (Dabholkar, 1992; Singh & Ceccarelli, 1995). In crop improvement, only the genetic component of

variation is important since only this component is transmitted to the next generation. A quantitative measure, which provides information about the correspondence between genotypic and phenotypic variance, is heritability (Dabholkar, 1992). Heritability assumes that individuals more closely related are more likely to resemble one another than distant ones (Falconer et al., 1996). Estimate of heritability assists breeders to allocate resources necessary to effectively select for desired traits and to achieve maximum genetic gain with little time and resources. There are different ways to calculate heritability. It may be estimated as broad-sense or narrow-sense, on single plant, individual plot or mean of entry (Nyquist & Baker, 1991). Some researchers reported that parent-offspring regression analysis has been used to estimate heritability in both crops and animals. Genetic advance explains the degree of gain obtained in a character under a particular selection pressure. High genetic advance coupled with high heritability estimates offers the most suitable condition for selection. It also indicates the presence of additive genes in the trait and further suggests reliable crop improvement through selection of such traits. Estimates of heritability with genetic advance are more reliable and meaningful than individual consideration of the parameters (Nwangburuka et al., 2012). According to Falconer and Mackay (1996), the relative importance of heredity in determining phenotypic values is called the heritability of the character. The extent of contribution of genotype to the phenotypic variation for a trait in a population is ordinarily expressed as the ratio of genetic variance to the total variance, i.e., phenotypic variance, for the trait; this ratio is known as heritability (Singh, 2005). Thus, heritability denotes the proportion of phenotypic variance that is due to genotype, i.e, heritable. The term heritability has been further divided into broad sense and narrow sense, depending whether it refers to the genotypic value or breeding value, respectively (Falconer, 1989; Holland et al., 2003). The ratio of genetic variance to phenotypic variance (σ_g^2 / σ_p^2) is called heritability in the broad sense

or genetic determination. It expresses the extent to which individual phenotypes are determined by the genotypes. A large percentage for a character is regarded as highly heritable whereas if it is smaller, some environmental agency is considered responsible for phenotypic manifestation of the character (Dabholkar, 1992). On the other hand, the ratio of additive variance to phenotypic variance (σ_A^2/σ_p^2) is called heritability in the narrow sense. The ratio of additive variance to phenotypic variance expresses the extent to which phenotypes are determined by the genes transmitted from the parents. The ratio also expresses the magnitude of genotypic variance in the population, which is mainly responsible for changing the genetic composition of a population through selection (Dabholkar, 1992; Falconer, 1989; Holland et al., 2003). Estimates of heritability serve as a useful guide to breeders. The knowledge of the relative heritability of the various traits and their genotypic and phenotypic correlation can aid in the design of efficient breeding systems where many traits need to be improved simultaneously (Jones, 1986). The breeder is able to appreciate the proportion of variation that is due to genotypic (broad sense heritability) or additive (narrow sense heritability) effects, that is, the heritable proportion of variation in the first case, and the proportion of genetic variation that is fixed in pure lines in the latter case (Singh, 2005). However, the type of gene action involved in the expression of a character has a significant role in determining heritability values. Characters that are controlled largely by genes acting in an additive fashion have higher heritability than characters governed by genes with large non-additive effects (Dabholkar, 1992; Falconer, 1989; Robinson & Hanson, 1963). According to Dabholkar (1992), it is important to note that heritability is a property not only of the character being studied, but also the population being sampled and the environmental circumstances to which individuals have been subjected. If heritability of a character is very high, for example, 0.8 or more, selection for the character should be fairly easy (Singh, 2005). This is because there would be a close correspondence

between the genotype and the phenotype due to a relatively smaller contribution of the environment to the phenotype. A high heritability implies that the genetic variation for a trait can be precisely assessed from phenotypic observations (broad-sense) and that the trait can be easily transmitted to the offspring of the selected genotypes (narrow-sense) (Marianne Banziger & Cooper, 2001; Falconer, 1989). But for a character with low heritability, say less than 0.4, selection may be difficult or impractical due to the masking effect of the environment on genotypic effects (Singh, 2005).

2.10 Genetic diversity and its relationship with heterosis/hybrid performance

Assessment of genetic diversity is important in plant breeding if there is to be improvement by selection. Genetic diversity among and within genera, species, subspecies, populations, and elite breeding materials is equally of interest in plant genetics and breeding. While plant taxonomists and germplasm banks are primarily interested in the higher levels of this hierarchy, plant breeders are mainly concerned with diversity among and within breeding populations and elite germplasm, because it largely determines the future prospects of success in breeding programs (Melchinger, 1999). One consequence of modern agricultural practices, which generally emphasizes maximum productivity with acceptable quality and uniformity, has been a reduction in genetic diversity (Lee, 1995). However, maize by virtue of its outcrossing nature and heterozygosity, possess broad genetic diversity. Detailed knowledge regarding genetic diversity and relationship among breeding materials is indispensable for the development of new maize inbred lines, establishment and assignment of maize inbred lines to heterotic groups, choice of testers and identification of promising combinations for exploitation of heterosis (Melchinger, 1999; Smith & Smith, 1992; Legesse et al., 2007; Xia et al., 2005). This applies particularly to hybrid breeding, where recognition and exploitation of heterotic patterns between different sources of germplasm are

important for success. Before 1970, established methods for measuring genetic diversity between taxonomic units have relied on pedigree analysis and morphological, physiological or cytological markers as well as biometric analysis of quantitative and qualitative traits, heterosis or segregation variance in crosses (Lee, 1995; Melchinger, 1999). Nevertheless, estimates of distance measures based on pedigree have several shortcomings. Some of the assumptions made in the calculation of pedigree relatedness may not be valid, particularly the assumptions of equal contribution from parents and lack of selection in derivation of new lines (Smith & Smith, 1989). Pedigree relationships often serve as standards to test the effectiveness of morphological in determining relationships among breeding lines and predicting heterosis (Tracy, 1994). Morphological traits have long been used to estimate systematic relationships in maize. Also morphology has proved useful for classifying maize races and populations (Goodman & Brown, 1988).

2.11 Over-expression of AGPase genes enhances seed weight in maize

The yields of cereal crops have increased mainly from improved agricultural practices and the use of superior cultivars developed through breeding efforts. Appropriately, the increased yields have received increasing attention from plant breeders, especially increases due to genetic causes. Many biochemical and molecular studies on starch synthesis have focused on identifying the ratelimiting enzymes to control metabolism. Historically, AGPase has received considerable attention because of its allosteric properties and its position in catalyzing the first unique step in starch synthesis (Hannah, 1997). AGPase is a tetrameric enzyme that consists of two large subunits and two small subunits in higher plants. Some studies have unveiled the functions of the different subunits. *E. coli* strains producing large subunit homotetramers from the potato had limited enzymatic activity, whereas those

synthesizing only small subunit homotetramers from the potato were catalytically active but much less sensitive to allosteric effectors (Iglesias et al., 1993). Removal of an N-terminal peptide from the large subunit from the potato decreased sensitivity to Pi inhibition (Laughlin et al. 1998). In maize the endospermspecific shrunken2 (*Sh2*) gene encodes the large subunit, while the small subunit is encoded by brittle2 (*Bt2*) (Bae et al., 1990; Hannah & Nelson, 1976). *E. coli* cells expressing only *Sh2* subunits had slightly higher AGPase activity than cells solely expressing *Bt2* subunits (Burger et al., 2003). An *E. coli* expression system utilizing potato tuber and maize endosperm mosaic AGPase revealed that the small subunit plays a vital role in allosteric regulation (Cross et al., 2004). AGPase plays an important role in starch synthesis. Transgenic and hybridization approaches have increased starch content. For example, (Stark et al., 1992) reported that over-expression of a non-regulated bacterial AGPase in transgenic potatoes and heterologous expression in transgenic tobacco calli both produced increased starch content compared with the wild-type. Subsequently, (Giroux et al., 1996) modified the allosteric properties of the maize endosperm AGPase heterotetramers by using *Sh2* revertant allele named *rev6* and *Bt2* gene encoded maize AGPase small subunits. These lines had 18% greater individual seed weight than lines expressing wild-type *Sh2*. Another example is Sh2hs33 (Greene & Hannah, 1998), which had a single-point mutation in *Sh2* that enhanced the stability of maize endosperm AGPase subunit interactions. Recently, the *E. coli* mutant *glgC* gene (*glgC16*) was introduced into maize and this increased AGPase activity under Pi-inhibitory conditions, and caused increased seed weight (Wang et al., 2007).

2.12 Quantification of RNA by Real-Time PCR

A reliable and robust method for measuring the expression of alternatively spliced transcripts is an important step in investigating the significance of each variant. So far,

accurate quantification of splice variants has been laborious and difficult due to the intrinsic limitations of conventional methods. The many advantages of real-time PCR have made this technique attractive to study its application in quantification of splice isoforms. Alternative splicing is a widespread process used in higher eukaryotes to regulate gene expression and functional diversification of proteins. A reliable and robust method for measuring the expression levels of splice variants is an important step in investigating the significance of each variant. Four methods are commonly used to quantify alternative transcripts: northern blotting (Sambrook et al., 1989), ribonuclease protection assay (Saccomanno, 1992), semi-quantitative RT–PCR (Ferre, 1992) and competitive RT–PCR (Becker-Andre & Hahlbrock, 1989). All these methods have their intrinsic limitations. Northern blotting is time-consuming, requires relatively large amounts of RNA, and is only suitable for determining relative concentrations of mRNA transcripts that occur in moderate to high abundance. RNase protection analysis is more sensitive than northern blotting, absolute RNA levels can be determined and small sequence variations can be detected. However, it is not sensitive enough to detect low abundance transcripts and the technique is also time-consuming. Semi-quantitative PCR reactions must be stopped and quantified in the early exponential phase, which is of course different for every sample depending on the initial target concentration. Quantitative Real-time PCR (qPCR) is an important method to measure gene expression level, and thus to obtain information about gene function in vivo. In general, there are two different methods of qPCR either based on intercalating dyes (e.g., SYBR Green I) or using a sequence specific detection probe (Horst & Peterhansel, 2007). The reverse transcription quantitative PCR (RTqPCR) has opened the possibility to investigate the biochemical and physiological changes for specific gene expression (Kim et al., 2003). The relative expression of a target gene can be normalized by a reference gene and by

comparing with the control sample can explain the fold changes in the gene of interest (Livak & Schmittgen, 2001).

In dyes based assays, the intercalating dye binds and detects nonspecifically any double-stranded DNA that accumulated during PCR like by- products and primer-dimers. In probe based assays, a labeled sequence-specific probe anneals to amplification product between the primer binding sites. Therefore, only the correct amplicon containing both primer and probe binding sites will generate a signal. Until now, the disadvantage of probe-based assays has been that they are less flexible than dye-based assays (Horst & Peterhansel, 2007). Competitive PCR has been commonly used, but its dynamic range is limited to a target-to-competitor ratio of about 1:10 to 10:1. Furthermore, different competitors must be designed for every new target and a large series of reactions with different competitor: target ratios have to be analyzed, which makes this quantification method labor intensive. Recently, a real-time quantitative PCR method was developed that overcomes the limitations associated with conventional quantification methods. Using this method, PCR product accumulation is continuously monitored during cycle progression by means of fluorescent detection chemistries. Real-time PCR is characterized by a large dynamic range of quantification, high accuracy, sensitivity and throughput capacity, and requires no post-amplification manipulation, thus avoiding possible carry-over contamination (Gibson et al., 1996). This technique may be very well suited to quantitate alternatively spliced transcripts, but so far only three reports using this technique for the study of alternative splicing have been published (Kafert et al., 1999). A reason for this might be the difficulty in creating two reliable standard curves for the intrapolation of unknown samples, a critical step in this kind of analysis. Knowledge of the copy number ratio between the corresponding dilution points of both standard curves is essential in order to compare the quantities of one transcript to another (Vandenbroucke et al., 2001).

CHAPTER 3: METHODOLOGY

3.1 Plant materials

For this study, a total of 12 corn advance lines were collected from the Institute of Biological Sciences (ISB), University of Malaya, Malaysia. In a previous project, a total of 12 top cross hybrids were formed using yellow sweet corn and white field corn. The parental lines included open-pollinated varieties (normal OPV parents), check varieties and inbred lines (donor parents). Parental field corn was collected from the International Maize and Wheat Improvement Centre (CIMMYT, Mexico) and parental sweet corn was collected from the International Institute of Tropical Agriculture (IITA, Nigeria) (Table 3.1). Prior to the appearance of silk, the developing ears were covered with a crystal clear plastic bag to make sure that the emerging silk was not contaminated with undesirable pollen. At anthesis, pollen was collected from desirable plants in the individual inbred lines using brown tassel bags, bulked for each inbred line and used to pollinate agronomically good plants in the open-pollinated varieties that served as female parents. A day prior to artificial crossing, the tassel of the male parent was covered with a brown tassel bag. This permitted fresh, uncontaminated pollen to be collected for crossing the next morning. At harvest, 5 clean cobs were selected from each line with good husk out of 26. These were de-husked, sun dried, shelled and put in separate envelopes and tagged. Therefore, all corn lines in this study were considered composite corn lines. The research project comprising three experiments was conducted in field and laboratory conditions at two locations with various climates (Malaysia and Iran). Initially, all of these twelve lines were grown for morpho-agronomic evaluation in the tropical environment (Kuantan, Malaysia). Finally, based on the morphological and physiochemical performance, six outstanding lines were selected for gene expression analysis. These six superior lines were selected and planted at a new test location in a dryland environment (Hajiabad, Iran) to consider the expression levels of the *Sh2* and

Bt2 genes in different growing conditions. The materials and methods are given for each experiment and a summary of the experiments is given in Table 3.2.

Table 3.1: Twelve corn advance lines and their parental origin

Genotype	Parental origin	Development location
UMNFJ 1	Mexico, Nigeria	ISB, FS, UM, Malaysia
UMNFJ 2	Mexico, Nigeria	ISB, FS, UM, Malaysia
UMNFJ 3	Mexico, Nigeria	ISB, FS, UM, Malaysia
UMNFJ 4	Mexico, Nigeria	ISB, FS, UM, Malaysia
UMNFJ 5	Mexico, Nigeria	ISB, FS, UM, Malaysia
UMNFJ 6	Mexico, Nigeria	ISB, FS, UM, Malaysia
UMNFJ 7	Mexico, Nigeria	ISB, FS, UM, Malaysia
UMNFJ 8	Mexico Nigeria	ISB, FS, UM, Malaysia
UMNFJ 9	Mexico, Nigeria	ISB, FS, UM, Malaysia
UMNFJ 10	Mexico, Nigeria	ISB, FS, UM, Malaysia
UMNFJ 11	Mexico, Nigeria	ISB, FS, UM, Malaysia
UMNFJ 12	Mexico, Nigeria	ISB, FS, UM, Malaysia

Table 3.2: Summary of experiments at the two test locations

Experiment	Number of corn lines	Experimental condition
Characterization of morpho-agronomic traits in a tropical environment	12	Field
Characterization of morpho-agronomic traits in a dryland environment	6	Field
Confirmation of the presence of *Sh2* and *Bt2* genes	12	Laboratory
Evaluation of the expression levels of *Sh2* and *Bt2* genes in leaf tissue at two test locations	6	Laboratory

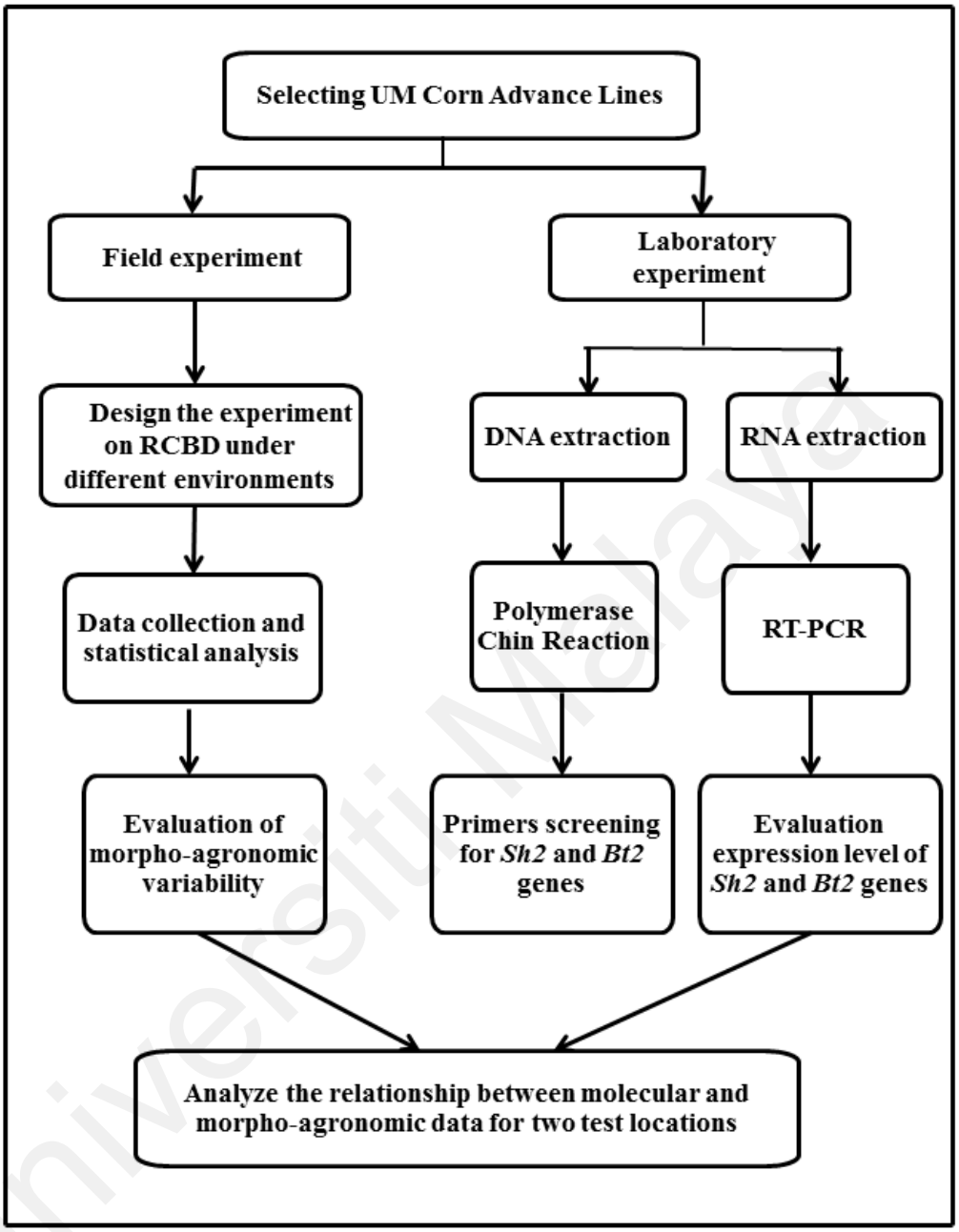

Figure 3.1: Flow chart of the research methodology

3.2 Evaluation of the morphological characteristics

3.2.1 Growing conditions

The experiment was laid out by Randomized Complete Block Design (RCBD) with three replications at each test location. The intercultural operations, i.e., weeding, water management and plant protection measures were followed for normal corn plant growth. Data for natural environmental conditions were collected for both test locations (Figures 3.2 and 3.3).

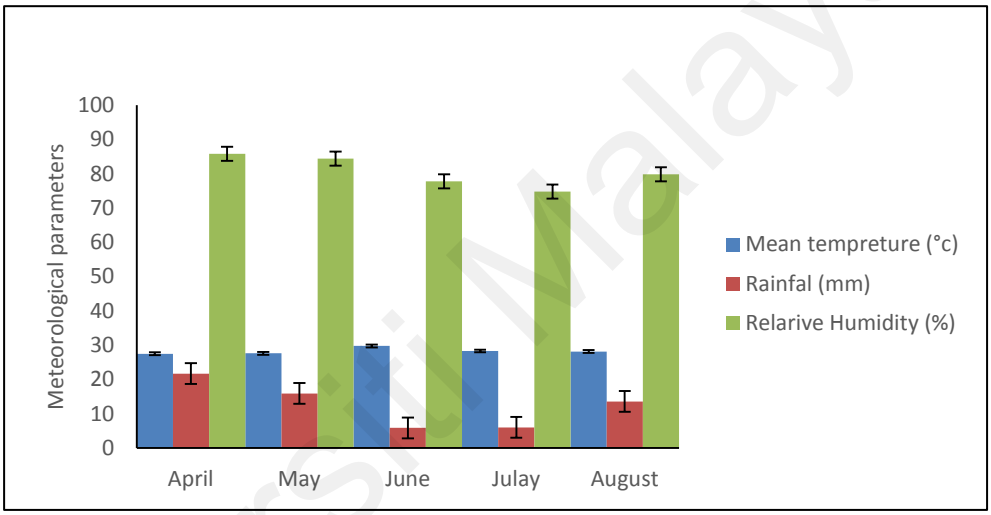

Figure 3.2: Meteorological information for the experimental site in Malaysia

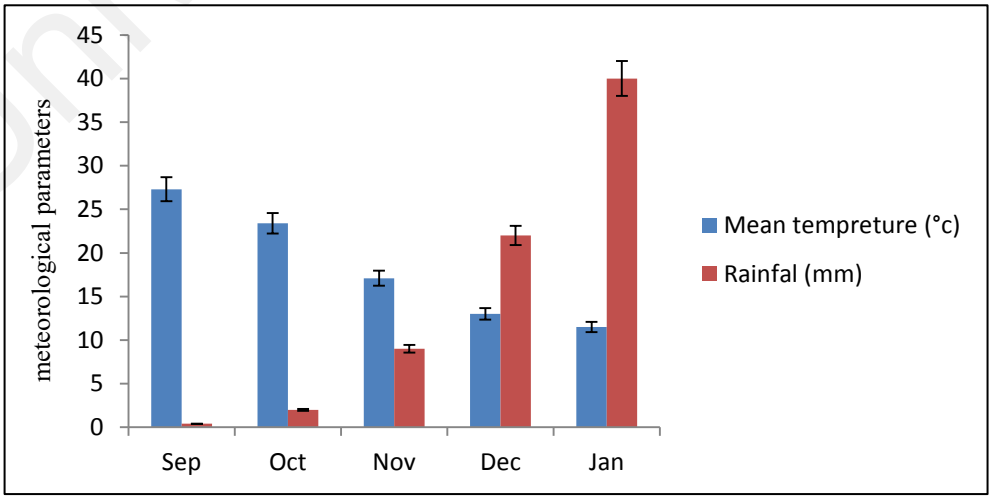

Figure 3.3: Meteorological information for the experimental site in Iran

3.2.2 Experimental design and management practice

The experiment was laid out with the Randomized Complete Block Design (RCBD) with three replications in Malaysia (tropical climate) and three replications in Iran (dryland climate). The experiment at the first location was from March 2015 until August 2015 to evaluate the morph-agronomic characterization. Initially, all twelve lines were grown for morph-agronomic evaluation. Then based on their morphological and physiochemical performance, six outstanding lines were selected to consider the same morphologic characteristics at the second test location (Iran). The experiment in Iran was from September 2015 until January 2016. In both Malaysia (Kuantan) and Iran (Hajiabad), the land was ploughed 4 times and levelled by laddering. To drain the excess rain water field, there were drainage channels around the plots. The plot size at both locations was the same, and it was 6 x 14 m. The plots were divided into three blocks four meters long and three meters wide. Grains were planted in rows 4m long. The distances between the blocks, rows (lines) and plants were 1 m, 75 cm and 50 cm respectively. The type of soil in Kuantan was strong, fine and blocky structured, clay loam, few fine roots, dark brown and pH = 6.70. The soil type in Iran was medium fine with loam texture and pH = 8.60. The plots at both locations were fertilized with the same amounts of N2, P2O5, and K2O at the rates of 120, 80 and 40 Kg ha-1 respectively. Nitrogen was applied in 3 equal splits. The first split of nitrogen was applied along with phosphorus at sowing. The second dose of nitrogen was applied 30 days after sowing and the third dose at crop tasseling stage. Two seeds were planted in each hill (planting hole) and plots were weeded three times, 15, 30 and 45 days after sowing. After sowing, no irrigation was required for the crops planted in Malaysia, but the crops planted in Iran were watered every 7 days. To adjust the population density in different plots, maize plant thinning was done to maintain plant-to-plant distance of approximately 50 cm. Moreover, during flowering, the tassel and silk were covered with

paper bags. According to the level of infestation and infection, insecticide/pesticide and disease control measures were adopted.

