Genetic Characterization of maize (*Zea mays* L.) Hybrids Using Molecular Markers

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Dedicated to

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CERTIFICATE – I

This is to certify that the thesis entitled ‘’***Genetic Characterization of maize (Zea mays L.) Hybrids Using Molecular Markers’’*** submitted in partial fulfilment of the requirements for the award of the degree of Master of Science in Agricultural (Seed Science and Technology) , to the Faculty of Post-graduate studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir is a record of bonafide research work carried out by **Mr. Mohammad Elyass Bhat (Regd. No. 887-M-2012-A)** under my supervision and guidance. No part of the thesis has been submitted for any other degree of diploma.

It is further certified that information received during the course of investigation has duly been acknowledged.

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CERTIFICATE – III

This is to certify that the thesis entitled *''Genetic Characteristics of maize (Zea Mays **L**.) Hybrids Using Molecular Markers''* submitted by **Mr. Mohammad Elyass Bhat (Regd. No. 887-M-2012-A)** in partial fulfilment of the requirements for the award of the degree of Master of Science in Agriculture (**Seed Science and Technology**) , to the Faculty of Post-graduate studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir was examined and approved by the **Advisory Committee** and **External Examiner** on_________________

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Abstract

Maize (Zea mays L.) is the second most important cereal crop after rice in Kashmir. Few promising hybrids have been released or introduced in the province by public and private organizations. Once put in seed chain, it is difficult through a bare comprehension to adjudge cultivar distinctiveness and genetic purity of hybrids in commercial seed lots. Morphological markers or descriptor traits could be used to characterize specific cultivars, however, this can only be done with little precision and sometimes may not be possible where genotypes are morphologically similar. Detection of parental (female) line impurity in hybrid seed is very important before exercising mass scale distribution of seed to farmers. For the purpose, use of codominant markers like SSRs becomes rather more feasible option. A study was carried out to identify the SSR markers which may be used in hybrid purity analysis. A set of seven parental lines and five hybrids were genotyped using 45 SSR markers. The markers Umc-1664, Umc-2372, Phi-061 were found polymorphic across corresponding parents of all the five hybrids. These markers in future may be used to identify parental seed mixture (homozygotes) in hybrid seed lots. Also, thirty one morphological characteristics were studied to discriminate among hybrids H_1, H_2, H_3, H_4 and H_5. Only nine of these traits differentiated the hybrids and were polymorphic including only a single trait that showed three phenotypic classes. Similarity coefficient of 0.74 was recorded between hybrids H_1 and H_3 based on all the thirty traits. In comparison to the morphological markers, SSRs revealed greater degree of polymorphism among hybrids. Maximum probability of identity was recorded for H_1 vs H_4 which was as low as 0.21. With the help of ten SSR markers, SSR barcode was developed which may be useful in characterization and identification of any given hybrid in open seed market or farmers fields. The polymorphic markers will help in hybrid purity testing while the SSR multi-locus profile generated in the present program is expected to discriminate a given hybrid from other hybrids and OPVs in a seed chain.

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1. Introduction

Maize (Zea mays L.) that belongs to tribe Maydea and family ÷poaceaeø is the second most important cereal crop in the world after wheat (Asif et al 2006). It holds a unique position in world agriculture and constitutes important part of human diet. Maize is used as an ingredient to poultry and livestock feed. Maize is produced on nearly 100 million hectares in developing countries with almost 70% of the total maize production in the developing world coming from low to lower middle income countries (FAOSTAT, 2010). A demand for maize is projected to get doubled by the year 2050 and the crop may attain highest production globally by 2025 (Rosergent *et al.,* 2009) In India the crop occupies an area of 8.55 million hectares and the production is 21.76 million tons with an average yield of 2.48 tones ha^{-1} (Anonymous, 2013). Maize is grown under wide range of agro climatic conditions varying from temperate to humid sub-tropical.