3.2.3 Data collection on quantitative traits

Quantitative data on tasseling date, silking date, plant height, ear number per plant, ear weight, ear length, kernels per ear, 1000 kernel weight and yield were recorded from ten guarded plants selected randomly and then averaged on a per plant basis. Agronomic parameters were measured, such as the date when half of the population appeared silk and half appeared tassel, as well as the flowering date for both male (tassel) and female (silk) inflorescences. Days to tasseling (DYTS) were documented as the number of days from planting to the time 50% of plants had fully emerged tassel. Days to silking (DYSK) were recorded as the number of days from planting to the time 50% of plants had completely extruded silk. Plant height was measured from the soil surface to the base of the leaf flag. Number of ears per plant and days to maturity were recorded after the physiological maturity stage (ripening stage). Once the plants in the plots attained physiological maturity, they were harvested manually. The husk covers that protect the corn grains were removed and the corn kernels were dried for a few days in the sun, after which they were weighed twice for accuracy. The grain filling period was calculated by deducting the maturation time from the flowering date. The field weight was measured by weighing the whole corn ear for each genotype. On the other hand, the grain weight is only the weight of grains alone without the husk cover and shell. Five hundred seeds were picked at random and then their weight was multiplied by two to measure the thousand grain weight. The ears were considered to be dry when their weight became constant.

3.2.4 Statistical analysis

The collected data were entered into Excel and was analyzed using both descriptive statistics including mean, standard deviation, and variance, frequency distribution and inferential statistics. The phenotypic correlation and genotypic correlation coefficients were collected using Steel and Torrie methods for all nine quantitative traits including tasseling date, silking date, plant height, ear number per plant, ear weight, ear length, kernel per ear, 1000 kernel weight and yield (Steel & Torrie, 1980).

Prior to data analysis all variables were subjected for normality test using Kolomogorove –Smirnove test and results indicated that all variables were distributed normally. Analysis of variance based on the RCBD was done to study the differences among genotypes for all characteristics for both environments. Means were compared by DMRT (Duncan multiple range test). The multivariate analysis, especially the principal component analysis (PCA) and cluster analysis was done to study the phenotypic and genotypic variation among selected corn advance lines (Cruz & Regazzi, 1994; Mardia et al., 1979). Cluster analysis was done based on UPGMA (between group linkages) to investigate distance, similarity and relatedness of genotypes or populations, so that similar genotypes can be classified into one group and dissimilar ones into distinct groups. Principal component analysis (PCA) was done to determine the variable independence and balanced weighting of each trait, which leads to an effective contribution of different characters on the basis of respective variation. Principal component analysis utilized to derive a 2 dimensional scatter plot of individuals, such that the geometrical distances among individuals in the plot reflect the genetic distances among them with minimal distortion.

In order to maintain, evaluate and utilize germplasm effectively, it is important to investigate the extent of genetic diversity available. Smith and Smith (1989) considered

morphological characterization as an important step in description and classification of crop germplasm because a breeding program mainly depends upon the magnitude of genetic variability. Following data analysis based on RCBD (Randomized Complete Block Design), genotypic and phenotypic parameters were collected to partition the gross (phenotypic) variability into the components due to genetic and non-genetic factors and to estimate the magnitude of these. Variance components (genotypic, phenotypic and error variance) were estimated using the formulae as follows: (Prasad et al., 1981; Wricke & Weber, 1986).

$$V_g = \frac{MSG - MSE}{r}$$

$$V_p = \left[MSG + MSE\right]$$

$$V_e = MSE$$

MSG, MSE and r are the mean squares of genotypes, mean squares of error and number of replications, respectively. Phenotypic (PCV) and genotypic (GCV) coefficient of variation were computed according to the following:

$$PCV = \left[\frac{\sqrt{V_p}}{\overline{X}}\right] \times 100$$

$$GCV = \left[\frac{\sqrt{V_g}}{\overline{X}}\right] \times 100$$

Where Vp, Vg and X are the phenotypic variances, genotypic variances and grand mean, Broad sense heritability (Hbs) is the ratio of the genotypic variance (Vg) to the phenotypic variance (Vp) as percent and it was estimated on genotypic mean basis

(Allard, 1999). Genetic advance (GA) expected and GA as percent of the mean assuming selection of the superior 5% of the genotypes were estimated in accordance with the methods in the following:

$$GA = K(S_P) \cdot Hbs$$

GA (as % of the mean) = (GA / x) x 100

(Fehr et al., 1987)

Where k is a constant (which varies depending upon the selection intensity and, if the latter is 5%, it stands at 2.06). Sp is the phenotypic standard deviation (√vp), Hbs is the heritability ratio and x refers to the season mean of the character (Ali et al., 2008; Sunday et al.).

Broad sense heritability (Hbs) can be estimated based on the procedure described by Poehlman (1994), as follows:

$$Hbs = \frac{\sigma_g^2}{\sigma_{ph}^2} \times \frac{100}{1}$$

Genotypic correlation was calculated using the following equation for all traits as follows:

$$r_G = \frac{\sigma_{G(X,Y)}}{\sqrt{\sigma_{G(X)}^2 \cdot \sigma_{G(Y)}^2}}$$

(Becker, 1992).

Where r_G is genetic correlation between the traits X and Y, $\sigma_{G(X,Y)}$ is genotypic covariance between the traits X and Y, $\sigma_{G(X)}^2$ is genotypic variance of the trait X, and

$\sigma^2_{G(Y)}$ is genotypic variance of the trait Y.

Combined analysis of variance also was applied to investigate the stability of all characteristics. All data analysis was done using SAS Ver 9.1 and SPSS Ver 22.

3.3 Evaluation the presence of *Sh2* and *Bt2* genes

3.3.1 DNA extraction

Twelve newly developed corn lines were planted in twelve different pots. After 2 weeks, young leaves of each plant were plucked, avoiding the hard parts of leaves and placed on aluminum foil then was transferred into liquid nitrogen immediately to freeze and stored at -80°C until to use for extraction the genomic DNA. Due to transfer the pots to laboratory and high speed for freezing process, for DNA extraction planting in pots were preferred than field. The leaf material was ground to a fine powder with a mortar and pestle that had been pre-cooled with liquid nitrogen and then total genomic DNA of each plant was extracted from 100 mg of fresh leaf tissue using a DNeasy Plant Mini Kit.

3.3.2 Evaluation of DNA quality and quantity

DNA is quantified by using Nano drop (NANODROP Spectrophotometer, ND-2000). Concentration range then can be measured by ND-2000 from 2ng/µl to 15,000 ng/µl dsDNA without dilution. Sample volumes used for measurement is only 1µℓ. Nucleotides, RNA, ssDNA, and dsDNA will all absorb at 260nm and contribute to the total absorbance. The ratio of absorbance at 260nm and 280nm is used to assess the purity of DNA and RNA. A ratio of around 1.8 is generally accepted as pure for DNA. The ratio of absorbance at 260/230 is used as a secondary measure of nucleic acid purity. The 260/230 values for 'pure' nucleic acid are often higher than the respective 260/280 values. Expected 260/230 values are commonly in the range of 2.0-2.2. DNA

quality is also evaluated by running the DNA on gel electrophoresis with 0.8% agarose gel. Three microliter (µl) of DNA samples was mixed with 3µl of 6X loading buffer on a para film paper and then loaded in the well of Agarose gel. The electrophoresis was operated at 70 V, 150 mA, 40 minutes in 1X TBE buffer and Hind III ladder was used.

3.3.3 Selection and design of oligonucleotide primers

Four primers were designed based on the full sequence of *Sh2* gene from NCBI (accession no. M81603.1) using Primer-BLAST program (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). General properties of designed primers were considered using Sequence Manipulation Suite software (http://www.bioinformatics.org/sms2/pcr_primer_stats.html). Furthermore, primer designing, four published primers were used for amplifying *Sh2* and *Bt2* genes shown in Table 3.3 and three primers were used as housekeeping genes (Table 3.4).

Table 3.3: Oligonucleotide Primer Sequences to amplify *Sh2* and *Bt2* gene

Gene	Primer	Application	Sequence (5' → 3')
Sh2	LH71 F LH71 R	Normal PCR	GGGAAGAAGCTTCAAAGCTAC GGATCCAAAGCAGGGTAATGG
Sh2	F2 F F2 R	Normal PCR	TGTCTGTTGAGGGGAAAGCC GGAGGGCATACTGGTGGAGA
Sh2	F4 F R4 R	Normal PCR	GGCACCCACCATCACCTATT TGAGGAGGGCATACTGGTGG
Sh2	F5 F F5 R	Normal PCR RT-PCR	TAATGCCAAAGCATCCAGGC TGTCGGCAAGAATGGAGCAA
Sh2	F6 F F6 R	Normal PCR	CGGCTGTGCATTTTGGAAGT GCCTGGATGCTTTGGCATTA
Sh2	sh2 F sh2 R	Normal PCR	GGGAGCGGACACCTATGAA AGCCTCTTGGATGCCCTTAC
bt2 (BT2a)	bt2a F bt2a R	Normal PCR RT-PCR	GCCGCTGCAAATGATTCAACATACC GCAGGCTTGGCACGCTTCTTTG
bt2 (BT2b)	bt2b F bt2b R	Normal PCR RT-PCR	ATAGCCTCAGCTTCGCCCAGGA CACGGGCATCGAGATGGACACT

Table 3.4: Oligonucleotide primer sequences to amplify housekeeping genes

Gene	Primer	Application	Sequence (5' → 3')
18S rRNA	Zm18s F Zm18s R	RT-PCR	GCCTTCGGGATCGGAGTAAT CCCCCAACTTTCGTTCTTGA
eEF-1a	eEF F eEF R	RT-PCR	CTTCATAGGAATGGAAGCTGCGGGTA CGACCACCTTGATCTTCATGCTGCTA
Actin	Actin F Actin R	RT-PCR	TTTCACTCTTGGTGTGAAGCAGAT GACTTCCTTCACGATTTCATCGTAA

3.3.4 Polymerase Chain Reaction (PCR) and Sequencing of genomic DNA

Polymerase Chain Reaction (PCR) amplification for each primer set was performed in a C1000 Thermal Cycler (Bio-Rad) in a total volume of 10µl reaction solution consisting of 1.5µl of DNA extracted from leaf tissues, 5µl of Gotaq Green master mix (Promega, USA) and 1µl of each primer (10mM). The PCR reactions were carried out as follows: initial denaturation at 95 C for 5min, 35 cycles of denaturation at 94°C for 30s, annealing temperature for 30s, and 1 min of extension step at 72°C. The program was then completed with a final extension at 72°C for 10 min. Following amplification, the presence of PCR products were verified *via* electrophoresis. Agarose gel (1.0%) was used in electrophoresis of PCR products. The gel electrophoresis was carried out at 80V, 175mA using 1 x TBE Running Buffer for 30 min. The gels were visualized under ultraviolet light (Alpha Imager Gel Documentation System, Siber Hegner, Germany). Also, the sequencing products were analyzed by an ABI 3730xl DNA analyzer (Applied Biosytems, USA).

3.4 Expression level of *Sh2* and *Bt2* genes in leaf and endosperm

3.4.1 RNA extraction

After screening the presence of *Sh2* and *Bt2* genes in these twelve corn lines, six superior lines based on the morphological performance were selected to carry out gene expression experiment. The leaves of plants were collected, preserved in liquid

nitrogen and stored at -80°C. Total leaf RNA was isolated using RNA extraction kit (SV Total RNA Isolation System, Promega Corporation, USA). The Total endosperm RNA was isolated from dissected mid-ear endosperm 20 DAP which was the period of active starch 'filling' (Shang-Jing et al., 2006) using Trizol Reagent (Invitrogen) according to the manufacturer's protocol. Total RNA was pooled from three individual kernels per ear. The quality and concentration of extracted total RNA were determined by nanodrop Spectrophotometer (Nano Drop 2000, Thermo Scientific, USA) at 260 nm.

3.4.2 Real-Time PCR

The Real time quantitative PCR amplification was carried out using CYBR green fluorescence in the RTqPCR (CFX96 Touch™ Real-Time PCR Detection System, Bio-Rad, USA) on a total of 20 μl reaction volume containing SYBR Green mix (GoTaq 1-step RTqPCR reaction mix, Promega Corporation, USA), Primer (Forward and reverse), Nuclease free water and RNA template (100 ng). Negative control (Without reverse transcriptase), No template control (without RNA) and Positive control (without primer) were included with each reaction set, assayed triplicate for 3 biological replicates. The thermal cycling condition was 48°C for 15 min (Reverse transcription), 95°C for 10 min (Reverse transcription inactivation) followed by 40 cycles of 95°C for 10 sec (Denaturation), 58°C /52°C /55°C (*18s*, *Bt2b* and *Sh2* respectively) for 30 sec (Annealing), 72°C for 30 sec (Extension). After amplification, the samples were kept at 95°C for 10 sec and 65°C for 5 sec then raised gradually by 0.5°C every 5 sec to obtain melt curve.

3.4.3 Data analysis with the $2^{-\Delta\Delta C}_{T}$ method

The amplification results from CFX Manager Software (Included with CFX96 Touch™ Real-Time PCR Detection System, Bio-Rad, USA) were exported to Excel file and the quantification of gene expression was conducted according to the relative quantification methods of Livak and Schmittgen (Livak & Schmittgen, 2001). The C_T values of *18s* gene was used as internal control (Endogenous reference) and C_T values of the parent line for each corn advance line were used as calibrator (Control). The effects hybridization on internal control, *Sh2* and *Bt2* genes were estimated using $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001) where same amount of RNA was used for external normalization.

3.4.3.1 Derivation of the $2^{-\Delta\Delta C}_{T}$ method

The equation that describes the exponential amplification of PCR is:

$X_n = X_0 \times (1 + E_X)^n$ (Murphy et al., 1990)

where X_n is the number of target molecules at cycle n of the reaction, X_0 is the initial number of target molecules. E_X is the efficiency of target amplification, and n is the number of cycles. The threshold cycle (C_T) indicates the fractional cycle number at which the amount of amplified target reaches a fixed threshold.

Thus,

$X_T = X_0 \times (1 + E_X)^{C_{T,X}} = K_X$ (Noonan et al., 1990)

Where X_T is the threshold number of target molecules, $C_{T,X}$ is the threshold cycle for target amplification, and K_X is a constant. A similar equation for the endogenous reference (internal control gene) reaction is:

$$R_T = R_0 \times (1 + E_R)^{C_{T,R}} = K_R \qquad \text{(Horikoshi et al., 1992)}$$

where R_T is the threshold number of reference molecules, R_0 is the initial number of reference molecules, E_R is the efficiency of reference amplification, $C_{T,R}$ is the threshold cycle for reference amplification, and K_R is a constant. Dividing X_T by R_T gives the expression

$$\frac{X_T}{R_T} = \frac{X_0 \times (1 + E_X)^{C_{T,X}}}{R_0 \times (1 + E_R)^{C_{T,R}}} = \frac{K_X}{K_R} = K \qquad \text{(Heid et al., 1996)}$$

For real-time amplification using TaqMan probes, the exact values of X_T and R_T depend on a number of factors including the reporter dye used in the probe, the sequence context effects on the fluorescence properties of the probe, the efficiency of probe cleavage, purity of the probe, and setting of the fluorescence threshold. Therefore, the constant K does not have to be equal to one. Assuming efficiencies of the target and the reference are the same,

$$E_X = E_R = E,$$

$$\frac{X_0}{R_0} \times (1 + E)^{C_{T,X} - C_{T,R}} = K, \qquad \text{(Winer et al., 1999)}$$

or

$$X_N \times (1 + E)^{\Delta C_T} = K \qquad \text{(Schmittgen et al., 2000)}$$

where X_N is equal to the normalized amount of target (X_0 / R_0) and ΔC_T is equal to the difference in threshold cycles for target and reference ($C_{T,X}$ - $C_{T,R}$).

Rearranging gives the expression

$$X_N = K \times (1 + E)^{-\Delta C_T} \qquad \text{(Schmittgen \& Zakrajsek, 2000)}$$

The final step is to divide the X_N for any sample q by the X_N for the calibrator (cb):

$$\frac{X_{N,q}}{X_{N,cb}} = \frac{K \times (1+E)^{-\Delta C_{T,q}}}{K \times (1+E)^{-\Delta C_{T,cb}}} = (1+E)^{-\Delta\Delta C_T} \qquad \text{(Chen \& Shyu, 1994)}$$

The amount of target, normalized to an endogenous reference and relative to a calibrator, is given by: $\text{amount of target} = 2^{-\Delta\Delta C_T}$ (Iyer et al., 1999)

3.4.3.2 Assumptions and applications of the $2^{-\Delta\Delta C}{}_T$ method

For the $\Delta\Delta C_T$ calculation to be valid, the amplification efficiencies of the target and reference must be approximately equal. A sensitive method for assessing if two amplicons have the same efficiency is to look at how ΔC_T varies with template dilution. If the absolute value of the slope is close to zero, the efficiencies of the target and reference genes are similar, and the $\Delta\Delta C_T$ calculation for the relative quantification of target may be used. If the efficiencies of the two amplicons are not equal, then the analysis may need to be performed via the absolute quantification method using standard curves. Alternatively, new primers can be designed and/or optimized to achieve a similar efficiency for the target and reference amplicons.

3.4.3.3 Selection of internal control and calibrator for the $2^{-\Delta\Delta C}{}_T$ method

The purpose of the internal control gene is to normalize the PCRs for the amount of RNA added to the reverse transcription reactions. It was found that standard housekeeping genes usually suffice as internal control genes. It is highly recommended that the internal control gene be properly validated for each experiment to determine that gene expression is unaffected by the experimental treatment. The choice of calibrator for the $\Delta\Delta C_T$ method depends on the type of gene expression experiment that one has planned. The simplest design is to use the untreated control as the calibrator. Using the $\Delta\Delta C_T$ method, the data are presented as the fold change in gene expression

normalized to an endogenous reference gene and relative to the untreated control. For the untreated control sample, $\Delta\Delta C_T$ equals zero and 2^0 equals one, so that the fold change in gene expression relative to the untreated control equals one, by definition. For the treated samples, evaluation of $2^{-\Delta\Delta C_T}$ indicates the fold change in gene expression relative to the untreated control. Similar analysis could be applied to study the time course of gene expression where the calibrator sample represents the amount of transcript that is expressed at time zero. Situations exist where one may not compare the change in gene expression relative to an untreated control, for example, if one wanted to determine the expression of a particular mRNA in an organ.

3.4.3.4 Data analysis using the $2^{-\Delta\Delta C_T}$ method

The C_T values provided from real-time PCR instrumentation are easily imported into a spreadsheet program such as Microsoft Excel. To demonstrate the analysis, data are reported from a quantitative gene expression experiment and a sample spreadsheet is described. Triplicate samples of cells were collected at each time point. Real-time PCR was performed on the corresponding RNA synthesized from each sample. The data were analyzed using Eq: amount of target $= 2^{-\Delta\Delta C_T}$, where $\Delta\Delta C_T = (C_{T,\text{Target}} - C_{T,18s})_{\text{Time x}} - (C_{T,\text{Target}} - C_{T,18s})_{\text{Time 0}}$. Time X is any time point and Time 0 represents the 1 X expression of the target gene normalized to *18s*. The mean C_T values for both the target and internal control genes were determined at time zero and were used in amount of target $= 2^{-\Delta\Delta C_T}$. The fold change in the target gene, normalized to *18s* and relative to the expression at time zero, was calculated for each sample using Eq: amount of target $= 2^{-\Delta\Delta C_T}$. The mean, SD, and CV are then determined from the triplicate samples at each time point. Using this analysis, the value of the mean fold change at time zero should be very close to one (i.e., since $2^0 = 1$). From this study, it was found the verification of the mean fold change at time zero to be a convenient method to check for

errors and variation among the triplicate samples. A value that is very different from one suggests a calculation error in the spreadsheet or a very high degree of experimental variation. In the preceding example, three separate RNA preparations were made for each time point and carried out through the analysis. Therefore, it made sense to treat each sample separately and average the results after the $2^{-\Delta\Delta C_T}$ calculation. When replicate PCRs are run on the same sample, it is more appropriate to average C_T data before performing the $2^{-\Delta\Delta C_T}$ calculation.

Exactly how the averaging is performed depends on if the target and reference are amplified in separate wells or in the same well. The variance estimated from the replicate C_T values is carried through to the final calculation of relative quantities using standard propagation of error methods. One difficulty is that C_T is exponentially related to copy number. Thus, in the final calculation, the error is estimated by evaluating the $2^{-\Delta\Delta C_T}$ term using $\Delta\Delta C_T$ plus the standard deviation and $\Delta\Delta C_T$ minus the standard deviation. This leads to a range of values that is asymmetrically distributed relative to the average value. The asymmetric distribution is a consequence of converting the results of an exponential process into a linear comparison of amounts. By using probes labeled with distinguishable reporter dyes, it is possible to run the target and reference amplifications in the same well.

Therefore, it makes sense to calculate ΔC_T separately for each well. These ΔC_T values can then be averaged before proceeding with the $2^{-\Delta\Delta C_T}$ calculation. Again, the estimated error was given as an asymmetric range of values, reflecting conversion of an exponential variable to a linear comparison. Subtraction of the average $\Delta C_{T,cb}$ to determine the $\Delta\Delta C_T$ value is treated as subtraction of an arbitrary constant. The C_T values for nonreplicated samples were carried through the entire $2^{-\Delta\Delta C_T}$ calculation before averaging. Alternatively, it is possible to report results with the calibrator

quantity defined as 1X without any error. In this case, the error estimated for the average $\Delta C_{T,cb}$ value must be propagated into each of the $\Delta\Delta C_T$ values for test samples.

CHAPTER 4: RESULTS

4.1 Quantitative characteristics

Descriptive statistics including mean and standard deviation were used to measure the quantitative traits, viz. tasseling date, silking date, plant height, ear number per plant, ear weight, ear length, kernels per ear, 1000 kernel weight and yield per plant as presented in Table 4.1 and Table 4.2. The tables show the variability in various corn lines at two different test locations (Malaysia and Iran). The most important traits (yield, kernels per ear and 1000 kernel weight) across the two locations exhibited high variation. The most highly variating traits in Malaysia were yield, 1000 kernel weight and kernel number per ear. Meanwhile, yield, kernel number per ear, plant height and 1000 kernel weight respectively displayed the highest variation in Iran.

Table 4.1: Basic statistics for the quantitative traits of twelve corn advance lines grown in Malaysia

Trait	Mean ± SD	Minimum	Maximum
Tasseling (d)	62.19 ± 4.74	54.00	71.00
Silking (d)	66.14 ± 2.75	62.00	73.00
Plant height (cm)	141.22 ± 13.01	123.32	171.48
Ear no./plant	3.34 ± 1.03	1.9	5.3
Ear wt. (g)	284.53 ± 33.76	205.48	334.21
Ear length (cm)	26.99 ± 7.66	9.51	40.54
No. kernels/ear	295.70 ± 31.12	251.65	346.15
1000 kernel wt. (g)	290.37 ± 41.12	164.93	357.24
Yield/plant	290.15 ± 117.87	150.03	627.96

Table 4.2: Basic statistics for the quantitative traits of six corn advance lines grown in Iran

Trait	Mean ± SD	Minimum	Maximum
Tasseling (d)	69.56 ± 1.54	67.00	72.00
Silking (d)	74.89 ± 1.28	73.00	77.00
Plant height (cm)	181.26 ± 15.97	138.80	203.50
Ear no./plant	2.31 ± 0.32	1.6	3.7
Ear wt. (g)	178.82 ± 3.25	172.80	181.70
Ear length (cm)	14.40 ± 1.07	12.50	15.40
No. kernels/ear	254.11 ± 33.84	191.60	298.80
1000 kernel wt. (g)	126.28 ± 8.71	110.00	135.00
Yield/plant	75.13 ± 20.13	102.02	41.41

4.2 Morphological and yield trait variability among corn lines

To compare all traits among twelve corn lines at the first location (Malaysia), analysis of variance was carried out based on randomized complete block design (RCBD). The results showed significant differences among all twelve corn lines at the p = 0.01 level, except for tasseling date (28.76 ns) and ear length (71.45 ns), which were not statistically significant (Table 4.3). Following the analysis of variance, Duncan's multiple range test was applied for a mean comparison among corn lines (Table 4.4). The mean comparison results indicate that the highest mean tasseling date was for UM 12 (M = 67.33 days) and the lowest was for UM 5 (M =58 days). However, there was no significant difference among corn lines for this trait. The highest mean silking date was observed for UM 2 (M = 69.33 days), which was not significantly different from UM 1, UM 3, UM 8, UM 9, UM 10, UM 11 and UM 12. Then UM 7 (M = 62 days) exhibited the lowest mean silking date, which was significantly different from UM 1, UM2, UM 3, UM 8, UM 9, UM 10, UM 11 and UM 12. In terms of plant height, the

highest mean was observed for UM 8 (M = 165.2 cm), which was significantly different from all corn lines except UM 5 and UM 12. The lowest mean plant height was seen in UM 9 (M = 128.9 cm) and this was significantly different from all corn lines except UM 5, UM 8 and UM 12. For the number of ears per plant, UM 4 (M = 4.47) had the highest mean, whereas UM 9 and UM 11 had the lowest means, but this trait was not significantly different among corn lines. The highest mean ear weight was observed for UM 1 (M = 327.5 g) and this was significantly different from UM 3, UM 4, UM 5, UM 6, UM 7, UM 8 and UM 9. The lowest mean ear weight was found for UM 9 (M = 237.7 g), which showed no significant difference from UM 3, UM 4, UM 7 and UM 8. The highest mean ear length was observed for UM 6 (M = 36.6 cm) and the lowest was for UM 5 (M = 20.5 cm). This trait showed no significant difference among corn lines. Regarding the number of kernels per ear, the highest mean was observed for UM 1 (M = 328.43), which was significantly different from UM 3, UM 5, UM 9 and UM 12. The lowest mean was for UM 5 (M = 263.21) with significant difference from UM 1, UM 6 and UM 11. For 1000 kernel weight, UM 1 (M = 349.1 g) showed the highest mean and it was significantly different from all corn lines except UM 11. The lowest mean for this trait was observed for UM 8 (M = 222.3 g) with significant difference from all corn lines except UM 4 and UM 7. For the last trait, yield per plant, the highest mean was seen for UM 1 (M = 504.7 g), which was significantly different from all corn lines except UM 2 and UM 3. The lowest mean for this trait was for UM 8 (M = 187.1 g) with insignificant difference from all corn lines except UM 1 and UM 2.