In Jammu and Kashmir State, maize is second most important crop after rice and is a staple food of people in tribal areas. Mostly maize is grown as rainfed crop in marginal hilly terrains of Kashmir Valley. The crop occupies an area of 0.1 million hectares with production and productivity of 0.15 million tones and 1.2 tones per hectare (Anonymous, 2012). The low productivity of maize as compared to national average has been probably due to poor diffusion of improved germplasm, rainfed ecology and non adoption of improved varieties. Maize due to its diversified uses enjoys huge demand, hybrid technology provides an effective way of achieving growth in production and productivity levels of the crop. Although, number of varieties have been released for valley basin and high altitude areas of J&K, however, few hybrids/ composites have become popular in maize growing regions and have successfully been grown by farmers with the aim of achieving high production. The quality of seed is major determinant to increased production (Agarwal and Gowdar, 2010).

Maize naturally is highly cross pollinated species, therefore, composite varieties loose the genetic purity after 4-5 yrs of cultivation in farmers field. In that context hybrid seed is replaced every year where loss in genetic purity of the seed may not be concern. However, practically quality of hybrid seed at least in terms of genetic purity rests on hybrid seed production technological standards. Most of the ordinary commercial seed production growers do have constraints to ensure high or 100% genetic purity of hybrids.

The maize hybrids are known for their improved yield performance, genetic uniformity, resistance to pests and diseases and are being preferred by the farmers. Thus, genetic purity of seed becomes much more important since seed is the initial lever for bolstering production potentiality and means of creating surplus in commercial markets (Srivastava and Jaffee, 1993). Hybrid corn has helped to achieve a quantum jump in yield. Before dispensing seeds to the farmer its purity testing is essential to ensure its trueness. It is estimated that for every percent impurity in hybrid seed the yield reduction can go upto100 kg ha^{-1} (Li Liu *et al*. 2000). In maize it is even higher as the reduction in the yield goes to 135 kg ha^{-1} (Mao *et al*. 1996). Moreover, the contamination reduces the genetic and physiological quality of seeds that consequently decreases the crop productivity (Kalnka *et al* 2006).

Since detailed morphological descriptors of the plants and seeds have been used for identification but this method is ambiguous and time consuming. The methods like grow out test normally demand considerable time and labour. Hybrid purity testing is best achieved by use of DNA markers. Molecular markers which could clearly distinguish the hybrids from its parental lines have been identified and developed in major agricultural and horticulture crops (Palave *et al*. 2010). SSR markers have proven to be preferred and robust molecular markers for purity identification, due to their high efficiency, reproducibility and simplicity (Wu *et al.*, 2010).

Genetic purity of commercial seed lots is of immense importance in realization of full yield potential of varieties per unit area. Therefore, present study was broadly aimed to identify the DNA based markers for hybrid purity testing. The study was pursued with the following objectives:

1. Morphological characterization of maize inbreds and hybrids
2. Molecular characterization and DNA finger printing of single cross maize hybrids.

Scientifically, a fertilized matured ovule together covered with seed coat is called seed. Structurally, a true seed is a fertilized matured ovule, consisting of an embryonic plant, a store of food and a protective seed coat, a store of food consists of cotyledons and endospermö. From seed technological point of view seed may be sexually produced matured ovule consisting of an intact embryo, endosperm and or cotyledon with protective covering (seed coat). It also refers to propagating materials of healthy seedlings, tuber, bulbs, rhizome, roots, cuttings, setts, slips, all types of grafts and vegetatively propagating materials used for production purpose. Thus seed is the most vital and crucial input for crop production, one of the ways to increase the productivity without adding appreciably to the extent of land now under cultivation by planting quality seed. Quality seed is an entity which meets the Minimum Seed Certification Standards in terms of physical purity, genetic purity, germination per cent, moisture content and seed health. The variety once released should maintain its seed quality in time and space and is a function of minimum seed quality standards prescribed for that variety and the floral biology of the crop concerned. The genetic purity in open pollinated varieties during multiplication stages is prone to contamination due to the phenomenon of out crossing with foreign pollen besides physical admixtures. Parental line contamination in hybrids is a major issue in purity of hybrid seed. Higher genetic purity is an essential prerequisite for the commercialization of any hybrid. The traditional way to assess the seed genetic purity in major food crops is grow-out test (GOT), where the crop is grown and evaluated at different stages of crop growth with the aid of available morphological descriptors. The process is time consuming, requires larger area for replicated trails and highly skilled personnel for making often subjective decision (Lucchese *et al.,* 1999).