Table 4.3: Mean square (MS) values from ANOVA for the yield and yield components of twelve corn advance lines grown in Malaysia

Source of variation	Tasseling (days)	Silking (days)	Plant height (cm)	Ear no. /plant	Ear wt. (g)	Ear length (cm)	No. kernels/ ear	1000 kernel wt. (g)	Yield/plant
Genotypes	28.76 ns	18.57**	430.44**	1.85**	2948.93**	71.44 ns	1843.49**	5021.21**	28260.5**
Block	40.11ns	6.361ns	118.47 ns	2.67*	369.49 ns	197.19*	2214.15*	285.92 ns	35049. 9**
Error	17.69	2.15	43.49	1.85	304.63	39.63	418.15	153.57	4788.36
F-value for Genotypes	1.63 ns	8.64**	9.90**	3.60**	9.68**	1.80 ns	4.41**	32.70**	5.90**
CV (%)	6.77	2.22	4.67	21.48	6.13	23.32	6.92	4.27	23.85
R2	0.51	0.82	0.84	0.69	0.83	0.58	0.73	0.94	0.78

* Significant at the 0.05 level, ** significant at the 0.01 level

Table 4.4: Comparison of the means of all traits among twelve corn advance lines (Malaysia) using Duncan's multiple range test

Genotypes	Tasseling (days)	Silking (days)	Plant height (cm)	Ear no. /plant	Ear wt. (g)	Ear length (cm)	No. kernel/ ear	1000 kernel wt. (g)	Yield/plant
UM1	66±4.04 a	68±1 a	134.69±2.29 c	4.33±0.73 a	327.55±3.74 a	31.46±5.85 a	328.43±7.59 a	349.15±8.87 a	504.75±102.03 a
UM 2	62.33±3.84 a	69.33±0.33 a	142.54±2.57 bc	4.4±0.75 a	310.66±8.99 abc	28.51±2.43 a	315.62±18.42 abc	313.62±6.17 b	428.35±61.05 ab
UM 3	61.33±3.84 a	67.33±1.2 ab	129.35±3.42 c	4.33±0.62 a	238.15±3.67 e	21.73±6.31 a	268.15±15.01 bc	295.58±5.42 bc	350.14±68.31 abc
UM 4	61.67±2.33 a	63.67±0.88 bcd	136.57±2.07 c	4.47±0.59 a	272.26±18.74 cde	28.12±4.02 a	288.4±3.54 abc	229.61±15.59 e	293.56±37.59 bc
UM 5	58±2.08 a	63±1 cd	159.56±8.56 a	2.8±0.38 a	282.36±8.57 bcd	20.53±3.35 a	263.21±9.02 c	286.43±7.27 bc	208.65±19.77 c
UM 6	59±1.53 a	63.67±0.88 bcd	135.66±2.75 c	2.9±0.26 a	282.84±8.34 bcd	36.6±2.26 a	319.21±4.13 ab	309.37±4.77 b	287.03±30.58 bc
UM 7	59.67±1.45 a	62±1.15 d	134.57±2.43 c	2.8±0.26 a	270.26±16.83 cde	26.45±3.69 a	286.73±3.79 abc	248.41±5.75 de	199.71±20.8 c
UM 8	66.33±2.03 a	68.67±0.88 a	165.2±6.64 a	2.73±0.47 a	254.08±9.94 de	30.46±2.37 a	300.68±29.89 abc	222.35±5.7 e	187.11±45.38 c
UM 9	59.33±2.03 a	65.67±1.33 abc	128.99±2.47 c	2.67±0.3 a	237.79±3.58 e	21.37±4.75 a	267.79±13.88 bc	295.22±3.86 bc	213.17±32.77 c
UM 10	61.33±2.19 a	67.67±0.33 a	140.88±3.98 bc	2.73±0.42 a	309±7.14 abc	26.85±2.19 a	313.96±18.69 abc	311.96±6.88 b	263.46±31.95 bc
UM 11	64±2.08 a	66.33±0.88 abc	132.69±3.07 c	2.67±0.41 a	325.55±2.33 ab	29.8±5.91 a	326.77±7.28 a	347.15±6.87 a	305.93±55.53 bc
UM 12	67.33±0.88 a	68.33±0.33 a	153.97±3 ab	3.23±0.09 a	303.84±13.66 abc	22±4.13 a	269.47±5.94 bc	275.62±4.31 cd	239.91±5.29 c

Values are mean ± SE of three replications; means with the same letter are not significantly different at p = 0.05 according to Duncan's multiple range test

To compare all traits among six corn lines at the second location (Iran), analysis of variance based on randomized complete block design (RCBD) was also done. Based on the results in Table 4.5, high variance in tasseling date, plant height, ear length, kernel number per ear, 1000 kernel weight, ear weight and yield per plant was observed among the six corn advance lines. According to the results, there was a significant difference among the six corn lines for all traits except silking date and ear number per plant, which showed no significant variation. Tasseling date, plant height, ear length, kernel number per ear and 1000 kernel weight displayed significance at the p=0.01 level, whereas ear weight (26.23) and yield per plant (916.85) were significant at the p=0.05 level (Table 4.5). Following the analysis of variance, Duncan's multiple range test was used for a comparison of the means among corn lines (Table 4.6). The results indicate that the highest mean tasseling date was for UM 11 (M = 71.33 days), which was not significantly different from UM 3, UM 4 and UM 6. The lowest mean tasseling date was observed for UM 4 and UM 6 (M =68.33 days), which was significantly different from UM 11. The highest mean silking date was for UM 11 (M = 76 days) and the lowest was for UM 3 and UM 4 (M = 74 days). However, there was no significant difference in this trait among the corn lines. For plant height the highest mean was for UM 11 (M = 198.6 cm), but this was not significantly different from other corn lines except UM 1. The lowest mean plant height was for UM 1 (M = 157.33 cm), which was significantly different from UM 2, UM 6 and UM 11. For the next trait, ear number per plant, UM 11 (M = 2.53) had the highest mean, whereas UM 2 (M =1.87) had the lowest mean. There was no significant difference between corn lines in terms of ear number per plant. The highest mean for ear weight was for UM 4 (M = 181.1 g) and it was significantly different from UM 11. The lowest mean for ear weight was for UM 11 (M = 174.77 g), which showed significant difference from UM 4 and UM 6. The highest mean for ear length was observed in UM 4 (M = 15.23 cm) with significant

difference from UM 2 and UM 11. The lowest mean was for UM 11 (M = 12.63 cm), which was significantly different from all corn lines except UM 2. For the kernel number per ear trait, the highest mean was obtained for UM 6 (M = 277.33) and this was significantly different from UM 2 and UM 11. The lowest mean was obtained for UM 11 (M = 209.93), which was significantly different from all corn lines except UM 2. For the 1000 kernel weight, UM 4 (M = 133.33 g) had the highest mean with significant difference from UM 2 and UM 11. The lowest mean for this trait was observed for UM 2 (M = 113.33 g), with significant difference from all corn lines except UM 11. For the last trait, yield per plant, the highest mean was seen for UM 3 (M = 87.16 g), but this was not significantly different from all other corn lines except UM 2. The lowest mean for this trait was for UM 2 (M = 45.34 g), which was significantly different from UM 1, UM 3, UM 4 and UM 6. Table 4.6 explains the mean comparison for the nine quantitative characteristics of six corn advance lines. Based on results in Tables 4.4 and 4.6, from the twelve corn lines in Malaysia and six lines in Iran, the highest range of yield per plant and other effective yield components was obtained by UM 1. Therefore, this line is useful for improving breeding programs.

Table 4.5: Mean square (MS) values from ANOVA for the yield and yield components of six corn advance lines grown in Iran

Source of variation	Tasseling (days)	Silking (days)	Plant height (cm)	Ear no. /plant	Ear wt. (g)	Ear length (cm)	No. kernels/ ear	1000 kernel wt.(g)	Yield/plant
Genotypes	3.96**	2.09 ns	589.07**	0.16 ns	26.24*	3.66**	3215.73**	234.59**	916.85*
Block	7.72**	2.06 ns	259.74 ns	0.12 ns	0.63ns	0.031ns	106.40 ns	7.39 ns	148.25 ns
Error	0.52	2.32	86.91	0.25	4.73	0.09	317.36	10.19	200.54
F-value for Gen	7.57**	0.90 ns	6.78**	0.62 ns	5.55*	38.22**	10.13**	23.02**	4.57*
CV (%)	1.038	2.034	5.14	21.69	1.22	2.15	7.01	2.53	18.85
R2	0.87	0.39	0.80	0.29	0.74	0.95	0.84	0.92	0.71

* Significant at the 0.05 level, ** significant at the 0.01 level

Table 4.6: Mean comparison of all traits among six corn advance lines (Iran) using Duncan's multiple range test

Genotypes	Tasseling (days)	Silking (days)	Plant height (cm)	Ear no. /plant	Ear wt. (g)	Ear length (cm)	No. kernels/ ear	1000 kernel wt. (g)	Yield/plant
UM 1	70.00±1.15 ab	75.00±0.58 a	157.33±11.23 b	2.37±0.19 a	180.1±0.59 ab	15.1±0.17 a	276.47±1.79 a	131.00±1.53 a	85.58±5.98 a
UM 2	70.00±0.58 ab	75.67±0.88 a	183.23±6.81 a	1.87±0.09 a	175.3±1.38 ab	13.4±0.21 b	213.93±4.52 b	113.33±2.03 b	45.34±3.19 b
UM 3	69.33±0.33 b	74.00±0.58 a	176.13±5.78 ab	2.4±0.06 a	180.6±0.82 ab	15.13±0.09 a	274.67±4.79 a	132.33±1.45 a	87.16±1.24 a
UM 4	68.33±0.88 b	74.00±0.58 a	182.2±1.64 ab	2.37±0.17 a	181.1±1.62 a	15.23±0.2 a	272.33±18.71 a	133.33±1.76 a	86.05±9.06 a
UM 6	68.33±0.88 b	74.67±0.88 a	190.07±2.93 a	2.33±0.07 a	181.03±0.57 a	14.9±0.2 a	277.33±6.3 a	131.00±1.73 a	84.72±2.64 a
UM 11	71.33±0.33 a	76.00±0.58 a	198.6±3.76 a	2.53±0.28 a	174.77±1.49 b	12.63±0.09 b	209.93±11.32 b	116.67±2.19 b	61.89±15.72 ab

Values are the mean ± SE of three replications; means with letters are not significantly different at p = 0.05 according to Duncan's multiple range test.

4.3 Principal component analysis

The quantitative data from the two locations were subjected to principal component analysis (PCA) in a multivariate analysis. Morphological characteristics were used to investigate the genetic variations among twelve corn lines at the first location (Malaysia). The results show that three principal components and factors with Eigen values greater than one explain 76.33% of the total variability. This signifies large variation among the genotypic traits under investigation. The first principal component (PC1) is related to kernel number per ear, ear length and ear weight, which explains 31.98% of the total variability (Table 4.7). The characteristics with the greatest positive weight on PC2 were tasseling date and silking date, and these components explain 25.48% of the total variance among corn lines. The characteristics with the greatest positive weight on the third component were plant height, ear number per plant and 1000 kernel weight. The last component was related to the maize plant yield, which explains 18.88% of the total variance.

Table 4.7: Principal components (PCs) of the morphological traits among twelve corn advance lines

Trait	1st component	2nd component	3rd component
No. kernels/ear	**0.95**	0.15	0.12
Ear length	**0.84**	-0.05	-0.07
Ear wt.	**0.74**	0.34	0.11
Silking	0.12	**0.89**	0.11
Tasseling	0.21	**0.88**	-0.16
Plant height	-0.13	0.38	**-0.83**
Ear no./plant	-0.14	0.43	**0.64**
1000 kernel wt.	0.56	0.07	**0.58**
Eigenvalue	3.04	1.80	1.27
Proportion σ2%	31.98	25.48	18.88
Cumulative σ2%	31.98	57.46	76.34

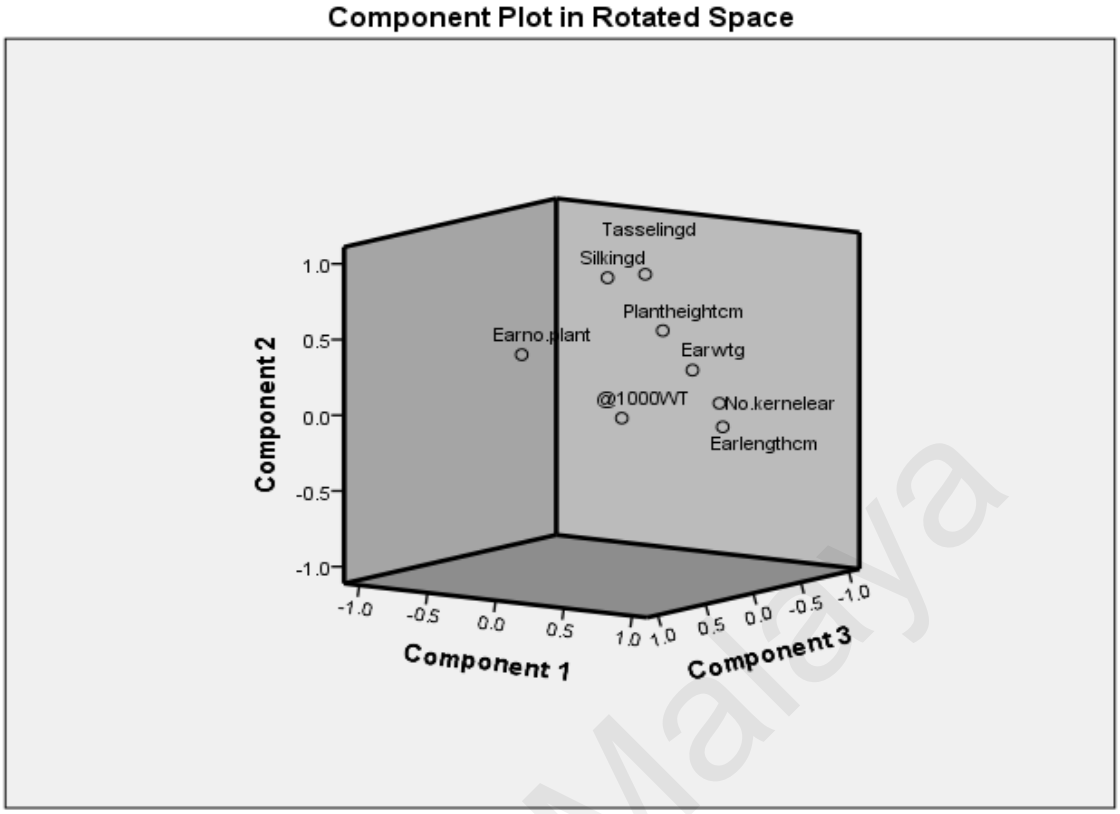

Figure 4.1: Scatter diagram of the three principal component scores for twelve corn advance lines

The results for the second location (Iran) show that two components with Eigen values greater than one extracted explain 86.62% of the total variability. This represents great variation among the genotypic traits under investigation. The first principal component (PC1) includes almost all traits except ear number per plant, explaining 70.60% of the total variability (Table 4.8). The characteristic with the greatest positive weight on PC2 was only ear number per plant and this component explains 16.02% of the total variance among corn lines.

Table 4.8: Principal components (PCs) of the morphological traits of six corn advance lines

Trait	1st component	2nd component
Ear length	**1.00**	0.02
Ear wt.	**0.98**	0.19
No .kernels/ ear	**0.98**	0.19
1000 kernel wt.	**0.93**	0.35
Silking	**-0.92**	-0.15
Tasseling	**-0.84**	0.10
Plant height	**-0.58**	0.34
Ear no./plant	0.14	**0.97**
Eigenvalue	5.65	1.28
Proportion σ2%	70.60	16.02
Cumulative σ2%	70.60	86.63

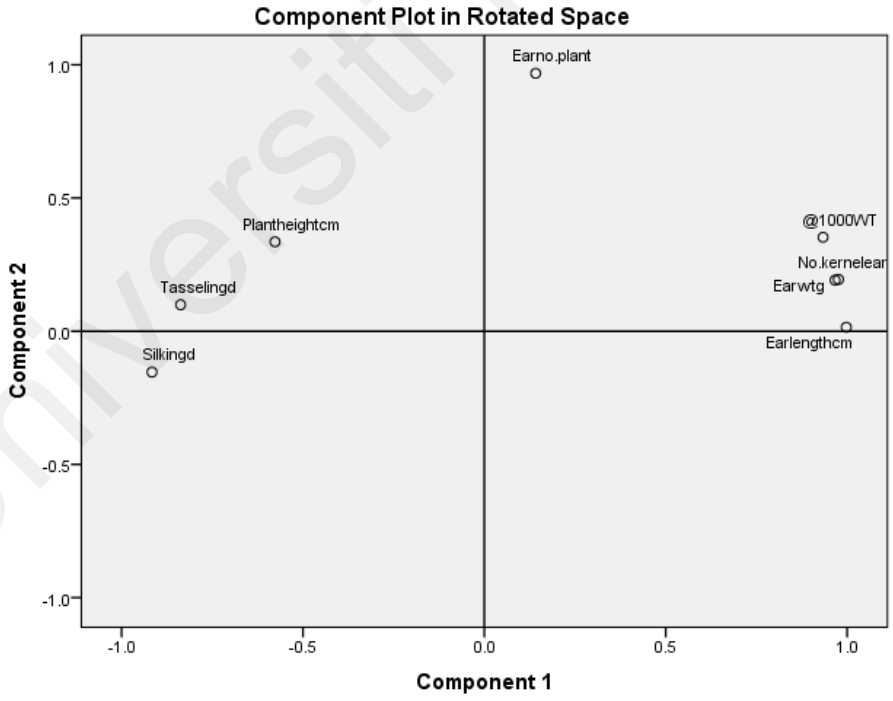

Figure 4.2: Scatter diagram of the first 2 PC scores for six corn advance lines

4.4 Cluster analysis

Cluster analysis by unweighted pair group method with arithmetic mean (UPGMA) was used to classify all corn advance lines into 3 main groups. A dendrogram based on the average linkage distance for twelve corn lines was also calculated and is presented in Figure 4.3.

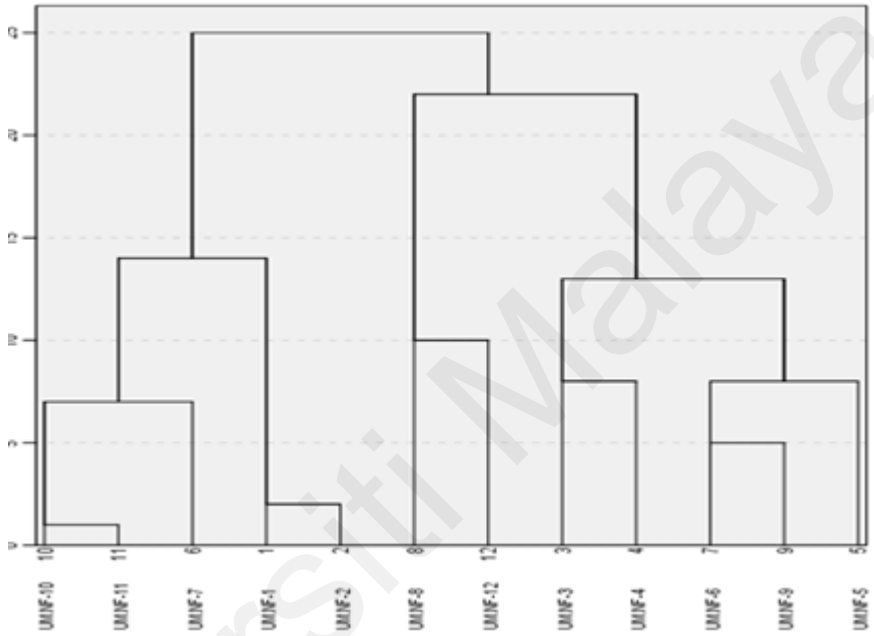

Figure 4.3: Dendrogram using average linkage between groups (Rescaled Distance Cluster Combine)**.**

For the second corn line growth location (Iran), a dendrogram based on the average linkage distance of six corn advance lines was calculated and is presented in Figure 4.4. Cluster analysis was used with the UPGMA method to divide all lines into 2 main groups.

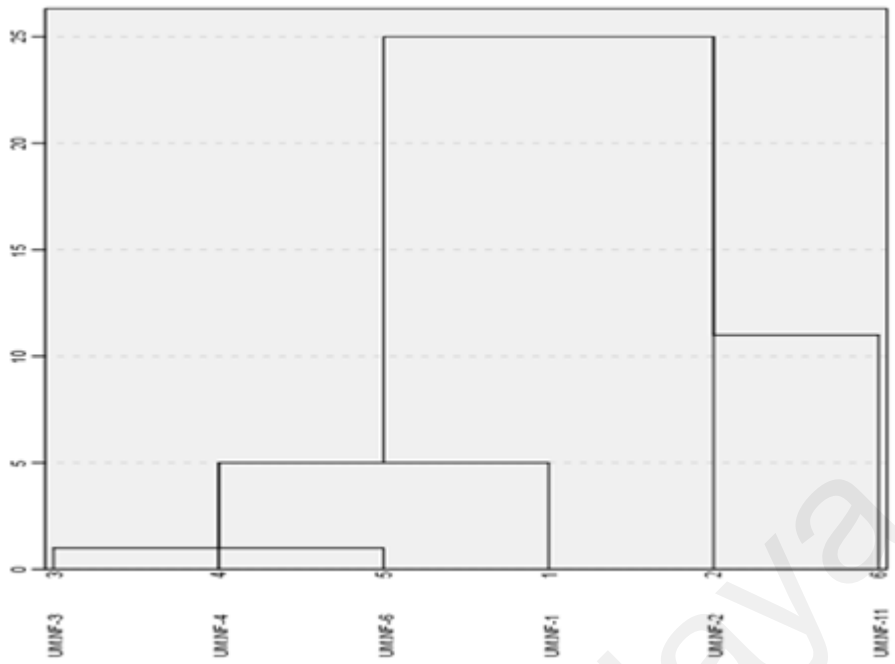

Figure 4.4: Dendrogram using average linkage (between groups) (Rescaled Distance Cluster Combine)

4.5 Genetic parameters of the yield and yield components of corn advance lines

The genotypic and phenotypic variances for both locations are presented in Table 4.9 and Table 4.10. In Malaysia, the genotypic coefficient of variability (Table 4.9) was the highest for yield per plant (30.48%), followed by ear number per plant (20.01%) and 1000 kernel weight (13.87%). The phenotypic coefficient of variability was also high for yield per plant (38.70%), then ear number per plant (29.35%) and ear length (26.26%). Almost all traits showed high heritability, except tasseling date and ear length. The maximum heritability was for 1000 kernel weight (91.35%) followed by plant height (74.78%) and ear weight (74.31%). The maximum genetic advance was observed for yield per plant (16116.36), followed by 1000 kernel weight (3342.31) and ear weight (1815.61). The minimum genetic advance was observed for ear number per plant (0.92) followed by tasseling date (7.60) and silking date (11.28). Higher heritability estimates correlating with good genetic advance expected in the next generation for 1000 kernel weight and ear weight indicate that these characteristics are supported by additive gene effects.

In Iran, there was a difference among genotypic and phenotypic variances for silking date, plant height, ear weight, kernel number per ear, 1000 kernel weight, and yield per plant, which indicates the environmental influence on these characteristics (Table 4.10). The genotypic coefficients of variability were the highest for yield per plant (20.56%), kernel number per ear (12.23%) and ear length (7.56%). The phenotypic coefficients of variability were the highest for yield per plant (27.89%), ear number per plant (14.52%) and kernel number per ear (14.09%). Almost all traits showed high heritability, except silking date (14.36%) and ear number per plant (19.23%). The maximum heritability was observed for ear length (92.58%), followed by 1000 kernel weight (88.01 %) and kernel number per ear (75.27%). The maximum genetic advance was observed for kernel number per ear (1990.12), followed by yield per plant (491.85) and plant height (344.80). The minimum genetic advance was observed for ear number per plant (0.04), followed by silking date (0.48) and tasseling date (2.36). Higher heritability estimates correlating with good genetic advance expected in the next generation for kernel number per ear indicate that this characteristic is supported by additive gene effects.