a. Morphological traits for varietal identification

International Plant Genetic Resource Institute (IPGRI) promotes a minimum set of morphological characters that entails genetic diversity at particular points in the genome corresponding to the observed characteristics recognized internationally for management of crop germplasm.

Seeds, seedlings and plants of various cultivars exhibit a wide range of morphological distinctness which is helpful in varietal identification and genetic purity testing. These

features specific to crops make them candidate markers for DUS testing. The characters which have been generally used in description of varieties include as follows:

b. Seed morphology

Morphological characters of seeds *viz.*, seed shape, seed size, colour and test weight are useful traits for varietal identification in number of crops.

Paukens (1975) reported that kernel colour (white, light yellow, bright yellow, dark yellow, red, *etc.*), length (short, medium and long), width (narrow, medium and wide) and thickness (thin, medium and thick) were used for determining the cultivar trueness and purity in maize.

Nagapadma *et al*. (1996) characterized the twenty-three maize inbred lines based on seed colour (yellow and white) and 100 grain weight (bold, medium and small).

Elsaeed (1967) reported varietal differences in Beladi and Rebya-34 of broad bean based on seed weight which was 192.7g and 317.1g, respectively per 500 seeds.

Shivasubramanian and Ramakrishnan (1978) reported that the shape and colour of kernel were useful in identifying rice varieties.

Vanagamudi *et al*. (1988) grouped the rice varieties based on the colour of silk integument (yellow, grey, brown, slightly reddish, red or violet), colour of hulled grain (white, brown, reddishbrown, red, yellow or orange), vitrous characters, length of hulled grain (short, medium, long and very long), shape, 1000 seed weight and presence or absence of pearl spot.

Paramesh (1983) classified 24 soybean genotypes based on the number of seeds per pod and 100 seed weight. The seeds were grouped in to small, medium and large seeded groups based on test weight.

Tiwari *et al*. (1978) grouped twenty one soybean varieties based on 100 seed weight into three groups *viz.*, small (10-15.5g), medium (16-19.5g) and bold (20g).

Mikhailov and Travyanko (1987) revealed that inheritance of seed coat colour in soybean is due to dominant genes.

Agarwal and Pawar (1990) revealed that soybean varieties could be distinguished from each other on the basis of morphological characters of seed *viz.*, seed size (bold , medium, small), seed coat colour (black and yellow).

Twenty two pea cultivars were grouped based on their variation in seed size (small, medium and large) seed colour (white, pale green, mottled green and grey) and seed shape (round and flattened) (Anon., 1992).

Morphological and biochemical characters *viz.*, seed colour, anthocyanin pigmentation of seedling and electrophoresis of seed esterase could be used to identify five sorghum hybrids and their parental lines (Anon., 1998).

Ponnuswamy *et al*. (2003) reported that 22 cotton genotypes showed wider variation for seed and seedling characters *viz.*, seed colour, seed shape, seed size, fuzzy nature of seed, 100 seed weight, seedling pigmentation, pigmentation of leaf petiole, number of gossypol glands and angle of petiole that makes with them.

Arunkumar *et al*. (2004) reported that 45 cultivars of pearl millet including fourteen hybrids and their parental lines were characterized using qualitative morphological characters of seed (seed colour as green, gray brown and brown), seedling characters and plant morphological characters.

c. Seedling characters

Seedling character like hypocotyl pigmentation and seedling pubescence were made use in varietal identification.

Hutchinson and Ramaiah (1938) gave the detailed description of plant characters, fuzzy nature and colour of the cotton genotypes.

Lirinde (1986) and Terao (1986) classified the rice genotypes on seedling characters *viz.*, seedling length, coleoptile and sheath colour and mesocotyl colour.

Chakrabarthy and Agarwal (1989a) developed seed keys for identification of 16 blackgram genotypes using seedling characters like pigmentation (strong, moderate, weak), stem hairiness (glabrous and pubescent), leaflet shape (lanceolate and ovate), hypocotyl and radicle length.

Venkata Reddy (1991) reported that soybean shoot length, root length and seedling length was used as criteria for distinguishing genotypes under laboratory condition.

Presence of anthocyanin pigmentation on seedling could be used to identify sorghum hybrids and their parental lines (Anon., 1998).

Ravikumar (1999) observed the differences in shoot length, root length and seedling length as criteria for distinguishing genotypes under laboratory condition in soybean varieties.

Ponnuswamy *et al.* (2003) reported that 22 cotton genotypes showed wider variation for seed and seedling characters *viz.,* seed colour, seed shape, seed size, and fuzzy nature of seed, 100 seed weight, seedling pigmentation and pigmentation of leaf petiole, number of gossypol glands and angle of petiole.

Bonow *et al.* (2007) studied the efficiency of morphological markers for the identification of commercial rice cultivars. They revealed that the morphological descriptors were useful, but not enough to characterize and distinguish the upland rice cultivars. Seeds and plants morphological characteristics after anthesis were most suitable for the characterization and discription of the cultivars.

d. Morphological characters of Plant

Rosta (1975) grouped the rice varieties based on leaf blade length, width of leaf, leaf colour, colour of ligule, colour of auricle and colour of flowers.

Agarwal (1984) classified cotton genotypes using field parameters, such as leaf colour, shape, hairiness and stem characters and flower characters.

Chaudhary *et al.* (1977) observed phenotypic variability for the number of days to initial flowering, number of branches per plant and plant height in sesame crop.

Gupta and Gupta (1977) also observed differences between 34 varieties of sesame for characters like 1000 seed weight, plant height, capsules per plant, seeds per capsule and capsule length.

Shahi and Pandey (1981) evaluated 21 soybean varieties and grouped on the basis of nature of maturity and number of days to 50 per cent flowering.

Chakrabarthy and Agarwal (1989b) suggested that morphological characters such as stem pigmentation (weak, strong, moderate) hairiness, leaflet shape (lanceolate, obovate) and cotyledonary leaf shape could be used for classifying black gram varieties.

Reddy *et al.* (1989) recorded variation in five soybean cultivars for days to maturity, period from flower initiation to maturity, plant height, seeds per plant and yield per plant.

Boerma *et al.* (1990) classified soybean cultivar of Colquitt, Gorden and Thomas based on morphological characters. Colquitt had determinate growth habit, purple flower, and tawny pubescence on pod and delayed or early maturity compared to other varieties.

Nickell and Thomas (1990) compared soybean cultivars of LN83-2356 with Morgan. LN83-2356 had purple flower, brown pubescence on stem, 3 days early maturity and 8 cm shorter than Morgan.

Muralikrishna *et al.* (1990) screened the cotton varieties based on morphological characters *viz.*, pigmentation, gossypol glands and plant height.

Ashwanikumar *et al.* (1993) also studied varietal identification of six pearl millet varieties through morphological characters and presented list of key characters on the basis of seed shape, leaf characters and head shape, *etc.*

Morphological characters *viz.*, pigmentation trichomes, gossypol glands and plant height at 21 days were used by Muralikrishna *et al*. (1992) to determine the genetic purity of cotton hybrid (H-8) and its male parent (small dwarf) and female parent (G-cot-10).