Table 4.9: Genetic parameters of the yield and yield components of twelve corn advance lines grown in Malaysia

	Tasseling (days)	Silking (days)	Plant height (cm)	Ear no. /plant	Ear wt.(g)	Ear length (cm)	No. kernels/ ear	1000 kernel wt. (g)	Yield/plant
Genotypic variance	3.69	5.47	128.99	0.45	881.43	10.61	475.11	1622.55	7824.05
Error variance	17.69	2.15	43.49	0.51	304.63	39.63	418.15	153.57	4788.36
Phenotypic variance	21.38	7.62	172.47	0.96	1186.07	50.24	893.26	1776.12	12612.41
Genotypic coefficient of variation %	3.09	3.54	8.04	20.01	10.43	12.07	7.37	13.87	30.49
Phenotypic coefficient of variation %	7.43	4.17	9.30	29.35	12.10	26.26	10.11	14.51	38.71
Heritability in broad sense %	17.26	71.81	74.78	46.44	74.31	21.11	53.18	91.35	62.03
Genetic advance	7.60	11.28	265.69	0.92	1815.61	21.85	978.58	3342.31	16116.36
Genetic advance expressed as mean percent	12.22	17.05	188.16	27.51	638.18	80.94	330.93	1151.33	5555.45

Table 4.10: Genetic parameters of the yield and yield components of six corn advance lines grown in Iran

	Tasseling (days)	Silking (days)	Plant height (cm)	Ear no. /plant	Ear wt. (g)	Ear length (cm)	No. kernels/ ear	1000 kernel wt. (g)	Yield/plant
Genotypic variance	1.14	0.23	167.38	0.02	7.17	1.19	966.12	74.80	238.77
Error variance	0.52	1.39	86.91	0.09	4.73	0.10	317.36	10.19	200.54
Phenotypic variance	1.67	1.62	254.30	0.11	11.90	1.28	1283.48	84.99	439.31
Genotypic coefficient of variation %	1.54	0.64	7.14	6.37	1.50	7.57	12.23	6.85	20.57
Phenotypic coefficient of variation %	1.86	1.70	8.80	14.52	1.93	7.86	14.10	7.30	27.90
Heritability in broad sense %	68.67	14.36	65.82	19.23	60.26	92.58	75.27	88.01	54.35
Genetic advance	2.36	0.48	344.80	0.04	14.77	2.44	1990.12	154.08	491.85
Genetic advance expressed as mean percent	3.39	0.64	190.29	1.92	8.26	16.98	783.20	122.09	654.76

4.6 Genotypic and phenotypic correlation coefficients

According to the phenotypic correlation (rp) results for the first location (Malaysia), tasseling date was correlated positively and significantly with silking date (0.53) and ear number per plant (0.56) at the $p<0.01$ level, and it was positively and significantly correlated with ear weight (0.37) at the $p<0.05$ level. The correlation coefficients are moderate (Table 4.11). The results show that with increasing tasseling date and silking date, the ear number per plant and ear weight increased. The phenotypic correlation (rp) shows that silking date was positively correlated with all traits except ear length (-0.17). This means that with increasing the silking date period, the ear length decreased. However, the phenotypic correlation of silking date with other traits was not significant except with yield per plant. The plant height trait showed a positive but insignificant correlation with ear weight and ear length, whereas it showed a negative and significant correlation at the $p<0.05$ level with 1000 kernel weight (-.37). Moreover, with increasing plant height, the 1000 kernel weight significantly decreased. The result for ear number per plant indicates that it had a positive and non-significant correlation with ear weight, ear length, kernel number per ear and 1000 kernel weight, but it showed a positive and significant correlation with yield per plant (0.82) at $p<0.01$. The ear weight had a positive and significant correlation with ear length (0.34) and kernel number per ear (0.38) at $p<0.05$ and a significant correlation at $p<0.01$ with 1000 kernel weight (0.45) and yield per plant (0.52). The phenotypic correlation of ear length was positive with all traits except silking date (-0.17). Ear length showed a positive and significant correlation with kernel number per ear (0.41) and ear weight (0.34) at the $p<0.05$ level. The result for kernel number per ear showed it was positively and significantly correlated with 1000 kernel weight (0.47) and yield per plant (0.45) at the $p<0.01$ level and with ear weight (0.41) and ear length (0.38) at the $p<0.05$ level. A positive and significant correlation was observed between 1000 kernel weight and yield per plant

(0.57), kernel number per ear (0.47) and ear weight (0.52) at the $p<0.01$ level, whereas 1000 kernel weight had a negative and significant correlation with plant height (-0.37) at the $p<0.05$ level. According to the phenotypic correlation results, the highest correlation of yield as the main component was observed with ear number per plant (0.82), which was significant at the $p<0.01$ level. Furthermore, yield was correlated positively and significantly with tasseling date (0.53), ear weight (0.45), kernel number per ear (0.45), and 1000 kernel weight (0.57) at $p<0.01$. Yield also had a positive and significant correlation with silking date (0.34) at the $p<0.05$ level, but the yield per plant had a negative and non-significant correlation with plant height (-0.24).

Table 4.11: Estimated of phenotypic (rp) correlation coefficients among different characteristic of corn advance lines grown in Malaysia

Characteristic	Tasseling (days)	Silking (days)	Plant height (cm)	Ear no. /plant	Ear wt. (g)	Ear length (cm)	No. kernels/ ear	1000 kernel wt. (g)	Yield /plant
Tasseling (days)	1								
Silking (days)	0.53**	1							
Plant height (cm)	0.23	0.17	1						
Ear no./plant	0.56**	0.20	-0.12	1					
Ear wt. (g)	0.37*	0.17	0.06	0.23	1				
Ear length (cm)	0.25	-0.17	0.06	0.25	0.34*	1			
No. kernels/ear	0.15	0.22	-0.06	0.04	0.38*	0.41*	1		
1000 kernel wt. (g)	0.05	0.24	-0.37*	0.09	0.52**	0.09	0.47**	1	
Yield/plant	0.53**	0.34*	-0.24	0.82**	0.45**	0.29	0.45**	0.57**	1

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

According to the genotypic correlation (rg) results (Table 4.12), tasseling date was correlated positively with all traits and significantly with silking date (0.73) at the $p<0.01$ level. The correlation coefficient was quite moderate. The results also demonstrate that with increasing tasseling duration, the silking date significantly increased. In terms of genotypic correlation (rg), silking date was positively correlated with all traits, but the genotypic correlation of silking date was not significant with other traits except tasseling date. Next, plant height showed a positive but insignificant correlation with tasseling date, silking date and ear weight, and a negative and insignificant correlation with the other traits. The results for ear number per plant indicate that it had a positive and non-significant correlation with silking date, tasseling date, ear weight, ear length, kernel number per ear and 1000 kernel weight, but it had a positive and significant correlation with yield per plant (0.77) at the $p<0.01$ level. Ear number per plant had a negative and non-significant correlation with plant height (-0.27). Ear weight showed a positive and non-significant correlation with all traits, whereas it had a positive and significant correlation with kernel number per ear (0.68) and 1000 kernel weight (0.61) at the $p<0.05$ level. The genotypic correlation for ear length was positive with all traits except plant height (-0.12) as well as positive and significant with kernel number per ear (0.86) at the $p<0.01$ level. Kernel number per ear was positively and significantly correlated with ear weight (0.68) at the $p<0.01$ level and ear length (0.86) at $p<0.01$, whereas kernel number per ear showed a negative and non-significant correlation with plant height (-0.20). A positive and significant correlation was observed between 1000 kernel weight and ear weight (0.61) and yield per plant (0.63) at the $p<0.05$ level, but it had a negative and non-significant correlation with plant height (-0.45). According to the genotypic correlation results, the highest relationship for yield as the main component was observed with ear number per plant (0.77) with significance at the $p<0.01$ level. Also, yield was correlated positively and

significantly with 1000 kernel weight (0.63) at the $p<0.05$ level. Yield additionally exhibited a positive and non-significant correlation with all other traits, but yield per plant had a negative and non-significant correlation with plant height (-0.40). According to the results, with increasing all traits, yield per plant increased, but increasing plant height due to the negative correlation with yield caused a decrease in yield per plant.

Table 4.12: Estimated genotypic (rg) correlation coefficients for different characteristics of corn advance lines in Malaysia

Characteristic	Tasseling (d)	Silking (d)	Plant height (cm)	Ear no. /plant	Ear wt. (g)	Ear length (cm)	No. kernels/ ear	1000 kernel wt. (g)	Yield/ plant
Tasseling (days)	1								
Silking (days)	0.73**	1							
Plant height (cm)	0.32	0.20	1						
Ear no./plant	0.21	0.29	-0.27	1					
Ear wt. (g)	0.40	0.28	0.04	0.09	1				
Ear length (cm)	0.17	0.03	-0.12	0.05	0.38	1			
No. kernels/ear	0.29	0.28	-0.20	0.08	0.68*	0.86**	1		
1000 kernel wt. (g)	0.02	0.28	-0.45	0.07	0.61*	0.18	0.51	1	
Yield/plant	0.29	0.44	-0.40	0.77**	0.52	0.34	0.55	0.63*	1

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

According to the phenotypic correlation (rp) results for the second location (Iran), tasseling date was correlated positively and significantly with silking date (0.62) at the $p<0.01$ level and positively but non-significantly correlated with ear number per plant. Tasseling date also had a negative and significant correlation with ear length (-0.51), kernel number per ear (-0.48) and 1000 kernel weight (-0.52) at the $p<0.05$ level (Table 4.13). Moreover, tasseling date was correlated negatively and non-significantly with plant height, ear weight and yield per plant. The results indicate that with increasing tasseling duration, the silking date, ear number per plant and ear weight increased but the other traits decreased. The phenotypic correlation (rp) signifies that silking date was negatively correlated with all traits except tasseling date (0.62) and ear number per plant (0.06), which means that with increasing the silking period, the tasseling date and ear number per plant increased. However, the phenotypic correlation of silking date was negative and significant with ear weight (-0.53) and kernel number per ear (-0.58) at the $p<0.05$ level. Next, plant height had a negative and non-significant correlation with all traits and a negative and significant correlation with ear length (-.49) at the $p<0.05$ level. Essentially, with increasing plant height, ear length significantly decreased. The results for ear number per plant indicate that it had a positive and non-significant correlation with all traits except plant height (-0.18), which was a negative correlation. Furthermore, ear number per plant exhibited a positive and significant correlation with yield per plant (0.72) at $p<0.01$. Ear weight had a positive and significant correlation with ear length (0.83), kernel number per ear (0.96), 1000 kernel weight (0.77) and yield per plant (0.66) at $p<0.01$. Moreover, ear weight showed a negative correlation with tasseling date (-0.46), silking date (-0.53) and plant height (-0.40). The phenotypic correlation of ear length was positive and significant with ear weight (0.83), kernel number per ear (0.85), 1000 kernel weight (0.67) and yield per plant (0.90) at $p<0.01$. The correlation was negative and significant with tasseling date (-0.51) and plant height

(-0.49) at the $p<0.05$ level. Kernel number per ear was positively and significantly correlated with 1000 kernel weight (0.81), yield per plant (0.73), ear weight (0.96) and ear length (0.85) at $p<0.01$. However, kernel number per ear was negatively and significantly correlated with tasseling date (-0.48) and silking date (-0.58) at the $p<0.05$ level. A positive and significant correlation was observed for 1000 kernel weight with ear weight (0.77), ear length (0.90), kernel number per ear (0.81) and yield per plant (0.84) at $P<0.01$, whereas 1000 kernel weight had a negative and significant correlation with tasseling date (-0.52) at the $p<0.05$ level. According to the phenotypic correlation results, the highest relationship for yield as the main component was observed with 1000 kernel weight (0.84) with significance at $p<0.01$. Yield was also correlated positively and significantly with ear number per plant (0.72), ear weight (0.73), ear length (0.67) and kernel number per ear (0.66) at $p<0.01$. Yield was also negatively and non-significantly correlated with tasseling date (-0.23), silking date (-0.35) and plant height (-0.42). Table 4.13 indicates that tasseling date, silking date and plant height were negatively correlated with most traits. It means that an increase in these three traits will decrease other traits, especially yield per plant, due to the reverse phenotypic correlation coefficients.

Table 4.13: Estimated phenotypic (rp) correlation coefficients for different characteristic of corn advance lines in Iran

Characteristic	Tasseling (days)	Silking (days)	Plant height (cm)	Ear no. /plant	Ear wt. (g)	Ear length (cm)	No. kernels/ ear	1000 kernel wt. (g)	Yield/plant
Tasseling (days)	1								
Silking (days)	0.62**	1							
Plant height (cm)	-0.21	-0.03	1						
Ear no./plant	0.18	0.06	-0.18	1					
Ear wt. (g)	-0.46	-0.53*	-0.40	0.01	1				
Ear length (cm)	-0.51*	-0.39	-0.49*	0.05	0.83**	1			
No. kernels/ear	-0.48*	-0.58*	-0.44	0.08	0.96**	0.85**	1		
1000 kernel wt. (g)	-0.52*	-0.42	-0.33	0.35	0.77**	0.90**	0.81**	1	
Yield/plant	-0.23	-0.35	-0.42	0.72**	0.66**	0.67**	0.73**	0.84**	1

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

According to the genotypic correlation (rg) results (Table 4.14), tasseling date was correlated positively with silking date, plant height and ear number per plant and it was positively and significantly correlated with silking date (0.82) at the $p<0.05$ level. The correlation coefficient was moderate. The results show that with increasing tasseling duration, the silking date significantly increased. But the tasseling date showed a negative correlation with ear weight, ear length, kernel number per ear, 1000 kernel weight and yield per plant. The genotypic correlation between tasseling date and ear weight (-0.83) was negative but significant at $p<0.05$. The genotypic correlation (rg) showed that silking date was negatively correlated with all traits except tasseling date and plant height. The correlation was also negative and significant with ear weight (-0.91), ear length (-0.91), kernel number per ear (-0.86), 1000 kernel weight (-0.89) and yield per plant (-0.81) at the $p<0.05$ level. The plant height trait had a negative and non-significant correlation with all traits except tasseling date and silking date, with which it had a positive but non-significant correlation. The result for ear number per plant indicates that it had a positive and non-significant correlation with all traits, but it showed a negative and non-significant correlation with silking date (-0.24). Ear weight showed a positive and significant correlation with ear length (0.97), kernel number per ear (0.99), 1000 kernel weight (0.98) and yield per plant (0.93) at $p<0.01$ but a negative and significant correlation with silking date (-0.91) at $p<0.05$. The genotypic correlation of ear length was positive and significant with ear weight (0.97), kernel number per ear (0.97), 1000 kernel weight (0.86) and yield per plant (0.94) at $p<0.01$. However, ear length had a negative and significant correlation with silking date (-0.91) at the $p<0.05$ level. The results indicate that kernel number per ear was positively and significantly correlated with ear weight (0.99), ear length (0.97), 1000 kernel weight (0.98) and yield per plant (0.94) at the $p<0.01$ level but negatively and significantly correlated with silking date (-0.86) at $p<0.05$. A positive and significant correlation was noted for 1000

kernel weight with ear weight (0.98), ear length (0.94), kernel number per ear (0.98) and yield per plant (0.98) at $p<0.01$, while 1000 kernel weight had a negative and significant correlation with silking date (-0.89) at $p<0.05$. Based on the genotypic correlation results, the highest relationship observed for yield as the main component was with 1000 kernel weight (0.98) with a positive and significant correlation at $p<0.01$. Moreover, yield was correlated positively and significantly with number of kernels per ear (0.94) and ear weight (0.93) at $p<0.01$ and with ear length (0.86) at the $p<0.05$ level. Yield also showed a negative and non-significant correlation with tasseling date and plant height as well as a negative and significant correlation with silking date (-0.81) at the $p<0.05$ level. Based on phenotypic and genotypic correlation coefficients at both locations (Iran and Malaysia), yield per plant had a negative correlation with plant height. Therefore, increasing plant height will decrease yield per plant. In maize production, short stature and higher grain yield plant are desirable.

Table 4.14: Estimated genotypic (rg) correlation coefficients for different characteristic of corn advance lines in Iran

Characteristic	Tasseling (days)	Silking (days)	Plant height (cm)	Ear no. /plant	Ear wt. (g)	Ear length (cm)	No. kernels/ ear	1000 kernel wt. (g)	Yield/plant
Tasseling (days)	1								
Silking (days)	.82*	1							
Plant height (cm)	0.13	0.34	1						
Ear no./plant	0.08	-0.24	0.09	1					
Ear wt. (g)	-.83*	-.91*	-0.46	0.32	1				
Ear length (cm)	-0.80	-.91*	-0.62	0.17	.97**	1			
No. kernel/ear	-0.75	-.86*	-0.55	0.34	.99**	.97**	1		
1000 kernel wt. (g)	-0.72	-.90*	-0.47	0.49	.98**	.94**	.98**	1	
Yield/plant	-0.60	-.81*	-0.41	0.64	.93**	.86*	.94**	.98**	1

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

4.7 Combined analysis of variance for the yield and yield components of six advance corn lines at the two test locations

One of the main objectives of the present study was to achieve yield stability. Yield stability is an important feature of breeding programs and in most genotype evaluation trials, the genotype × environment (GE) interaction appears to be a common phenomenon. The GE interaction complicates the selection of truly superior genotypes in breeding and performance testing programs. Therefore, the yield data underwent combined analysis of variance by partitioning the total variation into components related to genotype (G), environment (E) and GE interaction effects. Genotype was considered a fixed effect and environment a random effect. The main effect of E was tested by replication within the environment (R/E) as error and the main effect of G was tested against the GE interaction. Then the GE interaction was tested against the pooled error. Multiple testing of the main effect was performed using Duncan's multiple range test at the 5% probability level.

The mean squares from the combined variance analysis at the two locations showed highly significant variations in lines, the environment and interactions between the environment and lines for all the characteristics studied (Table 4.15). Line variation was significant at the $p<0.01$ level for silking date (MS = 11.89 days), plant height (MS = 334.69 cm), ear weight (MS = 1654.90 g), kernel number per ear (MS = 1528.81) and 1000 kernel weight (MS = 2458.67 g). However, line variation was not significant for tasseling date and ear number per plant. Environment variation was significant at the $p<0.01$ level for all characteristics. The environment and lines interacted significantly at the $p<0.01$ level for all characteristics, except tasseling date and ear length. Line variation was significant at the $p<0.05$ level for yield per plant as the main trait (MS = 11157.60 g), whereas the environment and interaction between the environment and line variation were significant at the $p<0.01$ level for yield per plant with MS = 738740.25 g

and MS = 12779.86 g respectively. The complexity of yield and yield components as quantitative traits is a result of different processes that occur during development. The more degrees of interaction, the more dissimilar the genetic systems that control the physiological processes are, which confirms adaptation to different environments. The results in Table 4.15 indicate that most traits showed instability across the two test locations. Based on this result, only tasseling date and ear length displayed consistency and stability among these quantitative traits. Therefore, the environment and lines interacted strongly and significantly at these two test locations.

Table 4.15: Mean squares of lines, the environment and interactions between the environment and lines for nine characteristics of six corn advance lines combined across two locations

Characteristic	Lines	Environment	Environment * Lines
Tasseling (days)	16.43	462.25**	4.78
Silking (days)	11.89**	650.25**	6.38*
Plant height (cm)	334.69**	19051.27**	312.46**
Ear no./plant	0.86	21.31**	1.37*
Ear wt. (g)	1654.91**	117002.76**	2035.66**
Ear length (cm)	36.17	2017.06**	37.93
No. kernels/ear	1528.82**	25908.12**	3442.16**
1000 kernel wt. (g)	2458.68**	295292.62**	3510.64**
Yield/plant	11157.60*	738740.25**	12779.86**

The mean values of the yield and yield components were compared to identify the differences between six corn advance lines examined at the two different test locations. The results are presented in Figures 4.5 to 4.13 and show highly significant differences among the lines for all characteristics under study. The mean comparison across the two test locations demonstrates that all yield components were in a higher range at the first location, except tasseling date, silking date and plant height. The yield of all lines grown

in Malaysia was higher and significantly different. At this location, the highest yield was from line UM 1 (504.74 g) and the lowest yield was from line UM 6 (287.03 g). At the second location, the highest yield was from line UM 3 (87.16 g) and the lowest yield was from UM 2 (45.33 g).

Tasseling date:

The mean comparison results from the Bonferroni test for tasseling date demonstrate that except UM 1, the other lines were significantly different between the two locations at the $p<0.05$ level. The highest difference was observed for UM 6 (9.33) followed by UM 3 (8.00), while the lowest difference was observed for UM 1 (4.00) and UM 4 (6.67), respectively. According to the results (Figure 4.5), it can be concluded that tasseling date was higher for the corn advance lines grown in Iran than those grown in Malaysia. A large proportion of the corn advance lines had earlier anthesis in Iran than in Malaysia. In maize production, higher grain yield and yield components, early maturity, short anthesis-silking interval and short plant stature are desirable.

Table 4.16: Mean differences in tasseling date at the two test locations for the six corn advance lines examined

Genotype	(I) Location	(J) Location	Mean difference	SE	p-value
UM1	**Iran**	Malaysia	4.00	2.68	0.15
UM 2	**Iran**	Malaysia	7.67*	2.68	0.01
UM 3	**Iran**	Malaysia	8.00*	2.68	0.01
UM 4	**Iran**	Malaysia	6.67*	2.68	0.02
UM 6	**Iran**	Malaysia	9.33*	2.68	0.002
UM 11	**Iran**	Malaysia	7.33*	2.68	0.01

*significant at the 0.05 level

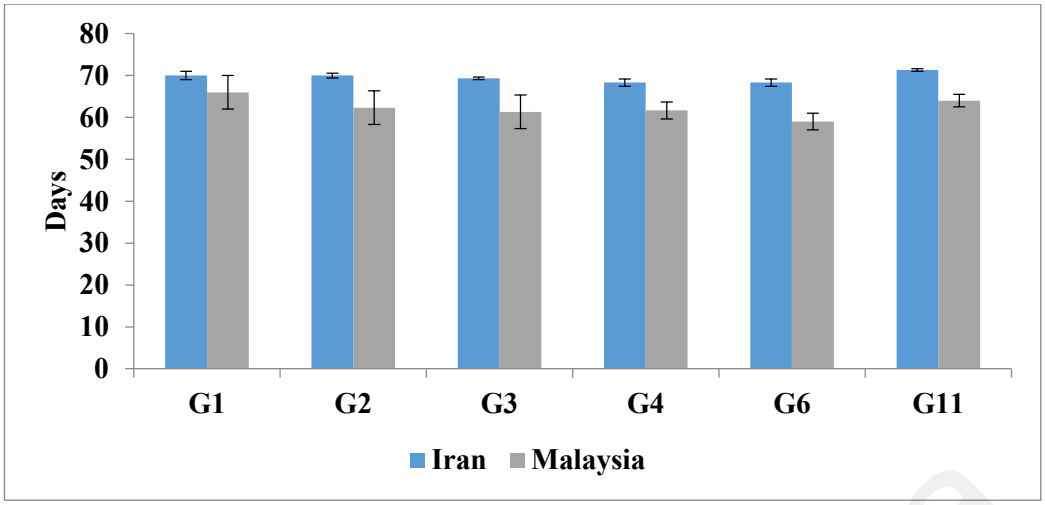

Figure 4.5: Mean comparison of tasseling date for the six corn advance lines examined at the two test locations

Silking date:

The mean comparison results from the Bonferroni test for silking date indicate that all lines were significantly different at the two locations at the $p<0.05$ level. The highest difference was observed for UM 6 (11.00) followed by UM 4 (10.33), while the lowest difference was observed for UM 2 (6.33) and UM 3 (6.67). Based on the results obtained (Figure 4.6), it is suggested that silking was higher for the corn advance lines grown in Iran compared to those grown in Malaysia. A large proportion of the corn advance lines had earlier and longer silking periods in Iran than in Malaysia. Silking is the stage when the crop is most sensitive to water and heat stress, which can reduce the kernel number (Westgate et al., 2004). In the silking stage, the total number of kernels per ear is determined; however, kernel abortion during early grain filling can reduce the kernel number (Milander, 2015).

Table 4.17: Mean difference in silking date of the six corn advance lines examined at the two test locations

Genotype	(I) Location	(J) Location	Mean difference	SE	p-value
UM1	Iran	Malaysia	7.00*	1.156	<0.001
UM 2	Iran	Malaysia	6.33*	1.156	<0.001
UM 3	Iran	Malaysia	6.67*	1.156	<0.001
UM 4	Iran	Malaysia	10.33*	1.156	<0.001
UM 6	Iran	Malaysia	11.00*	1.156	<0.001
UM 11	Iran	Malaysia	9.67*	1.156	<0.001

*significant at the 0.05 level

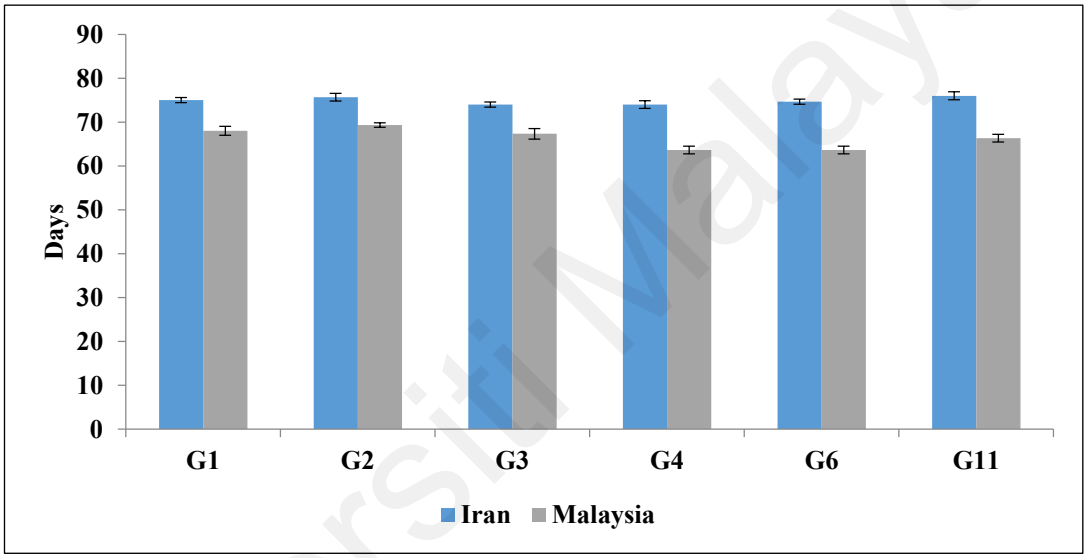

Figure 4.6: Mean comparison of silking date for the six corn advance lines examined at the two test locations

Plant height:

The mean comparison results from the Bonferroni test for plant height show that all lines were significantly different at the two locations at the p<0.05 level. The highest difference was observed for UM 11 (65.9) followed by UM 6 (54.4), while the lowest difference was observed for UM 1 (22.64) and UM 2 (40.69) respectively. According to the results (Figure 4.7), it can be said that plant height exhibited a greater range for the corn advance lines grown in Iran compared to Malaysia.

Table 4.18: Mean difference in plant height for the six corn advance lines examined at the two test locations

Genotype	(I) Location	(J) Location	Mean difference	SE	p-value
UM1	Iran	Malaysia	22.640*	5.97	<0.001
UM 2	Iran	Malaysia	40.690*	5.97	<0.001
UM 3	Iran	Malaysia	46.786*	5.97	<0.001
UM 4	Iran	Malaysia	45.626*	5.97	<0.001
UM 6	Iran	Malaysia	54.403*	5.97	<0.001
UM 11	Iran	Malaysia	65.907*	5.97	<0.001

*significant at the 0.05 level

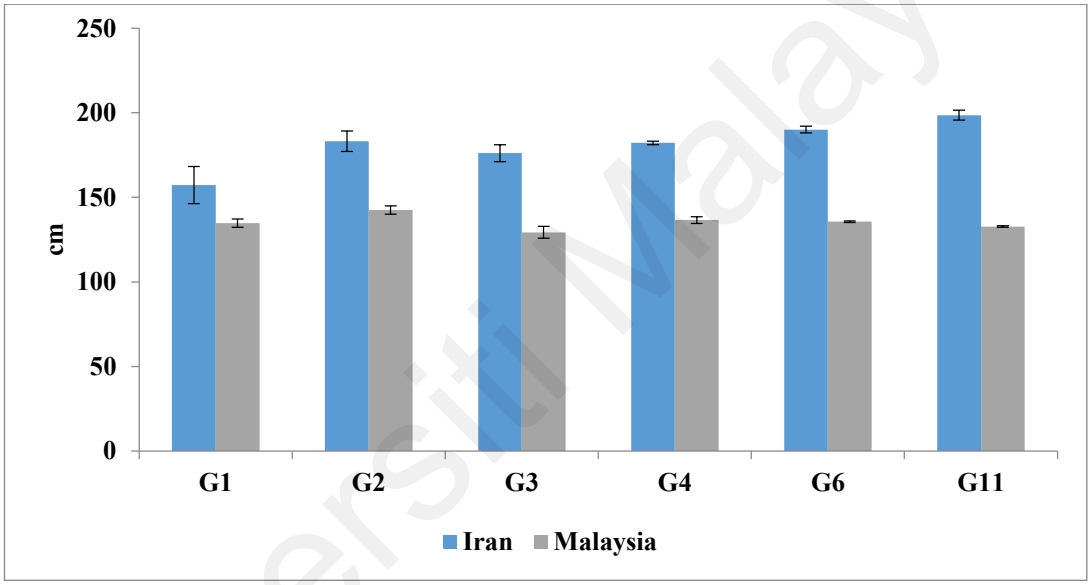

Figure 4.7: Mean comparison of plant height for the six corn advance lines examined at the two test locations

Ear number per plant:

The mean comparison results from the Bonferroni test for ear number per plant show that except UM 6 and UM 11, the other lines were significantly different at the two locations at the $p<0.05$ level. The highest difference was observed for UM 2 (2.53) followed by UM 4 (2.1), while the lowest difference was observed for UM 11 (0.13) and UM 6 (0.57), respectively. Based on the results (Figure 4.8), it is suggested that ear number per plant was higher for the corn advance lines grown in Malaysia compared to Iran.

Table 4.19: Mean difference in ear number per plant for the six corn advance lines examined at the two test locations

Genotype	(I) Location	(J) Location	Mean difference	SE	p-value
UM1	Iran	Malaysia	-1.97*	0.57	0.003
UM 2	Iran	Malaysia	-2.53*	0.57	<0.001
UM 3	Iran	Malaysia	-1.93*	0.57	0.003
UM 4	Iran	Malaysia	-2.10*	0.57	0.002
UM 6	Iran	Malaysia	-0.57	0.57	0.335
UM 11	Iran	Malaysia	-0.13	0.57	0.819

*significant at the 0.05 level

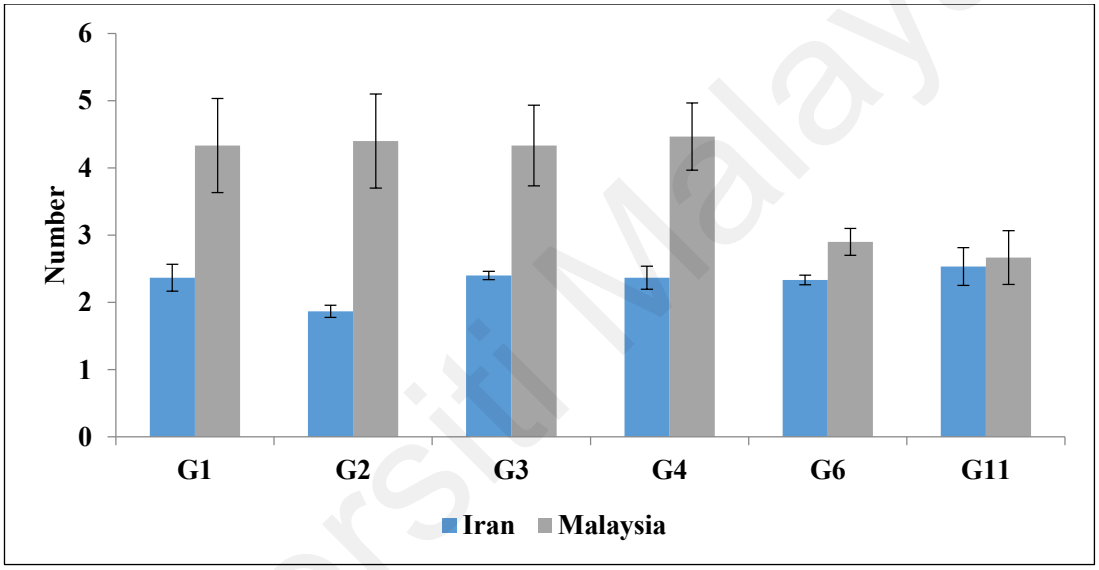

Figure 4.8: Mean comparison of ear number per plant for the six corn advance lines examined at the two test locations

Ear weight:

The mean comparison results from the Bonferroni test for ear weight demonstrate that all corn lines were significantly different at the two locations at the $p<0.05$ level. The highest difference was observed for UM 11 (150.78) followed by UM 1 (147.45), while the lowest difference was observed for UM 3 (57.55) and UM 4 (91.16), respectively. According to the results (Figure 4.9), ear weight was greater for the corn advance lines grown in Malaysia than in Iran.

Table 4.20: Mean difference in ear weight for the six corn advance lines examined at the two test locations

Genotype	(I) Location	(J) Location	Mean difference	SE	p-value
UM1	Iran	Malaysia	-147.45*	10.04	<0.001
UM 2	Iran	Malaysia	-135.36*	10.04	<0.001
UM 3	Iran	Malaysia	-57.55*	10.04	<0.001
UM 4	Iran	Malaysia	-91.16*	10.04	<0.001
UM 6	Iran	Malaysia	-101.81*	10.04	<0.001
UM 11	Iran	Malaysia	-150.78*	10.04	<0.001

*significant at the 0.05 level

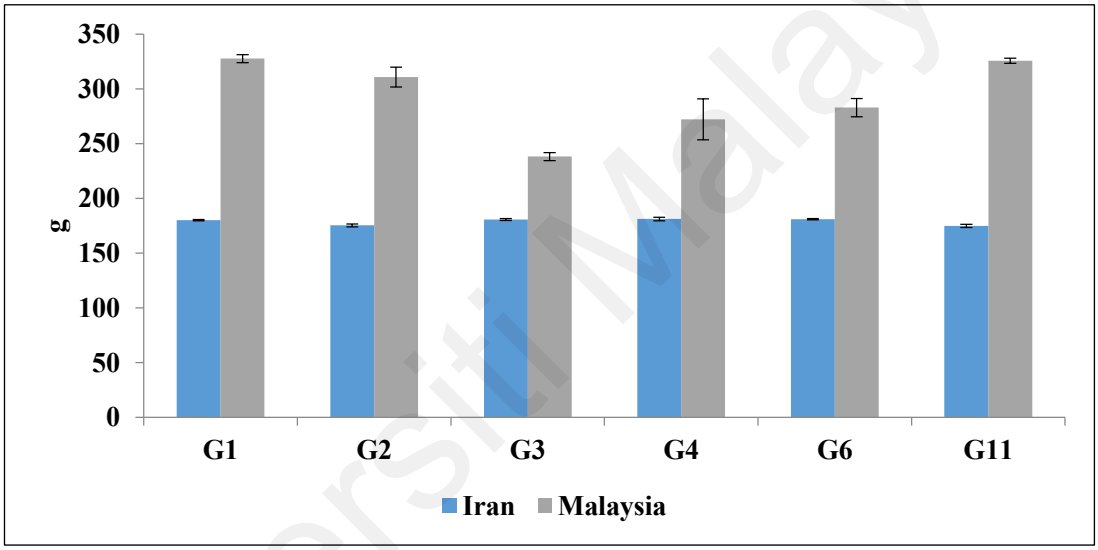

Figure 4.9: Mean comparison of ear weight for the six corn advance lines examined at the two test locations

Ear length:

According to the mean comparison results from the Bonferroni test for ear length, except UM 3 the other lines were significantly different at the two locations at the $p<0.05$ level. The highest difference was observed for UM 6 (21.70) and UM 11 (17.16), while the lowest difference was observed for UM 3 (6.6) and UM 4 (12.89). The results (Figure 4.10) represent that ear length was greater for the corn advance lines grown in Malaysia compared to those grown in Iran.

Table 4.21: Mean difference in ear length for the six corn advance lines examined at the two test locations

Genotype	(I) Location	(J) Location	Mean difference	SE	p-value
UM1	Iran	Malaysia	-16.36*	4.51	0.002
UM 2	Iran	Malaysia	-15.11*	4.51	0.003
UM 3	Iran	Malaysia	-6.60	4.51	0.159
UM 4	Iran	Malaysia	-12.89*	4.51	0.01
UM 6	Iran	Malaysia	-21.70*	4.51	<0.001
UM 11	Iran	Malaysia	-17.16*	4.51	0.001

*significant at the 0.05 level

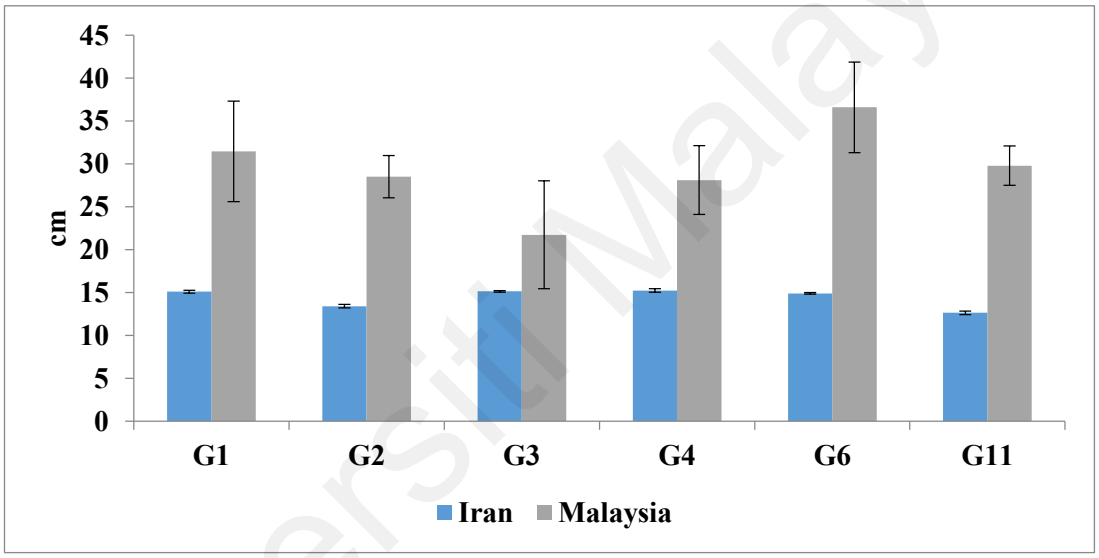

Figure 4.10: Mean comparison of ear length for the six corn advance lines examined at the two test locations

Kernel number per ear:

The mean comparison results from the Bonferroni test for number of kernels per ear show that except UM 3 and UM 4, the other lines were significantly different at the two locations at the $p<0.05$ level. The highest difference was noted for UM 11 (116.8) followed by UM 2 (101.6), while the lowest difference was observed for UM 3 (6.52) and UM 4 (16.07). According to the results in Figure 4.11, it can be concluded that the number of kernels per ear was higher for the corn advance lines grown in Malaysia than Iran, except for UM 3 that exhibited a higher range in Iran compared to Malaysia.

Table 4.22: Mean difference in kernel number per ear for the six corn advance lines examined at the two test locations

Genotype	(I) Location	(J) Location	Mean difference	SE	p-value
UM1	Iran	Malaysia	-51.97*	13.72	0.001
UM 2	Iran	Malaysia	-101.69*	13.72	<0.001
UM 3	Iran	Malaysia	6.52	13.72	0.64
UM 4	Iran	Malaysia	-16.07	13.72	0.255
UM 6	Iran	Malaysia	-41.88*	13.72	0.006
UM 11	Iran	Malaysia	-116.83*	13.72	<0.001

*significant at the 0.05 level

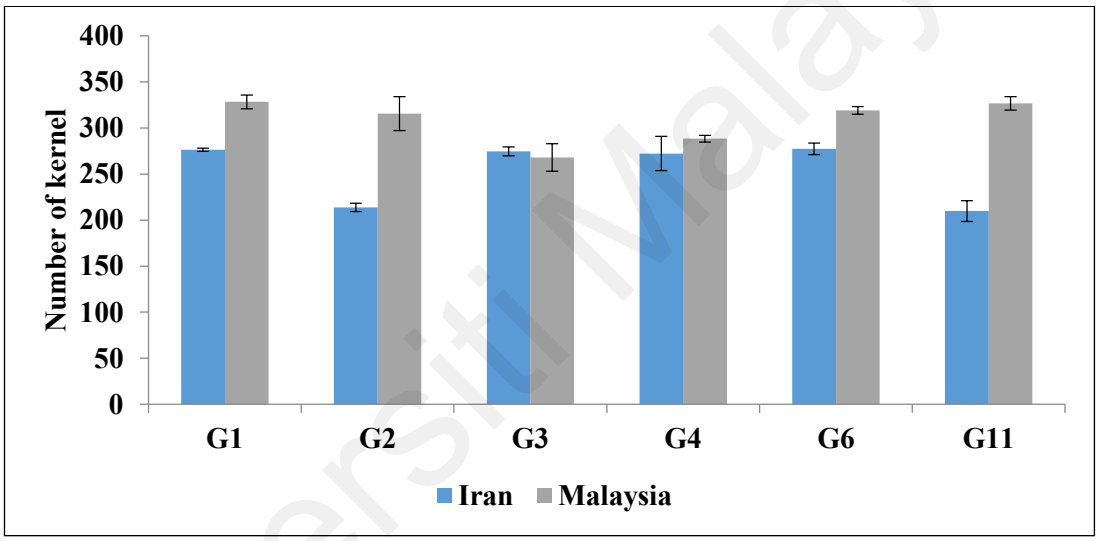

Figure 4.11: Mean comparison of kernel number per ear for the six corn advance lines examined at the two test locations

1000 kernel weight:

According to the mean comparison results from the Bonferroni test for 1000 kernel weight, all corn lines were significantly different at the two locations at $p<0.05$. The highest difference was observed for UM 1 (218.15) followed by UM 2 (200.29), while the lowest difference was observed for UM 4 (96.27) and UM 3 (163.24). Based on the results (Figure 4.12), it can be concluded that the 1000 kernel weight was higher for the corn advance lines grown in Malaysia compared to Iran.

Table 4.23: Mean difference in 1000 kernel weight for the six corn advance lines examined at the two test locations

Genotype	(I) Location	(J) Location	Mean difference	SE	p-value
UM1	Iran	Malaysia	-218.15*	7.95	<0.001
UM 2	Iran	Malaysia	-200.29*	7.95	<0.001
UM 3	Iran	Malaysia	-163.24*	7.95	<0.001
UM 4	Iran	Malaysia	-96.27*	7.95	<0.001
UM 6	Iran	Malaysia	-178.37*	7.95	<0.001
UM 11	Iran	Malaysia	-230.49*	7.95	<0.001

*significant at the 0.05 level

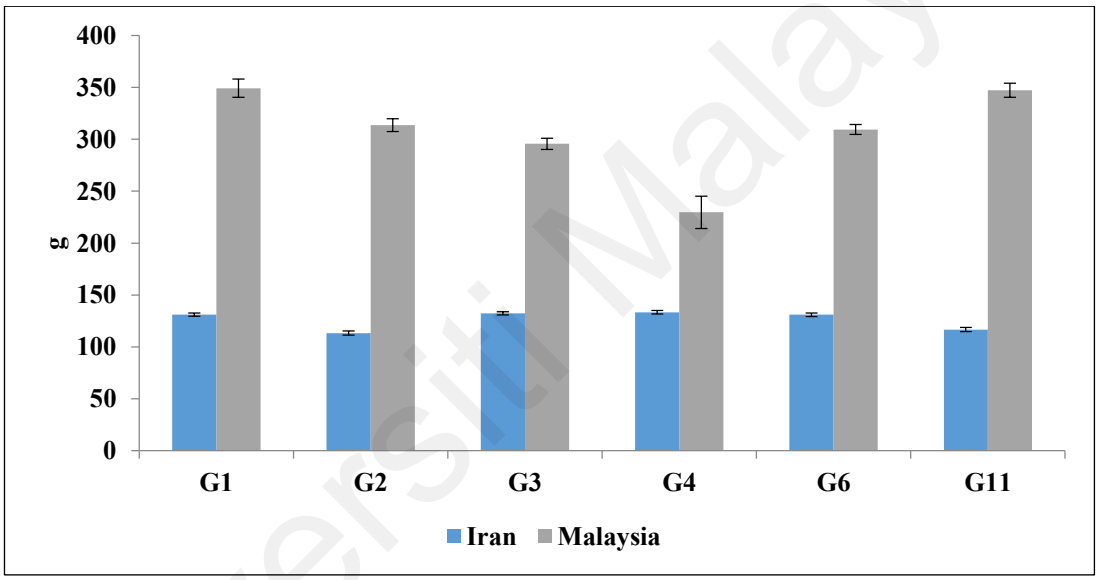

Figure 4.12: Mean comparison of 1000 kernel weight for the six corn advance lines examined at the two test locations

Yield per plant:

The mean comparison results from the Bonferroni test for yield per plant show that all corn lines were significantly different at the two locations at the p<0.05 level. The highest difference was observed for UM 1 (419.17) followed by UM 2 (383.01), while the lowest difference was observed for UM 6 (202.31) and UM 4 (207.5). Based on the results (Figure 4.13), it can be said that the yield per plant was higher for the corn advance lines grown in Malaysia compared to those grown Iran.

Table 4.24: Mean difference in yield per plant for the six corn advance lines examined at the two test locations

Genotype	(I) Location	(J) Location	Mean difference	SE	p-value
UM1	Iran	Malaysia	-419.17*	46.75	<0.001
UM 2	Iran	Malaysia	-383.01*	46.75	<0.001
UM 3	Iran	Malaysia	-262.98*	46.75	<0.001
UM 4	Iran	Malaysia	-207.50*	46.75	<0.001
UM 6	Iran	Malaysia	-202.31*	46.75	<0.001
UM 11	Iran	Malaysia	-244.03*	46.75	<0.001

*significant at the 0.05 level

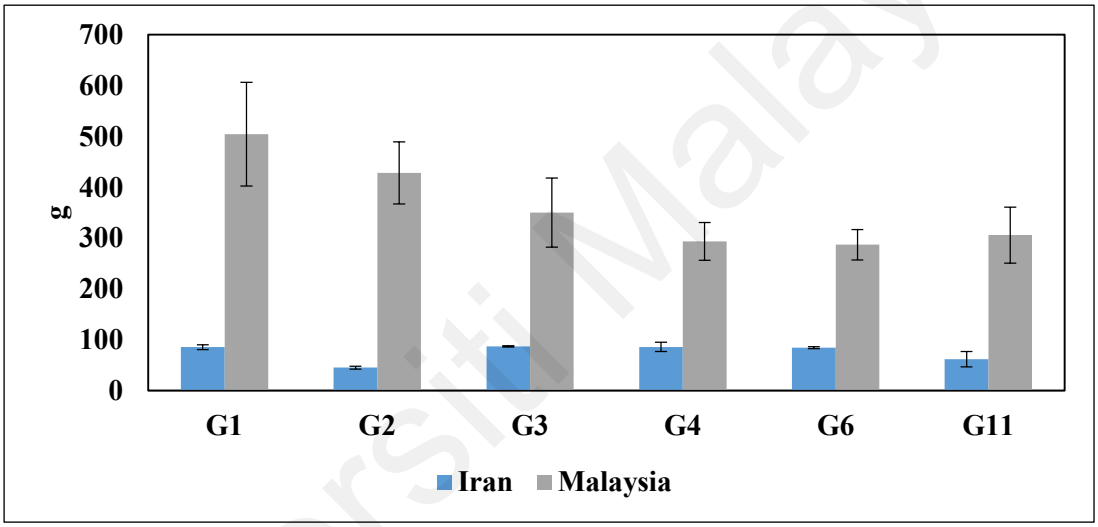

Figure 4.13: Mean comparison of yield per plant for the six corn advance lines examined at the two test locations

This study evaluated the morpho-agronomic traits of twelve new advance corn lines in Malaysia and compared the performance of six lines grown in Malaysia (tropical climate) and Iran (dryland climate). In general, the morpho-agronomic variability revealed that selecting these economic traits is effective in developing high-yielding corn lines. The cluster analysis results can be applied to further breeding programs for maize improvement. Genetic advances expected in the next generation for kernel number per ear, 1000 kernel weight and yield per plant indicate that these characteristics are supported by additive genetic effects, so these characteristics would be best for phenotypic selection at both locations. The phenotypic and genotypic correlation

coefficients revealed that selecting short plant stature is desirable to increase the grain yield in maize production. The UM composite corn lines generally failed in the temperate testing in Iran. Despite some research reporting that the yield of maize in the tropics is lower than that realizable in temperate countries, it was observed that the UM composite corn lines exhibited extensive heterosis exploitation in tropical climates rather than dryland climates and the yield of these corn lines in a temperate country is lower than in the tropics. A combined analysis showed that tasseling date and ear length were consistent and stabile at both test locations. This indicates that consistent and continuous selection for higher grain yield based on these traits is imperative.