Rajendra kumar (1992) characterized four hybrids and their parental lines of sunflower genotypes based on morphological traits. The variability exits for various characters among sunflower genotypes gave ample opportunity to generate identification of keys for existing hybrids and their parental lines at different stages of plant growth to conform the genetic purity standards.

Jawaharlal (1994) differentiated cotton genotypes using field parameters such as leaf colour, leaf shape, hairiness on the leaf and stem, leaf nectaries, flower characters, petal colour, pollen colour, petal spot, boll size boll shape and leaf petiole pigmentation.

Jayaramaiah *et al.* (1995) reported that days to 50 per cent flowering, days to maturity, plant height, stem girth, head diameter were found useful in characterization of sunflower genotypes.

Mudzana *et al.* (1995) reported that the morphological characters such as plant height, foliage colour, number of days to 50 per cent flowering, flower length, pod length, pod breadth and number of seeds could be used for variety discription of faba beans.

Bonetti *et al*., (1995) reported that 17 bean cultivars were grouped based on leaf colour (very light green, light green, medium green, dark green and very dark green) pod length (very short, medium, long and very long), maturity (early, medium and late) and time of flowering (early, medium and late).

Sunflower genotypes were characterized and grouped based on morphological characters like stem and petiole pigmentation, leaf shape, head shape and seed thickness. It is possible to group the varieties effectively by using these characters. Sunflower genotypes can also be successfully grouped based on bract shape and leaf shape which were found to be stable characters (Anon., 1996).

Patil *et al*. (1996) studied the sunflower genotypes and found that leaf area, harvest index, number of filled seeds and per cent autogamy showed higher differences among genotypes and the characters like days to 50 per cent flowering, days to maturity and number of leaves did not differ significantly.

Ezhilkumar (1999) characterized cotton genotypes based on stem hairiness, angle of petiole, pigmentation on petiole, bract size, petal colour and leaf lobes and these characters showed clear cut variation among the genotypes.

Miljanovic *et al*. (2000) assessed morphological variability for *Helianthus* species and reported that the characters such as number of leaves per stem, length of bracts, the length of ray flower and angle between lateral veins and mid rib were found to be useful in characterizing the two *Helianthus* species *viz*., *Helianthus gigantons and helianthus maximiliani.*

Roy and Mishra (2000) reported that plant height, leaf width and number of leaves showed higher genetic variability in full sib families, whereas the estimates of genetic variance for days to first flower, days to 50 per cent flowering, days to maturity, head diameter, leaf length, 100 seed weight and seed yield per plant were higher in half sib families of sunflower.

Jayalakshmi *et al*. (2001) characterized 29 sunflower hybrids based on head diameter, 100 seed weight and seed yield and the range they observed for these parameters varied from 11.0 to 19.9 cm, 3.1 to 7.4 g and 647 to 2788 kg/ha, respectively.

Manjula *et al.* (2001) observed significant differences among the sunflower genotypes based on plant height, days to first flowering, days to 50 per cent flowering and days to maturity.

Muralidharan and Manivannan (2001) characterized 19 groundnut genotypes based on number of flowers, number of primary and secondary branches, kernel weight and kernel shape. The bold kernel varieties had distinct morphological differences for other quantitative characters.

Sankarpandian (2002) reported that four cowpea varieties were grouped based on pod shape, seed colour and leaf shape.

Upadhyaya *et al.,* (2002) evaluated 1956 chick pea accessions for flower colour (white and light flower), plant colour and seed colour (orange and yellow orange).

Yadav and Shrivasthava (2002) characterized chickpea varieties based on seed colour (brown, light brown, dark brown, reddish brown, salmon white and green), seed size (bold, medium and small), stem pigmentation (strong, medium and absent), flower colour (white, deep pink, pink and light pink), foliage colour (dark green, green and light green), plant height (tall, medium and dwarf), poding habit (single and double), pod number per plant (low, medium and high), number of locules per pod (one, two and three) and duration (early, medium and late).