4.8 Successful amplification of DNA isolated from the corn advance lines

The average DNA concentration obtained was 0.6µg/µl with purity of $A^{260/280}$ 1.80 for each corn line. The polymerase chain reaction (PCR) assay using gene-specific primers showed that the *Sh2* gene, a transcript of the *Bt2* gene (*Bt2b*) and *18s* gene could be amplified from DNA isolated using the DNeasy Plant Mini Kit (Figures 4.14, 4.15 and 4.16). However, another transcript of *Bt2* (*Bt2a*) could not be amplified with the DNA isolated from these corn advance lines. Based on these results, a quantitative real-time PCR was performed to find the expression level of each of these two genes in the UM composite corn lines.

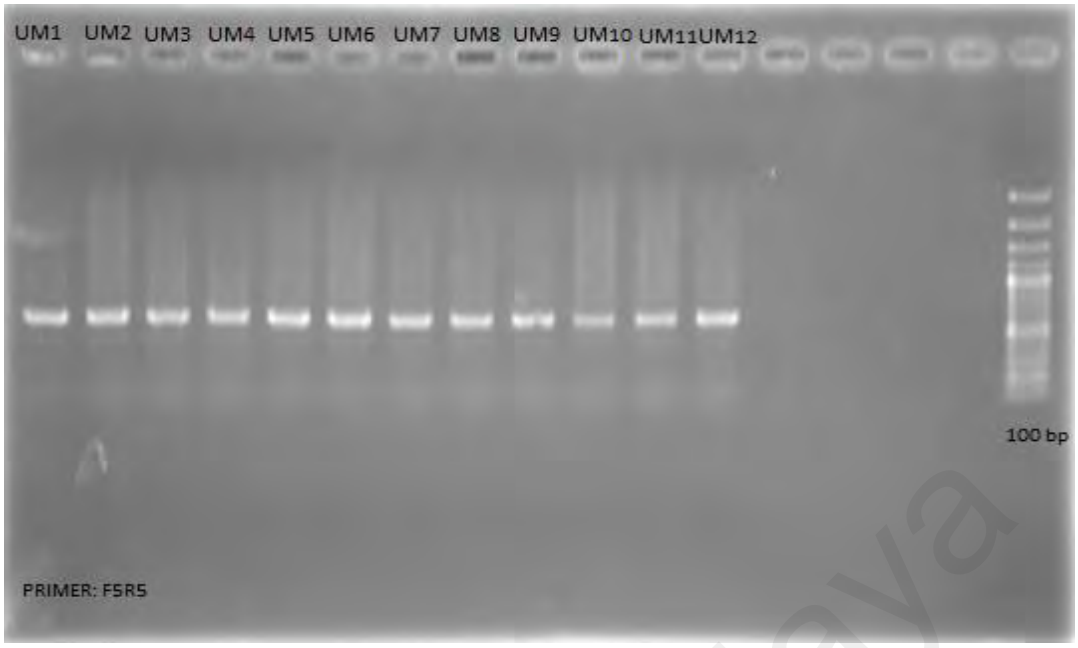

Figure 4.14: Gel image showing the presence of the *Sh2* gene in 12 UM corn advance lines

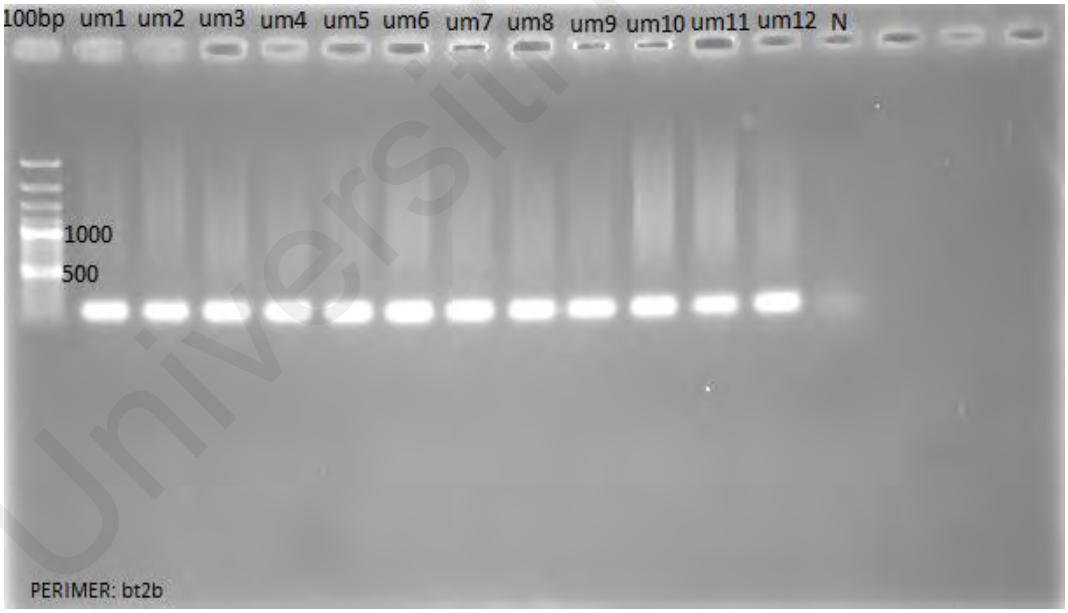

Figure 4.15: Gel image showing the presence of the *Bt2b* gene in 12 UM corn advance lines

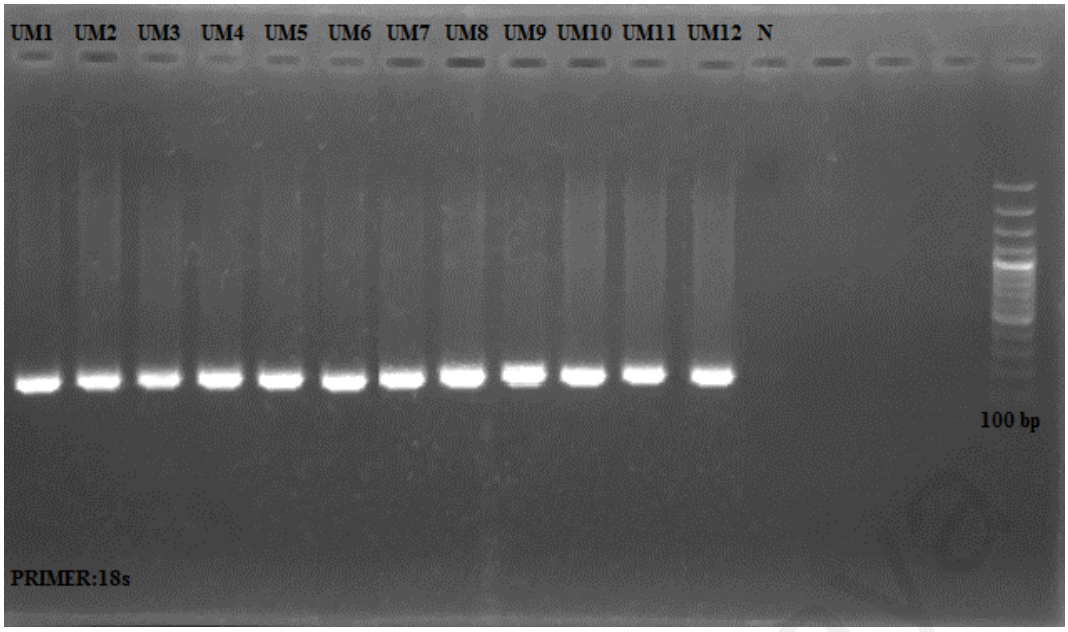

Figure 4.16: Gel image showing the presence of the 18s gene in 12 UM corn advance lines

4.9 C_T values of the *Sh2*, *Bt2b* and *18s* genes in the leaf tissue of six corn advance lines at the two test locations

Quantitative real-time PCR was used to measure the RNA transcription levels of various genes containing *Sh2*, *Bt2b* and *18s* as housekeeping genes in the leaf tissue of six composite corn lines. To compare the different RNA transcription levels, the C_T values were measured. C_T is defined as the number of cycles needed for the fluorescence to reach a specific detection threshold level and is inversely correlated with the amount of template nucleic acid present in the reaction. Initially, the average C_T values of the *Sh2*, *Bt2b* and *18s* genes were measured to analyse the transcript levels of these genes in leaf tissue. The C_T values of the *18s* gene were 7.85 and 8.14 in leaf tissue at the first and second locations, respectively. The C_T values of the *Sh2* and *Bt2b* genes in leaf tissue were 28.22 and 30.47 in Malaysia and 28.60 and 30.81 in Iran. The C_T values (starting materials) of *Bt2b* were similar and those of *Sh2* were also similar in the RNA obtained from leaf tissue at the two test locations.

Table 4.25: Mean C_T values for the leaf tissue of six corn advance lines in Malaysia

Leaf	*Sh2*	*18s*	*Bt2b*
Mean	28.10	7.85	30.50
Standard deviation	1.40	0.99	1.50

Table 4.26: Mean C_T values for the leaf tissue of six corn advance lines in Iran

Leaf	*Sh2*	*18s*	*Bt2b*
Mean	28.60	8.14	30.82
Standard deviation	1.24	0.75	1.78

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4.10 Relative transcription levels of the *Sh2* and *Bt2b* genes in the leaf tissue of six corn advance lines

Quantitative real-time PCR was used to measure the RNA transcription levels of the *Sh2* and *Bt2b* genes in the leaf tissue of six composite corn lines. To study the relative expression of the *Sh2* and *Bt2b* genes, the *18s* gene was used as an internal control and the parent line of each corn advance line served as a calibrator. The calibrators demonstrated a unique fold (almost 1.00) measurement, while the *Sh2* and *Bt2b* genes demonstrated different expression levels in the leaf tissue at each location. The *Bt2b* gene presented upregulate expression levels in the leaf tissue of all six corn advance lines at the first location (Figure 4.17). The highest expression level of the *Bt2b* gene was observed for UM 11 with 3.94 fold, followed by UM 3 (3.36), UM 6 (3.32), UM 4 (2.84) and UM 1 (2.21). The lowest expression level of the *Bt2b* gene was seen for UM 2 with 1.76 fold at the first location.

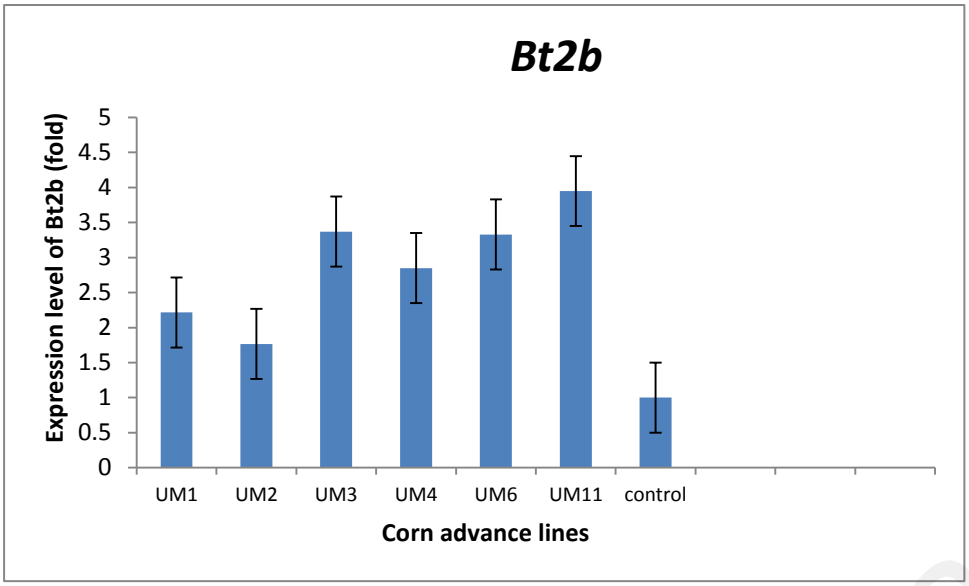

Figure 4.17: Relative transcription levels of the *Bt2b* gene in the leaf tissue of six corn advance lines in Malaysia

At the second location (Iran), the *Bt2b* gene exhibited upregulation in the leaf tissue of all six corn advance lines (Figure 4.18). The highest expression level of the *Bt2b* gene was observed in UM 11 with 4.08 fold, followed by UM 6 (3.52), UM 3 (3.03), UM 4 (2.62) and UM 1 (2.29). The lowest expression level of the *Bt2b* gene was found in UM 2, with 1.66 fold at the second location.

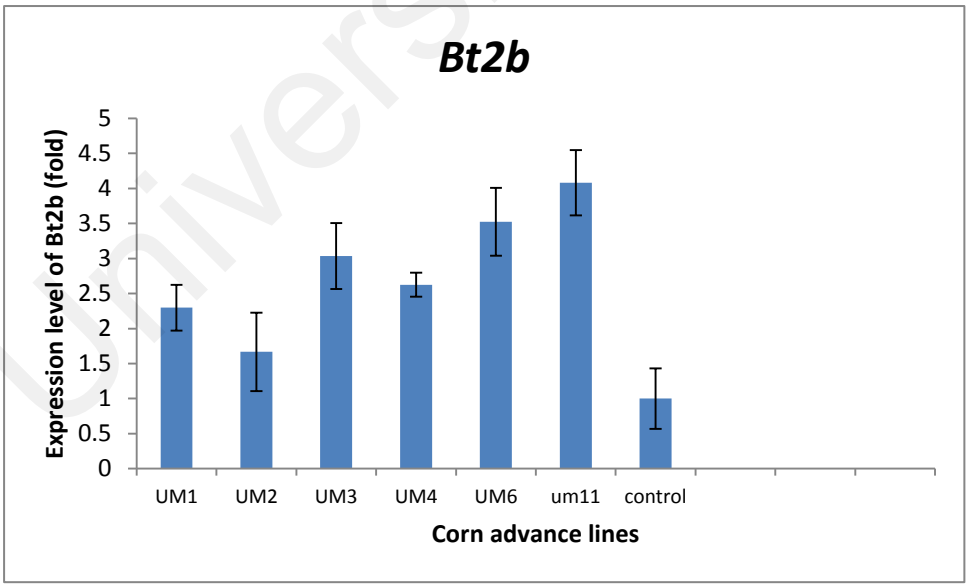

Figure 4.18: Relative transcript levels of the *Bt2b* gene in the leaf tissue of six corn advance lines in Iran

The relative transcription levels of the *Sh2* gene presented upregulation in the leaf tissue of all six corn advance lines at the first location. The highest expression

level of the *Sh2* gene was observed in UM 1 with 6.96 fold, followed by UM 4 (6.29), UM 11 (6.11), UM 6 (5.74) and UM 2 (5.32). The lowest expression level of the *Sh2* gene was seen in UM 3 with 4.54 fold at the first location (Malaysia), as presented in Figure 4.19.

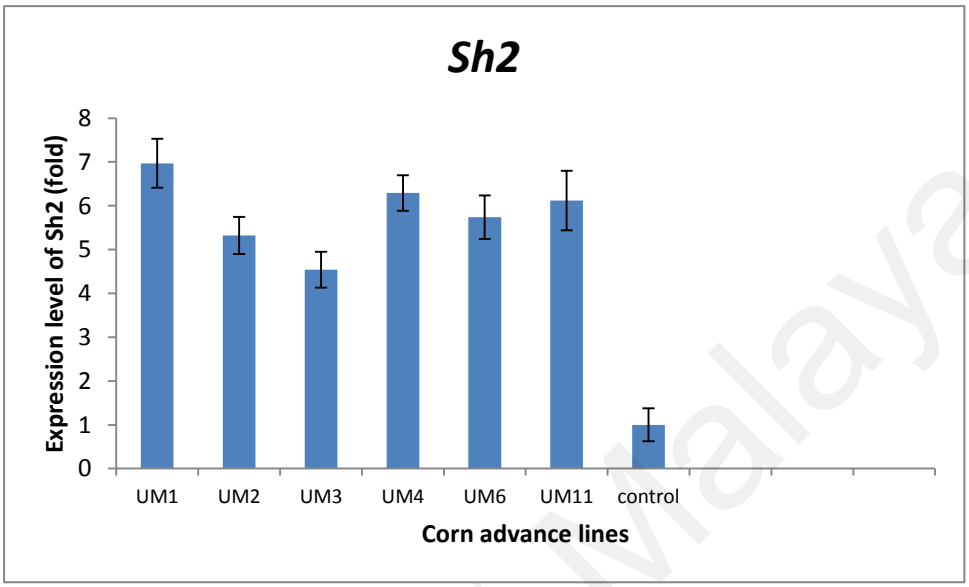

Figure 4.19: Relative transcription levels of the *Sh2* gene in the leaf tissue of six corn advance lines in Malaysia

At the second location (Iran), the relative transcription level of the *Sh2* gene presented upregulation in the leaves of all six corn advance lines. The highest expression level of the *Sh2* gene was observed in UM 1 with 6.4 fold, followed by UM 11 (6.06), UM 6 (5.65), UM 4 (5.26) and UM 2 (4.6). The lowest expression level of the *Sh2* gene was in UM 3 with 4.03 fold in Iran. The results are presented in Figure 4.20. A comparison of the *Bt2b* and *Sh2* gene expression levels indicates that the relative transcription level of the *Sh2* gene in the leaf tissue of all six corn advance lines was in a higher range than the *Bt2b* gene in both Malaysia and Iran.

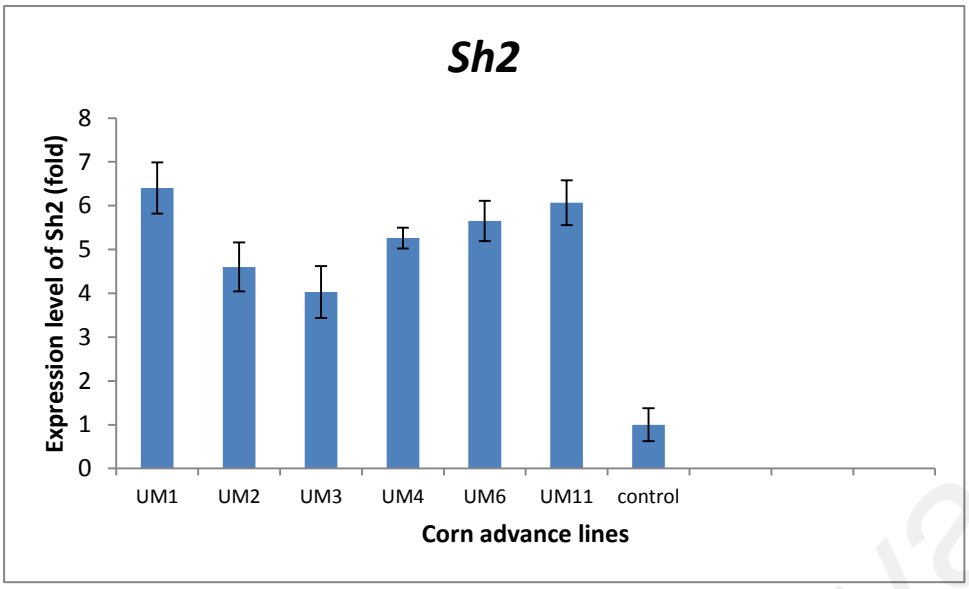

Figure 4.20: Relative transcription levels of the *Sh2* gene in the leaf tissue of six corn advance lines in Iran

To compare the expression levels of the *Bt2* and *Sh2* genes in the leaf tissue of six corn lines, analysis of variance was done. The results show the variability of these two genes in different lines at the two test locations (Table 4.27). The expression levels of the *Bt2* gene displayed significant differences among the six corn advance lines at both locations. Significance was at the $p<0.05$ level in Malaysia and $p<0.01$ among six corn advance lines at the second location (Iran). Meanwhile the expression level of *Sh2* showed significant differences at the $p<0.05$ level among six corn advance lines in Malaysia, whereas the expression level of *Sh2* was significantly different at $p<0.01$ among six corn advance lines in Iran. Following the analysis of variance, Duncan's multiple range test was applied for a mean comparison among corn advance lines (Table 4.28). The mean comparison results indicate that the highest mean expression level of the *Bt2*b gene in leaf tissue belonged to UM 11 (4.11), which was significantly different from UM 1 and UM 2 in Malaysia. In Iran, the highest mean expression level of *Bt2*b belonged to UM 11 (4.19) and it was significantly different from UM 1, UM 2 and UM 4. Meanwhile, the highest mean expression level of *Sh2* in leaf tissue belonged to UM 1 in Malaysia and Iran, with 6.97 and 6.42 fold, respectively, whereby the *Sh2* gene in UM 1 was significantly different from UM 3 at both locations.

Table 4.27: Mean square values (MS) from ANOVA for *Bt2b* and *Sh2* expression in leaf tissue among six corn advance lines at the two test locations

Source of variation	*Bt2b* Leaf (Malaysia)	*Bt2b* Leaf (Iran)	*Sh2* Leaf (Malaysia)	*Sh2* Leaf (Iran)
Genotype	2.122 *	2.365 **	2.130*	2.288**
Error	0.662	0.325	0.755	0.650
F-value for Genotype	4.84*	7.26**	3.73*	3.52**
CV (%)	22.464	19.667	12.892	14.981
R2	0.668	0.751	0.608	0.594

Table 4.28: Mean comparison of the *Sh2* and *Bt2b* gene expression levels in leaf tissue among six corn advance lines at the two test locations using Duncan's multiple range test

	Bt2b (Malaysia)	*Bt2b* (Iran)	*Sh2* (Malaysia)	*Sh2* (Iran)
UM1	2.22±0.114 b	2.30±0.006 bc	6.97±0.028 a	6.42±0.35 a
UM2	1.80±0.252 b	1.71±0.279 c	5.33±0.178 ab	4.72±0.693 ab
UM3	3.37±0.081 ab	3.04±0.051 abc	4.57±0.35 b	4.10±0.511 b
UM4	2.86±0.185 ab	2.63±0.147 bc	6.38±0.755 ab	5.27±0.122 ab
UM6	3.33±0.071 ab	3.55±0.298 ab	5.77±0.396 ab	5.69±0.414 ab
UM11	4.11±0.869 a	4.19±0.679 a	6.16±0.51 ab	6.11±0.501 ab

4.11 C_T values of the *Sh2*, *Bt2b* and *18s* genes in the endosperm tissue of six corn advance lines at the two test locations

Quantitative real-time PCR was used to measure the RNA transcription levels of various genes including *Sh2*, *Bt2b* and *18s* as housekeeping genes in the endosperm tissue of six composite corn lines. To compare the different RNA transcription levels, the C_T values were measured. Initially, the average C_T values of *Sh2*, *Bt2b* and *18s* were measured to analyse the transcription levels of these genes in endosperm tissue. The C_T values of the *18s* gene were 4.67 and 4.79 in the endosperm tissue of six corn advance lines grown in Malaysia and Iran, respectively. The C_T values of *Sh2* and *Bt2b* in the leaf tissue of six corn advance lines grown in Malaysia were 25.02 and 30.17, respectively, and 25.85 and 31.50 in Iran, respectively. The

difference in starting materials at the two locations led to investigating the relative expression of different exon fragments at the two locations.

Table 4.29: Mean C_T values for the endosperm tissue of six corn advance lines in Malaysia

Leaf	***Sh2***	***18s***	***Bt2b***
Mean	24.93	4.67	30.20
Standard deviation	0.76	0.54	0.71

Table 4.30: Mean C_T values for the endosperm tissue of six corn advance lines in Iran

Leaf	***Sh2***	***18s***	***Bt2b***
Mean	25.85	4.79	31.52
Standard deviation	0.60	0.48	0.60

4.12 Relative transcription levels of the *Sh2* and *Bt2b* genes in the endosperm tissue of six corn advance lines

Quantitative real-time PCR was used to measure the RNA transcription levels of the *Sh2* and *Bt2b* genes in the endosperm tissue of six composite corn lines. To study the relative expression of the *Sh2* and *Bt2b* genes, the *18s* gene was used as internal control and the parent line of each advance line served as a calibrator. The calibrators demonstrated a unique fold (almost 1.00) measurement, while the *Sh2* and *Bt2b* genes demonstrated different levels of expression in the endosperm tissue at each location. The *Bt2b* gene presented upregulated expression levels in the endosperm tissue of all six corn advance lines grown in Malaysia (Figure 4.21). The highest expression level of *Bt2b* was observed in UM 1 with 7.36 fold, followed by UM 3 (6.04), UM 11 (5.89), UM 2 (5.43) and UM 6 (5.15). The lowest expression level of *Bt2b* was observed in UM 4 with 3.93 for this line grown in Malaysia (Figure 4.21).

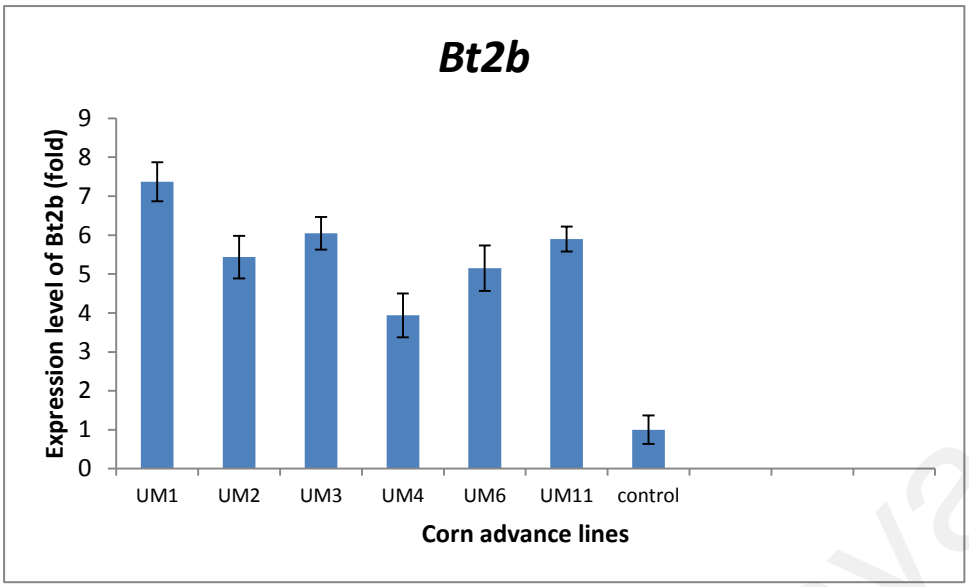

Figure 4.21: Relative transcription levels of the *Bt2b* gene in the endosperm tissue of six corn advance lines in Malaysia

The *Bt2b* gene showed upregulation in the endosperm tissue of all six corn advance lines grown at the second location (Iran) (Figure 4.22). The highest expression level of *Bt2b* was observed in UM 1 with 3.96 fold, followed by UM 3 (2.81), UM 2 (2.38), UM 6 (2.17) and UM 4 (1.96). The lowest expression level of *Bt2b* was observed in UM 11 with 1.69 fold for this line grown in Iran. The results present impressive expression levels of the *Bt2b* gene in the endosperm of the six corn advance lines in Malaysia in contrast to the leaf tissue of these lines at the same location. Nonetheless, the results indicate a non-significant difference in the expression levels of *Bt2b* in the leaf and endosperm tissue of six corn advance lines in Iran.

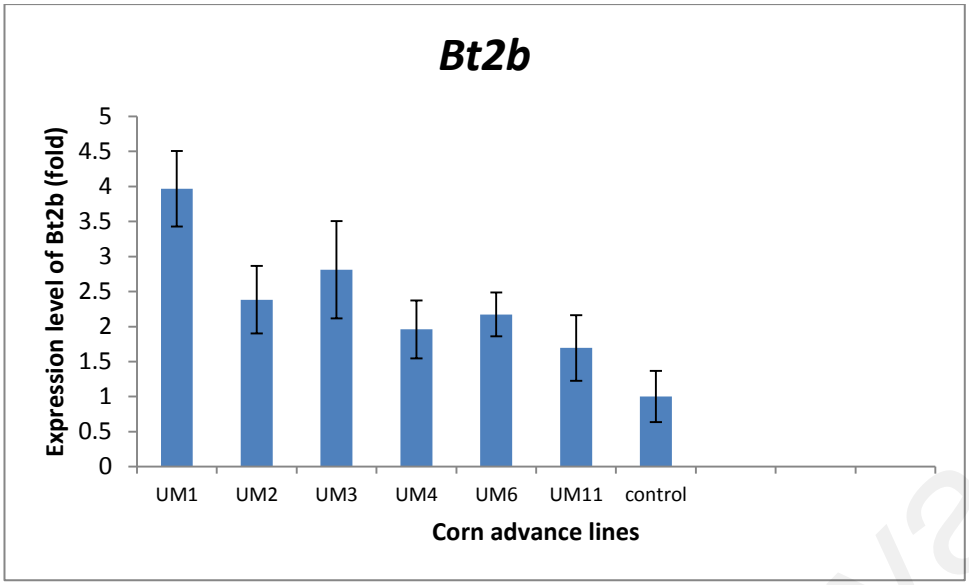

Figure 4.22: Relative transcription levels of the *Bt2b* gene in the endosperm tissue of six corn advance lines in Iran

In the endosperm tissue of the six corn advance lines grown in Malaysia, the relative transcription levels of the *Sh2* gene presented upregulation (Figure 4.23). The highest expression level of *Sh2* was observed in UM 2 with 5.96 fold, followed by UM 6 (5.2), UM 4 (4.44), UM 11 (4.21) and UM 3 (4.18). The lowest expression level of *Sh2* was observed in UM 1 with 3.12 fold at the first location (Malaysia), as presented in Figure 4.23. A comparison of the relative transcription levels in the *Bt2b* and *Sh2* genes at the first location indicate that the expression levels of *Bt2b* in the endosperm tissue of all six corn advance lines were in a higher range than the transcription levels of *Sh2* at this location.

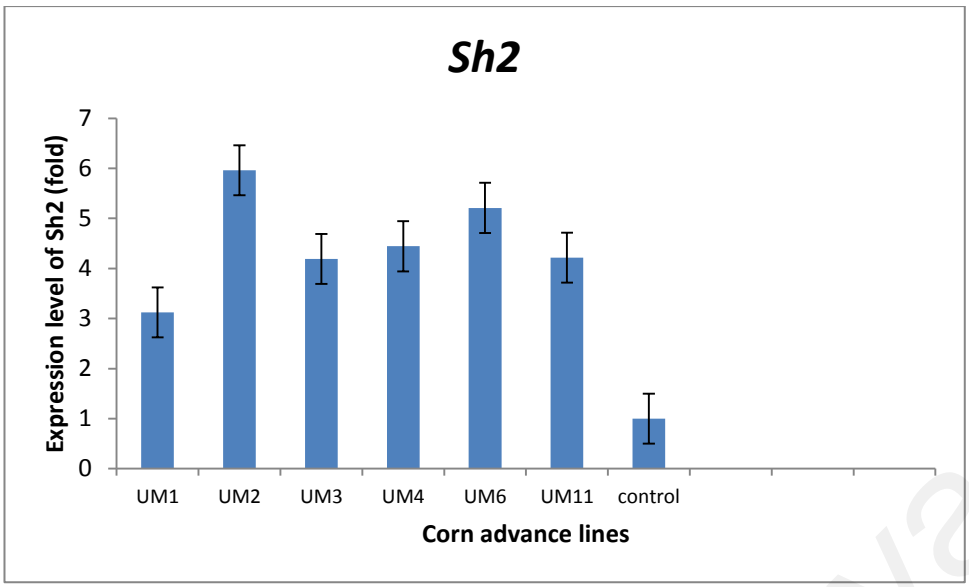

Figure 4.23: Relative transcription levels of the *Sh2* gene in the endosperm tissue of six corn advance lines in Malaysia

For the six corn advance lines grown in Iran, the relative transcription levels of the *Sh2* gene showed upregulation in endosperm tissue (Figure 4.24). The highest expression levels of the *Sh2* gene were observed in UM 3 with 3.05 fold, followed by UM 6 (2.8), UM 2 (2.41), UM 11 (2.09) and UM 4 (1.89). The lowest expression level of *Sh2* was observed in UM 1 with 1.73 fold in Iran, as presented in Figure 4.24. The results for the second location do not show any significant difference between the expression levels of the *Bt2b* and *Sh2* genes.

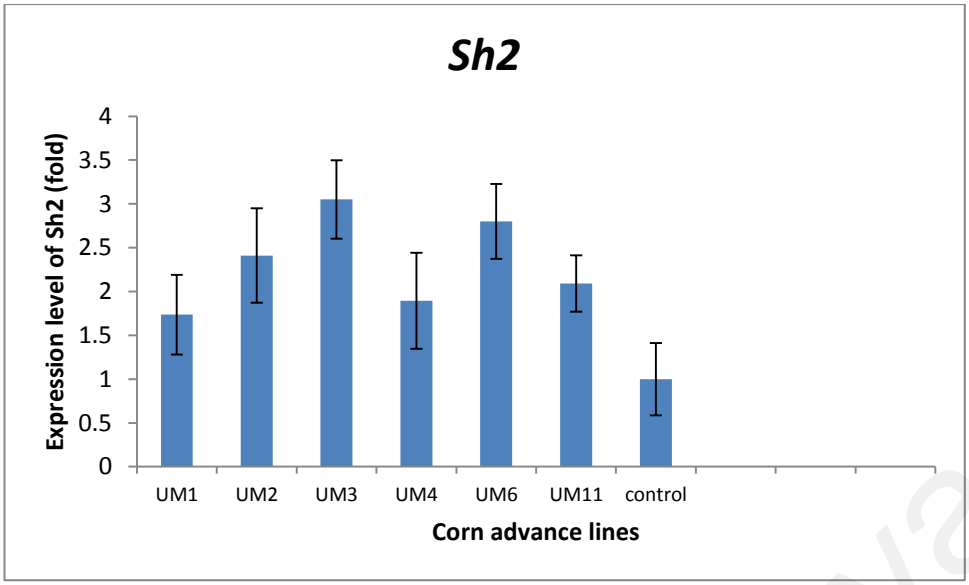

Figure 4.24: Relative transcription levels of the *Sh2* gene in the endosperm tissue of six corn advance lines in Iran

To compare the expression levels of the *Bt2* and *Sh2* genes in the endosperm tissue of six corn lines, analysis of variance was done. The results indicate variability in different corn advance lines at the two test locations (Table 4.31). The expression levels of the *Bt2* gene were significantly different among the six corn advance lines at both locations. Significance was at the $p<0.05$ level in Malaysia and at the $p<0.01$ level for six corn advance lines grown in Iran. While the expression level of *Sh2* showed significant differences at the $p<0.01$ level among six corn advance lines grown in Malaysia, the expression level of *Sh2* showed non-significant difference among six corn advance lines grown in Iran. Following the analysis of variance, Duncan's multiple range test was used for a mean comparison among corn advance lines (Table 4.32). The mean comparison results for the corn advance lines grown in Malaysia indicate that the highest mean expression level of the *Bt2*b gene in endosperm tissue was found in UM 1 (7.46), which was significantly different from UM 4. At the second location (Iran), the highest mean expression level of *Bt2*b was found in UM 1 (4.04), which was significantly different from UM 2, UM 4, UM 6 and UM 11. Meanwhile, the highest mean expression level of the *Sh2* gene in the endosperm tissue of the corn advance lines

grown in Malaysia was obtained for UM 2 (5.99) and it was significantly different from UM 1, UM 3, UM 4 and UM 11. Also in Iran, the highest mean expression level of *Sh2* belonged to UM 3 (3.19), which showed no significant difference from the other corn advance lines in Iran.

Table 4.31: Mean square values (MS) from ANOVA for the *Bt2* and *Sh2* expression levels in the endosperm tissue of six corn advance lines at the two test locations

Source of variation	*Bt2* endosperm (Malaysia)	*Bt2* endosperm (Iran)	*Sh2* endosperm (Malaysia)	*Sh2* endosperm (Iran)
Genotype	3.88*	2.07**	2.87**	0.97ns
Error	1.06	0.24	0.48	0.57
F-value for Genotype	3.41*	8.67**	12.39**	1.71ns
CV (%)	18.56	19.348	10.604	31.50
R2	0.59	0.783	0.837	0.42

Table 4.32: Mean comparison of the *Sh2* and *Bt2* gene expression levels in the endosperm tissue of six corn advance lines at the two test locations using Duncan's multiple range test

	***Bt2b* (Malaysia)**	***Bt2b* (Iran)**	***Sh2* (Malaysia)**	***Sh2* (Iran)**
UM1	7.46±0.873 a	4.04±0.541 a	3.15±0.264 c	1.77±0.255 a
UM2	5.45±0.3 ab	2.39±0.055 b	5.99±0.372 a	2.42±0.106 a
UM3	6.05±0.074 ab	2.84±0.273 ab	4.19±0.058 bc	3.19±0.703 a
UM4	4.00±0.497 b	1.97±0.127 b	4.45±0.066 b	1.94±0.294 a
UM6	5.33±1.03 ab	2.18±0.077 b	5.26±0.494 ab	2.94±0.681 a
UM11	5.91±0.272 ab	1.75±0.291 b	4.22±0.064 bc	2.10±0.13 a

According to the correlation coefficient results, the expression level of the *Bt2* gene in leaf tissue showed a negative and non-significant correlation with all morphological traits except plant height (0.08) and 1000 kernel weight (0.02) at both test locations (Table 4.33). The expression level of the *Sh2* gene in leaf tissue also showed non-significant correlation with all morphological traits, but it had a positive correlation with the ear number per plant, ear weight, ear length, kernel number per ear, 1000 kernel

weight and yield. Meanwhile the correlation coefficient results indicate that the expression level of *Bt2* in endosperm tissue was significantly and positively correlated with ear number per plant (0.69), ear weight (0.87), ear length (0.87), kernel number per ear (0.75), 1000 kernel weight (0.93) and yield (0.90) at the $p<0.01$ level for both locations. The results also indicate that with increasing expression level of the *Bt2* gene in endosperm tissue, the ear number per plant, ear weight, ear length, kernel number per ear, 1000 kernel weight and yield increased. However, the expression level of the *Bt2* gene in endosperm tissue showed negative and significant correlations with tasseling date (-0.69), silking date (-0.72) and plant height (-0.92). Moreover, the correlation coefficient results indicate that the expression level of *Sh2* in endosperm tissue was significantly and positively correlated with ear weight (0.74), ear length (0.75) and 1000 kernel weight (0.71) at the $p<0.01$ level, while it was significantly and positively correlated with ear number per plant (0.63) and yield (0.66) at the $p<0.05$ level for both locations. According to the results, with increasing expression levels of the *Sh2* gene in endosperm tissue, the ear number per plant, ear weight, ear length, 1000 kernel weight and yield increased. In this study, the expression level of the *Sh2* gene was upregulated in leaf and endosperm tissue via a hybridization program. Using the transgenic technique will significantly increase the expression level of this yield-related gene. The expression level of *Sh2* in endosperm tissue showed negative and significant correlations with tasseling date (-0.89), silking date (-0.79) and plant height (-0.70). Based on the correlation results, increasing the tasseling date, silking date and plant height significantly decreased the expression levels of the *Bt2* and *Sh2* genes in endosperm tissue. Hence, it seems logical to select short plant stature with short periods of anthesis and silking to express high levels of *Bt2* and *Sh2* genes in endosperm tissue.

Table 4.33: Correlation coefficients of the *Sh2* and *Bt2* gene expression levels and morphological traits among six corn advance lines at the two test locations

	Tasselling Date	Silking Date	Plant height	Ear no/plant	Ear wt. g	Ear length	No.kernels/ear	1000 kernel wt.	Yield
***Bt2* (Leaf)**	-0.057	-0.14	0.08	-0.18	-0.04	-0.06	-0.08	0.02	-0.17
***Bt2* (Endosperm)**	-0.69*	-0.72**	-0.92**	0.69*	0.87**	0.87**	0.75**	0.93**	0.90**
***Sh2* (Leaf)**	-0.08	-0.278	-0.28	0.243	0.44	0.45	0.38	0.31	0.33
***Sh2* (Endosperm)**	-0.89**	-0.79**	-0.70*	0.63*	0.74**	0.75**	0.55	0.71**	0.66*

* Correlation is significant at the 0.05 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed)

In general, the detection and expression of two important yield-related genes (*Sh2* and *Bt2*) in leaf and endosperm tissue were done by polymerase chain reaction and real-time PCR. The screening results indicate that the *Sh2*, *Bt2* and *18s* genes were amplified by the gene-specific primers. The expression levels of *Sh2* and *Bt2* in leaf and endosperm tissue were upregulated in new advance corn lines. The upregulation of these yield-related genes increases the starch synthetized in new advance corn lines. Accordingly, in this study increased starch and grain yield were obtained due to genetics.

An analysis of the relationships between the corn lines' morpho-agronomic variability and expression levels of the *Sh2* and *Bt2* genes in leaf and endosperm tissue revealed that some of the traits were significantly and strongly correlated with the expression level of *Sh2* and *Bt2* in endosperm tissue. Therefore, with improved breeding programs based on these traits it is possible to increase the expression levels of these two yield-related genes. Transgenic programs and cross transformation approaches can increase the starch content by modifying the expression levels of yield-related genes.

CHAPTER 5: DISCUSSION

5.1 Variability of UM corn advance lines and selection of suitable criteria by characterizing the morphological traits

Since the grain yield in maize is quantitative in nature and polygenically controlled, effective yield improvement and simultaneous yield component improvement are imperative (Bello & Olaoye, 2009). Selection on the basis of the grain yield character alone is usually not very effective and efficient. However, selection based on the yield's component characteristics may be more efficient and reliable (Alvi et al., 2003). The most important traits, i.e. kernels per ear, 1000 kernel weight and yield across the two locations exhibited high variation (Tables 4.1 and 4.2). This generally reveals that the selection for these economic traits is effective in developing high yielding varieties of maize. The first-order yield components of maize consist of the number of ears (or ears per plant), kernels per ear and kernel weight. Fageria et al. (2006) reported that the first-order yield components are sometimes referred to as primary components and have a direct effect on final yield as well as indirect effects on yield components that develop later. Yield components that can be considered second-order or secondary are those that indirectly effect yield through their effect on first-order components. Secondary components consist of rows per ear, ear length, kernels per row, and ear circumference (Fageria et al., 2006).

The analysis of variance results showed significant differences among the corn advance lines grown in Malaysia at the $p = 0.01$ level, except tasseling date and ear length, which were not significant. The analysis of variance for corn advance lines grown in Iran showed significant differences for all traits, except silking date and ear number per plant, which showed insignificant variation. At both locations, significant differences were observed among the corn advance lines for most traits evaluated. Tables 4.3 and 4.5 indicate the presence of high genetic variability in the inbred lines. Morpho-agronomic trait variations among the CIMMYT QPM genotypes have been

previously reported by several investigators (Akande & Lamidi, 2006; Hadji, 2004; Vasal et al., 1993). Furthermore, Prasanna et al. (2001) noted that the genetic variability for most traits in maize is very high and amenable to enhancements (Prasanna et al., 2001). The wide range between the minimum and maximum values of each trait further confirms the presence of substantial variation among the corn advance lines studied. Such variation is an opportunity for maize breeders to improve traits of interest through selection, hybridization and recombination of desirable genotypes. The mean comparisons of twelve corn advance lines grown in Malaysia based on Duncan's multiple range test showed that the highest mean belonged to line UM 1 for most traits, such as ear weight, ear length, kernel number per ear, 1000 kernel weight and yield per plant. For important traits like 1000 kernel weight and yield per plant, the lowest mean was observed for UM 8 (Table 4.4). At the second location (Iran), the highest and lowest means for the main traits belonged to UM 4 and UM 2, respectively (Table 4.6). Subdividing the variance into its components assists genetic resource conservation and utilization and it enables planning for use of appropriate gene pools in crop improvement for specific plant attributes (Bekele, 1984, 1985; Pecetti et al., 1992; Pecetti & Damania, 1996).

Higher grain yield was observed in the corn advance lines grown in Malaysia compared to those grown in Iran. This can be attributed to the high differences in temperature and humidity at the two test locations. For the experiments in Iran, irrigation to field capacity was done during planting and whenever the soil moisture dropped to a minimum, while the experiments in Malaysia were done exclusively in rain conditions. Significant location effects were observed from the combined analysis of variance (Table 4.15) as a result of differences in growing conditions between Malaysia and Iran. A large proportion of the corn advance lines had earlier anthesis in Iran but higher yield in Malaysia (Figures 4.5 and 4.13). In maize production, higher grain yield

and yield components, early maturity, short anthesis-silking interval and short plant stature are desirable. Most of these features were observed in the corn advance lines grown in Malaysia. All of these corn advance lines were developed at the Institute of Biological Sciences (ISB), University of Malaya, Malaysia. Evidently, the UM corn advance lines evaluated are more suitable for a tropical climate compared to dryland. A large proportion of the corn advance lines had earlier and longer silking periods in Iran than in Malaysia (Figure 4.6). Silking is the stage when the crop is most sensitive to water and heat stress, which can reduce the kernel number (Westgate et al., 2004). The total number of kernels per ear is determined in the silking stage; however, kernel abortion during early grain filling can reduce the kernel number (Milander, 2015). Significant environment x line interaction is not desirable for most traits (Table 4.15), as it indicates dissimilar performance ranks of the corn advance lines at the two test locations. This indicates that more breeding efforts should be dedicated to the development and selection of these corn advance lines for dryland environments. Knowledge of the relationship level among traits is important in designing effective selection programs for crop improvement (Krivanek et al., 2007). At the first location (Malaysia), the genotypic and phenotypic correlation coefficients generally showed a similar direction, but the genotypic correlation coefficients were higher than the corresponding phenotypic correlation coefficients, which may be due to environmental effects (Tables 4.11 and 4.12). According to the phenotypic correlation results, the highest relationship for yield as the main component was observed with ear number per plant with significance at $p<0.01$. Yield was also correlated positively and significantly with tasseling date, ear weight, kernel number per ear and 1000 kernel weight at $p<0.01$. Yield was additionally correlated positively and significantly with silking date at $p<0.05$, but the yield per plant had a negative and non-significant correlation with plant height. According to the genotypic correlation results, the highest correlation of yield as

the main component was observed with ear number per plant with significance at $p<0.01$. Yield was correlated positively and significantly with 1000 kernel weight at the $p<0.05$ level. It also exhibited positive and non-significant correlation with all other traits, but yield per plant had a negative and non-significant correlation with plant height. The phenotypic and genotypic correlation results indicate that with increasing all traits, yield per plant increased, but increasing plant height due to the negative phenotypic and genotypic correlations with yield decreased the yield per plant. Hence, at the first location (Malaysia), based on the genotypic correlation, it seems logical to select the corn lines with the lowest plant height for lodging resistance associated with high yield. This phenomenon is desirable and very important for maize breeders because they complicate the simultaneous improvement of high-yield and short plant stature lodging resistant lines. The lack of genotypic correlation between grain yield and tasseling and silking date indicates the absence of a functional relationship between these traits. Bolanos and Edmeades (1996) noted that the correlation between grain yield and anthesis-silking interval is weak under optimal management conditions but very strong under stress. The positive correlation of grain yield with its components, such as ear diameter and length, thousand kernel weight and number of kernels per row has also been reported previously (Bolanos & Edmeades, 1996). The correlation coefficient values observed in the current experiments are in agreement with the range of values reported by Hallauer and Miranda (1988). The traits that showed positive and significant correlation with grain yield can be improved simultaneously during selection for higher grain yield. These traits can also be used as secondary traits during indirect selection for grain yield. The correlation coefficients at the second location (Iran) demonstrated that the genotypic correlation coefficients were greater than the corresponding phenotypic correlation coefficients. The genotypic and phenotypic correlation coefficients displayed a similar direction (Tables 4.13 and 4.14). According

to the phenotypic correlation results for Iran, the highest relationship for yield as the main component was observed with 1000 kernel weight with significance at the $p<0.01$ level. Moreover, yield was correlated positively and significantly with ear number per plant, ear weight, ear length and kernel number per ear at $p<0.01$. Yield also showed negative and non-significant correlation with tasseling date, silking date and plant height. Based on these result, with increasing all traits, yield per plant increased; however, increasing the tasseling date, silking date and plant height due to the negative correlation with yield decreased the yield per plant. Therefore, in Iran, it seems logical to select plants with short tasseling period and silking date as well as short corn lines to lodge resistance associated with high yield. The genotypic correlation results for the corn advance lines grown in Iran showed that the highest relationship for yield as the main component was observed with 1000 kernel weight and it was positive and significant at the $p<0.01$ level. Yield was also correlated positively and significantly with kernel number per ear and ear weight at level $p<0.01$ and with ear length at $p<0.05$. Yield showed negative and non-significant correlation with tasseling date and plant height besides negative and significant correlation with silking date at the $p<0.05$ level. The results indicate that with increasing all traits, yield per plant increased, but increasing the tasseling duration, silking date and plant height on account of the negative correlation with yield decreased the yield per plant. According to the genotypic correlation in Iran, it seems logical to select plants with short tasseling and silking periods. Genetic traits, such as variance components, coefficients of variability, heritability and genetic advance provide estimates of genetic variation in the quantitative traits. Higher error variance was observed in the corn advance lines grown in Malaysia than those grown in Iran (Tables 4.9 and 4.10) as a result of smaller plot size, which increased the experimental error. There is a difference among the genotypic and phenotypic variance of all traits at both locations (Malaysia and Iran), which

indicates the influence of the environment on these characteristics (Tables 4.9 and 4.10). Significantly higher genetic variability was observed for most traits, indicating the potential to exploit the lines selected at both locations. This phenomenon is also evident from the fact that σ_g^2 is greater than σ_e^2 for most traits. In Malaysia the genotypic coefficient of variability was the highest for yield per plant, ear number per plant and 1000 kernel weight. The phenotypic coefficient of variability was also high for yield per plant, ear number per plant and ear length. Yield per plant and ear number per plant had high phenotypic and genotypic coefficient of variation values at this location. In Iran, the genotypic coefficient of variation was the highest for yield per plant, kernel number per ear and ear length. The phenotypic coefficient of variation was the highest for yield per plant, ear number per plant and kernel number per ear. Yield per plant, kernel number per ear and ear length had high phenotypic and genotypic coefficient of variation values at this location. In most cases, the traits with higher phenotypic coefficients of variation also had higher genetic coefficients of variation, indicating the minimum environmental effect on the phenotypic expression of the traits.

The broad sense heritability (H2) estimates were high for most traits evaluated at both locations, indicating that these traits are highly heritable. At the first location, higher heritability estimates correlated with good estimates of genetic advance expected in the next generation for 1000 kernel weight and ear weight and at the second location for kernel number per ear indicate that these characteristics are supported by additive genetic effects. Therefore, further selection would be effective on the set of lines evaluated in this experiment. Characteristic selection is fairly easy if its heritability is high; but for a characteristic with low heritability, selection may be difficult or impractical due to the masking effect of the environment on genotypic effects (Singh, 2005). Hallauer and Miranda (1988) summarized numerous estimates of heritability in maize and reported values ranging from 0.3 to 0.7. According to Johnson et al.,

heritability by itself provides no indication of the extent of genetic progress that would result from selecting the best individuals. They suggested that the estimates of heritability and genetic advance should always be considered simultaneously (Johnson et al., 1955). In addition, (Assefa et al., 1999) suggested that the genetic coefficients of variation together with heritability estimates would give the best picture of genetic advance to be expected from selection. Therefore, traits that exhibit high genotypic coefficients of variation, heritability and genetic advance in mean percent would be useful as a base for selection. In this study, the three mentioned parameters had larger values for traits such as 1000 kernel weight (Table 4.9). Simple selection of plants with greater 1000 kernel weight, for example, may lead to successfully improving the trait. As such, development of UM corn advance lines should focus on 1000 kernel weight as a selection criterion to increase the yield per plant. Moreover, this trait had positive correlation with grain yield. If the heritability of a characteristic is very high, for instance 0.8 or more, the selection of the characteristic should be fairly easy (Singh, 2005). This is because there would be a close correspondence between the genotype and phenotype due to a relatively smaller contribution of the environmental effect on the phenotype. High heritability implies that the genetic variation for a trait can be assessed precisely from phenotypic observations (broad sense) and that the trait can be easily transmitted to the offspring of the selected genotype (narrow sense) (Marianne Banziger & Cooper, 2001; Falconer, 1989). However, for a characteristic with low heritability, for instance with a value below 0.4, selection may be difficult or impractical due to the masking effect of the environment on the genotype (Singh, 2005).

The broad morphological variation among lines was further substantiated by principal component analysis (PCA) (Tables 4.7 and 4.8). The variations in all traits were dissected into three PCs, which accounted for 76.33% of the variability existing

among the lines at the first location (Malaysia). However, traits such as kernel number per ear, ear length, ear weight, silking date and tasseling date were the major contributors, respectively. At the second location (Iran), two components with Eigen values greater than 1 were extracted and these showed 86.62% of the total variability. In Iran, the first principal component (PC1) of ear length, ear weight, kernel number per ear, 1000 kernel weight, silking date, tasseling date and plant height indicated 70.6% of the total variability. (Beyene et al., 2006) reported that 71.8% of the total variation in 62 traditional Ethiopian highland maize accessions was explained by the first four PCs. These authors reported that ear height, number of kernels per row and 1000 kernel weight were the traits predominantly contributing to the total variation. The major role of the morphological traits in phenotypic variation observed in this study is also consistent with the work of Alika et al. (1992). Genetic distances estimated based on morpho-agronomic data revealed considerable variability among the inbred lines. Cluster analysis also confirmed the presence of variation among the lines. The dendrogram showed the resolution power of morphological and agronomic traits for grouping maize inbred lines. Cluster analysis can identify differences among genotypes for the breeder via genotype classification (Sabaghnia et al., 2012). Based on the average linkage distance, the twelve corn advance lines were grouped into 3 main groups. In Iran, the six corn advance lines were divided into 2 main groups. At both test locations UM 2 and UM 11 belonged to the same group, and UM 3, UM 4 and UM 6 were also in the same group at both locations. UM 1 in Malaysia was in a distinct group from UM 3, UM 4 and UM 6. The grouping of the twelve corn advance lines by multivariate methods in this study is of practical value to breeders. Representative lines may be chosen from particular groups for hybrid programs with other approved varieties. Several potentially important agronomic types have been identified and these may be exploited for genetic potential to transfer the desirable genes. This facilitates

assembling a core collection of accessions from a large genetic resource collection (Clements & Cowling, 1994; Frankel, 1984; Tolbert et al., 1979). Lucchin et al., 2003 clustered 20 Italian flint maize landraces into groups according to morphological and agronomic traits. Beyene et al. (2006) classified 62 traditional highland maize accessions into three groups using 15 morphological traits. In the diversity analysis of 45 maize inbreds, Gerdes and Tracy (1994) successfully grouped closely related inbred lines by morphology. However, reports consistently indicate that morphological markers have shortcomings in that they are highly influenced by prevailing environmental conditions (Bernardo, 1992; Beyene et al., 2005; Gerdes & Tracy, 1994).

5.2 Detection of the *Sh2* and *Bt2b* genes in composite corn lines by gene-specific primers

The rate-limiting step in starch synthesis entails the synthesis of ADP-Glc from Glc-1-P and ATP by AGPase (Russell et al., 1993). AGPase is a heterotetramer composed of two large subunits encoded by *Sh2* and two small subunits encoded by *Bt2*. Homozygous *Sh2* or *Bt2* kernels collapse (shrink) during the maturation process, becoming angular (brittle) at maturity when they contain very little starch and much greater amounts of Suc (Cameron & Teas, 1954; Laughnan, 1953). Whereas the presence of two different subunits is typical for higher plants, where both photosynthetic and non-photosynthetic bacteria have only a single AGPase gene. All genes have evolved from a common ancestor and substantial sequence similarity remains between *Sh2* and *Bt2*, although it is less than the similarity between *Bt2* and genes encoding small subunits in other species (Smith-White & Preiss, 1992). This study clarifies the expression levels of shrunken-2 (*Sh2*) and brittle-2 (*Bt2*) genes that encode the large and small subunits of ADP-glucose pyrophosphorylase in the leaf and endosperm tissue of UM corn advance lines, respectively. Initially, six primer pairs were tested to amplify the *Sh2* gene. Most of these six loci failed to amplify the

genomic DNA of at least one of the corn advance lines or provided irreproducible banding patterns and were subsequently rejected. Among four designed primers and two published primers used to amplify the *Sh2* gene, the F5 primer successfully amplified the genomic DNA of twelve UM composite corn lines. Detecting the *Sh2* gene with the F2 and F4 primers generated multiple bands, indicating that these two primers are not specific for *Sh2* gene detection. The F6 primer was not able to amplify the *Sh2* gene in all 12 corn lines. Furthermore, two published primers (*LH71* and *Sh2*) generated multiple bands to amplify the *Sh2* gene in the genomic DNA of twelve composite corn lines. Giroux et al. (1996) reported using the *LH71* primer to amplify DNA isolated from the leaves of *Sh2* revertants, while (Huang et al., 2014) reported functions of multiple genes that encoded ADP-Glucose pyrophosphorylase subunits using the *Sh2* primer to amplify the *Sh2* gene in maize endosperm, embryo and leaf. Since the *Bt2* gene generates two transcripts referred to as *Bt2a* and *Bt2b* (Rosti & Denyer, 2007), two primers were used to amplify the *Bt2a* and *Bt2b* transcripts. As shown in Figure 4.15, only one transcript of the *Bt2* gene was amplified from the genomic DNA of composite corn lines. This might be related to the accumulation of the *Bt2* gene in the embryo. Huang et al. (2014) reported detecting the *Bt2a* transcript only in endosperm, but *Bt2a* was not present in maize plant leaves. Endosperm mutations including brittle2 (*Bt2*) and shrunken2 (*Sh2*) alter the starch pathway such that kernel sugars, particularly sucrose, accumulate at the expense of starch. The detection of *Bt2* and *Sh2* in UM composite lines demonstrates that these two genes were upregulated successfully by the UM composite corn lines rather than the parent lines.

Zhang et al., 2007 investigated and compared the relationships between the starch synthesis rates and the activities of enzymes responsible for starch biosynthesis in developing grains of normal, pop, sweet and waxy corn throughout

the grain filling period. Mutant characterization was first limited to cytological descriptions, later on to biochemical quantifications of metabolites or enzyme activities, and only more recently to the cloning of the underlying genes. For example, the shrunken2 (*Sh2*) mutant was isolated in 1949 (Mains, 1949). The lack of starch in mature kernels was demonstrated in 1953 (Laughnan, 1953), the absence of ADP-Glc pyrophosphorylase (AGPase) activity was demonstrated in 1966 (Tsai & Nelson, 1966), and the mutated gene was isolated in 1990 (Bhave et al., 1990). Among all the developmental and biosynthetic pathways of the maize kernel, the one leading to starch is certainly the best characterized. The majority of genes involved in the major steps of starch synthesis have been identified by mutant analysis. Cossegal et al. 2008 reported the order along the pathway leading from Suc via activated hexoses to the starch polymer: Miniature1 (Mn1) codes the cell wall invertase IncW2, Shrunken1 (Sh1) and Sucrose synthase1 (Sus1) code Suc synthase, *Sh2* and Brittle2 (*Bt2*) code AGPase, Bt1 codes an ADP-Glc transporter, Waxy1 (Wx1) codes the granule-bound starch synthase GBSSI, Sugary2 (Su2) and Dull1 (Du1) code the soluble starch synthases SSIIa and SSIIIa respectively, Amylose extender1 (Ae1) codes the branching enzyme BEIIb, and Su1 codes the debranching enzyme ISAI (Cossegal et al., 2008; Hannah, 2005). The rate-limiting step in starch synthesis is the synthesis of ADP-Glc from Glc-1-P and ATP by AGPase (Russell et al., 1993). AGPase is a heterotetramer composed of two large subunits encoded by *Sh2* and two small subunits encoded by *Bt2*. The authors speculated that a cytosolic AGPase has the advantage of committing carbon to starch synthesis rather than other metabolic pathways in the presence of high Suc levels. Zhang and Dong (2007) reported that the rates of starch synthesis had some correlation with the activities of sucrose synthase (SS), soluble starch synthase (SSS), granule-bound starch synthase (GBSS) and starch-branching enzyme (SBE)

during the grain filling process. No correlation was found between the rates of starch synthesis and the activities of ADP-glucose pyrophosphorylase (AGPase) and starch-debranching enzyme (DBE). Sucrose synthase (SS) activity appears to play a major role in starch biosynthesis in maize. Granule-bound starch synthase (GBSS) is responsible for amylose synthesis, especially in the later period. Soluble starch synthase (SSS) and starch-branching enzyme (SBE) are associated with amylopectin biosynthesis (Zhang et al., 2007).

Based on the gene-specific primers that successfully amplified the *Sh2* and *Bt2*b genes from the total genomic DNA of the corn advance lines, reverse transcription-polymerase chain reaction (RT-PCR) experiments were done. In the RT-PCR experiments, the *Sh2* and *Bt2*b expression levels in the leaf and endosperm of corn advance lines were detected. The results indicate that these two subunits of ADP-glucose pyrophosphorylase (AGPase) were upregulated in the leaf and endosperm tissue of composite corn lines rather than their parental lines, whereas the gene-specific primer for *Bt2*a could not amplify the genomic DNA of these corn lines. Cossegal et al. (2008) reported that *Bt2*a was preferentially but not specifically expressed in endosperm, but *Bt2*b was expressed in almost all tissues tested. Huang et al. (2014) reported that *Bt2*b, an alternative transcript coding for a plastidial isoform, was expressed in almost all tissues tested with a pattern very similar to that of Agpslzm. However, *Bt2*a, the classical transcript coding for a cytoplasmic isoform, was almost exclusively expressed in the developing endosperm.

5.3 Expression level of *Sh2* and *Bt2*b genes in six composite corn lines by quantitative real-time PCR

In this study, the *Bt2* and *Sh2* genes that encode the enzyme AGPase were selected. Their overexpression impact was investigated on the UM corn advance lines at the two test locations. Laughnan (1953) stated that there are many mutants

in maize that produce striking differences from the normal in terms of texture, form and amount of endosperm. Since an overwhelming proportion of normal endosperm in maize is composed of reserve carbohydrates, principally starch, it is anticipated that studies on the action of such mutant forms would be of significance for the problem of carbohydrate biosynthesis. Hannah (2012) reported that most attempts to enhance starch accumulation in plants have focused on engineering AGPase activity. A heat-stable and orthophosphate-insensitive maize AGPase was recently shown to increase the starch yield in maize by up to 64% (Hannah et al., 2012). The expression levels of *Sh2* and *Bt2*b genes in newly developed UM corn advance lines were evaluated to consider the possibility of improving the starch content and increasing overall yield by multigene engineering. As a requirement to select a proper internal control gene, three housekeeping genes were tested including *18s*, *actin* and *eEF1*. As numerous studies have reported, housekeeping gene expression can vary considerably (Warrington et al., 2000). From the results it was observed that *18s* had the lowest CT value among the three housekeeping genes. Also in this investigation, the *18s* gene expressed uniformly in different corn lines, which led to using *18s* as internal control. A comprehensive literature analysis of expression studies indicates that 18S, GAPD, ACTB and 28S rRNA were used as single control genes for normalization in more than 90% of cases (Suzuki et al., 2000). Moreover, Huang et al. (2014) reported using *18s* as a housekeeping gene for expression analysis in maize. To estimate the effect of hybridization and experimental treatment (different test locations) on the expression of an endogenous reference or internal control, the method proposed by Livakand (2001) was used. This method was also used to estimate the effect of hybridization on the target genes (*Sh2* and *Bt2*). The highest expression level of *Sh2* in leaf tissues was observed in UM 1 among the six corn advance lines tested at both locations

(Malaysia and Iran). The expression was 6.96 fold and 6.4 fold, respectively (Figures 4.19 and 4.20). Based on Duncan's multiple range test, the highest mean calculated for 1000 kernel weight and yield per plant also belonged to UM 1 (M= 504.7 g) and it showed significant difference among the corn advance lines (Table 4.4). The upregulation of the *Sh2* gene in 1000 kernel weight and yield per plant in UM 1 can be due to the positive correlation between the expression level of *Sh2* and 1000 kernel weight and yield per plant (Table 4.33). The highest expression level of *Bt2*b in leaf tissue at both locations (Malaysia and Iran) was observed in UM 11, with 3.94 fold and 4.08 fold, respectively (Figures 4.17 and 4.18). The comparison of *Bt2b* and *Sh2* gene expression levels indicate that the relative transcript level of *Sh2* in the leaf tissue of all six corn advance lines was in a higher range than the transcript level of *Bt2b* at both locations. As expected, *Bt2* was weakly expressed in leaf but strongly in endosperm. Cossegal et al. (2008) reported that *Bt2* was strongly expressed in kernels, moderately in ears and tassel, and weakly in leaves. The expression level of *Bt2*b also showed a negative correlation with yield per plant (Table 4.33). The results of comparing the expression level of *Bt2*b in endosperm tissue indicate that among six corn advance lines, the highest expression level in both Malaysia and Iran was observed in UM 1 with 7.36 fold and 3.96 fold, respectively (Figures 4.21 and 4.22). Based on Duncan's multiple range test, the highest mean of 1000 kernel weight and yield per plant was for UM 1 (M= 504.7 gr) with significant differences among corn advance lines (Table 4.4). The upregulation of the *Bt2* gene for 1000 kernel weight and yield per plant in UM 1 can be due to the significant and positive correlation between the expression level of *Bt2* and 1000 kernel weight and yield per plant (Table 4.33). The results present the more impressive expression level of *Bt2b* in the endosperm of the six corn advance lines in Malaysia compared to the leaf of these lines at the same location.

Nonetheless, the results indicate significant differences in the expression levels of *Bt2b* in the leaf and endosperm tissue of the six corn advance lines at both test locations.

The highest expression level of the *Sh2* gene in the endosperm tissue of corn advance lines grown in Malaysia was observed in UM 2 with 5.96 fold, while for Iran it was observed in UM 3 with 3.05 fold (Figures 4.23 and 4.24). The results in Table 4.31 indicate there was no significant difference in the expression levels of *Sh2* in the endosperm tissue of six corn advance lines grown in Iran. A comparison of the relative transcript levels for *Bt2b* and *Sh2* in endosperm tissue indicates that the expression level of *Bt2b* was in a higher range than that of *Sh2* among six corn advance lines in Iran. According to the results, the *Sh2* gene was strongly expressed in leaf tissue but moderately expressed in endosperm tissue. Based on the correlation results, increasing the tasseling period, silking period and plant height significantly decreased the expression levels of the *Bt2* and *Sh2* genes in endosperm tissue. Hence, it seems logical to select corn lines with short periods of anthesis and silking and the short plant stature to express high levels of *Bt2* and *Sh2* in endosperm tissue to increase the overall yield.

Higher expression levels of the *Bt2* and *Sh2* genes were observed in the endosperm and leaf tissue of corn lines grown in Malaysia compared to those grown in Iran (Table 4.32). This may be due to the high differences in temperature at the two test locations. Giroux et al., 1996 modified the allosteric properties of maize endosperm AGP and achieved increased maize seed weight of approximately 15%. Hence, AGP represents an important target for genetic manipulation. Of significance to heat-induced yield loss is the fact that maize endosperm AGP is heat-labile (Hannah et al., 1980; Preiss et al., 1971). Maize endosperm AGP loses 96% of its activity when

heated in vitro at 57°C for 5 min. This result is in sharp contrast to potato AGP that is fully stable at 60°C (Okita et al., 1990; Sowokinos & Preiss, 1982). Singletary et al., 1994 extended studies on the heat lability of maize endosperm AGP by monitoring seed weight and various enzymic activities throughout the growth of in vitro-developed kernels at various temperatures. Seed weight decreased as temperature increased from 22°C to 36°C. Although elevated temperature impacted AGP and soluble starch synthase activities most negatively, Singletary et al., 1994 concluded that AGP is likely more important in the premature cessation of starch deposition. Likewise, Duke et al., 1996 monitored a number of starch synthetic enzymes and their transcripts in kernels developed at elevated temperatures, to which AGP activity was most sensitive. Duke and Doehlert postulated that AGP might have a faster turnover rate under heat stress conditions compared with other enzymes assayed. Although the maize investigations point to the heat lability of AGP as the major cause of heat-induced weight loss, studies on wheat suggest that loss of soluble starch synthase activity is the cause of heat-induced seed weight loss (Hawker & Jenner, 1993; Keeling et al., 1994) .

The transformation approach for crosses was used to produce new hybrid corn lines expressing two transgenes relevant to starch biosynthesis, specifically in leaf and endosperm tissue. From this study, the morpho-agronomic traits and evaluation of transcript levels of the *Sh2* and *Bt2* genes that encode the enzyme AGPase to enhance starch accumulation in plants and subsequently increase the overall maize yield provide a basis for plant breeding and crossbreeding programs. Studying the genetic variability and contribution of yield components to the yield of corn advance lines can improve starch-dependent agronomic traits. The composite corn lines obtained could be used in future maize breeding programs to improve the quantity and quality of starch, or produce tailored starches for specific downstream applications. Information from the

genetic diversity analyses of the corn advance lines can be used for effective utilization of the advance lines in breeding programs to form heterotic populations and develop desirable varieties. The corn advance lines used in this study are generally found to be useful sources of genetic variability for the development of new genotypes in tropical environments.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The research reported in this thesis was conducted on twelve corn advance lines developed through the hybridization of yellow sweet corn and white field corn. Therefore, all corn lines in this study are considered composite corn lines. The project was conducted in field and laboratory conditions at two test locations (Malaysia and Iran) to estimate the genetic variability of the corn lines on the basis of morphological and agronomic characteristics. The reasons for low yield were considered by analysing the characteristics contributing to yield. Finally, the transcript levels of two genes that encode the enzyme AGPase to enhance starch accumulation in the maize plant were evaluated.

Twelve corn advance lines were assessed for various agro-morphological traits in field condition in RCBD in a tropical environment (Malaysia). Data were recorded for nine quantitative traits. Out of the twelve corn advance lines, six were selected on the basis of yield per plant from Duncan's multiple range test (DMRT) for gene expression analysis. Moreover, these six superior lines were selected for planting in a dryland environment (Iran) and to consider the expression levels of the *Sh2* and *Bt2* genes in different growing conditions. All twelve corn advance lines under study were grouped into three main clusters on the basis of average linkage. Furthermore, variance was studied by principal component analysis (PCA). The first three principal components and factors with Eigenvalues over 1 explained 76% of the total variance at the first location. At the second location, two components with Eigenvalues greater than 1 were extracted and these two components explained 87% of the total variance. High variance was observed for yield, 1000 kernel weight and kernel number per ear at both locations. The genotypic and phenotypic correlation coefficient results among different characteristics revealed that yield per plant was significantly and positively correlated

with ear number per plant and 1000 kernel weight for six corn advance lines grown in Malaysia. Meanwhile, significant and positive genotypic and phenotypic correlation was observed between yield per plant and ear weight, ear length, kernel number per ear and 1000 kernel weight for six corn advance lines grown in Iran. Continuous improvement in maize is imperative for amplified competition for the crop. This can be achieved through the effective selection of suitable parent materials with significant genetic variability.

6.2 Recommendations

The gene expression experiments on the leaf and endosperm tissue of six crossed lines (made from field corn and sweet corn) were done to measure the RNA transcription levels of various genes including *Sh2* and *Bt2* as well as *18s* as a housekeeping gene by real-time quantitative PCR at two test locations. Initially, eleven primer pairs were tested to use in the gene expression experiments. Out of these eleven loci, three primer pairs successfully amplified the *Sh2*, *Bt2* and *18s* genes from the genomic DNA of the UM corn advance lines. The results from this study revealed the upregulation of the expression of *Sh2* and *Bt2* genes in leaf and endosperm tissues at two different test locations. Analysis of variance for these two genes showed significant differences in the leaf and endosperm tissue of six corn lines grown in Malaysia. Analysis of variance was also done for the expression levels of these two genes in the leaf tissue of six corn lines grown in Iran, which indicated significant differences. However, the variation of the *Bt2* gene in endosperm tissue showed significant difference but the variation of *Sh2* in this tissue showed non-significant difference among the six corn advance lines grown in Iran. The correlation coefficient between the expression levels of *Sh2* and *Bt2* and the morphological traits showed that the expression level of *Bt2* in leaf tissue was not significantly associated with any trait,

whereas the expression of this gene in endosperm tissue was significantly and positively associated with ear number per plant, ear weight, ear length, kernel number per ear, 1000 kernel weight and yield. Similarly, the expression of *Sh2* in leaf tissue showed no significant correlation with any trait, whereas the expression of this gene in endosperm tissue was significantly and positively associated with ear number per plant, ear weight, ear length, 1000 kernel weight and yield. The upregulation level of *Sh2* and *Bt2* in UM corn advance lines suggested that this is a good resource for maize development.

As future work, more breeding efforts should be devoted to the development of corn advance lines with better field performance in tropical and dryland environments. Furthermore, the expression level of *Sh2* and *Bt2* genes in embryo of UM corn advance lines should be considered. The classical transcript of the *Bt2* gene (*Bt2a*) that codes a cytoplasmic isoform will almost exclusively be expressed in the developing endosperm, whereas *Bt2b*, an alternative transcript coding for a plastidial isoform will be expressed in almost all tissues. More specifically, for the classical transcript (*Bt2a*), new gene-specific primers will be designed and the expression level of *Bt2a* in different tissues of corn advance lines will be measured. Besides, the expression level of the sugary genes in corn advance lines can be measured and compared with the parental lines.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

Publication:

Abna, F., Golam, F., & Bhassu, S. (2012). Estimation of genetic diversity of mungbean (Vigna radiata L. Wilczek) in Malaysian tropical environment. *African Journal of Microbiology Research*, 6(8), 1770-1775.

Abna, F., Noorma Wati Haron, N., & Danaee, M. (2018). Evaluation of Genetic Variability for Yield and Morphological Traits in Corn Advance Lines under Tropical and Dryland. Environments *International Journal of Agricultural Sustainability*.

Abna, F., Noorma Wati Haron, N., & Danaee, M. (2018). Confirmation of Sh2 and Bt2 Genes and Their Expression Level in Newly Developed Composite Corn Lines under Tropical and Dryland Environments. *International Journal of Agriculture and Biology.*

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Fatemeh Abna, Subha Bhassu, &Golam Faruq. (2012). Genetic Diversity Analysis in Different Varieties of Mungbean (Vigna radiata (L.) Wilczek) based on Quantitative Traits Using Multivariate Methods. Proceeding of the 2nd Annual International Conference on Advances in Biotechnology (Bio Tech 2012), Bangkok, Thailand.