Rajendra Prasad *et al.* (2003) characterized the 10 sunflower varieties, hybrids and their parental lines based on the leaf petiole pigmentation (absent and present), stem

pigmentation (weak, medium and dense), number of leaves per plant (low, medium and high), days to 50 percent flowering (early, medium and late) and seed characteristics.

Murali Mohan Reddy *et al.* (2004) characterized the castor genotypes based on the seed colour (white, maroon, brown, dark chocolate and black), 100 seed weight (low, medium and high), capsule dehiscence (non dehiscent, partially dehiscent and dehiscent), number of nodes on main stem (low, medium, high and very high), leaf shape, leaf colour, leaf length (small, medium and large) and other morphological characteristics.

Tarasatyavathi *et al.* (2004) reported that 75 soybean varieties were characterized based on leaf shape (lanceolate, pointed ovate, round ovate and triangular), leaf colour intensity (light, medium and dark), flower colour (white and violet), pod pubescence (absent and present), plant height (short, medium and tall), days to flowering (early, medium and late) and days to maturity (early, medium and late). Mate and Shelar (2006) characterized 16 sorghum hybrids based on plant height (very short and very tall), days 50 per cent flowering (very early and late) anther colour, stigma anthocyanin, colour of straw and glume colour at maturity.

Monica *et al*. (2007) classified 19 varieties of rice belonging to non basmati groups based on fifty two morphological descriptors at different stages of plant growth. Though the cultivars showed overlapping of descriptor expression in various combination traits, still the identity of all the cultivars in non-basmati as well as basmati group could be established individually and would be helpful for registration/ protection of these varieties.

Anon (2008) characterized two cotton hybrids based on leaf colour, days to 50 per cent flowering, petal colour, filament colouration, boll colour and boll shape.

e. Genetic purity

Smith and Smith (1989) collected the morphological data for 57 traits in 31 inbred lines of the US Corn Belt at three locations over two years. Repeatability were highest for the most tassel and ear characters with some vegetative traits also having high repeatability. They suggested that morphological characters that have high repeatability can form reliable descriptors of a phenotype.

Negut and Sarca (1994) reported that the percentage of maternal selfed and out crossed plants in seed lots of hybrid varieties with different contamination levels were estimated by electrophoretic analysis of eight-enzyme system in coleoptile tissue of five-day-old seedlings and by morphological analysis of plants grown out in plots. Field determinations of contamination were performed at the seedling stage, anthesis, and maturity.

There were small differences between electrophoretic and morphological analysis at high contamination levels (the former indicating two ó three per cent greater contamination) and results suggested that data on field-grown plants should be taken at maturity. Among the advantages of electrophoresis over the use of morphological traits were rapidity of the method, ability to determine contamination percentage before harvest and the independence of the method from environmental interactions.

J. Ininda et al *(2005)* studied genetic diversity for gray leaf spot resistance in maize using SSR markers. Forty-one genotypes comprising of collections from Kenya, CIMMYT, IITA and South Africa were identified to be resistant to gray leaf spot. The genotypes were analysed for variability by using twenty-eight microsatellite markers covering the entire maize genome. The results indicated diversity among lines for selected markers.

Daniel et al. (2012) set an experiment to determine genetic purity of commercial hybrids and their inbred lines using SSR markers. Seedlings of four F1 hybrids and four inbred lines were grown in the screen house of IITA for DNA extraction using Dellaporta method with some modifications. Six Simple Sequence Repeat (SSR) markers were used for Polymerase Chain Reaction (PCR) using Touch-Down PCR profile. Genetic purity level of inbred lines ranged between 91.3% and 98.7% while the hybrids ranged between 81.3% and 95%. SSR markers were found powerful biotechnological tool capable of detecting genetic purity status of Nigerian maize hybrids.

Saxena et al (2010) carried out molecular characterization of hybrids with the help of 148 simple sequence repeat (SSR) markers, including 32 novel markers reported here for the first time, on 159 A (cytoplasmic male sterile), B (maintainer) and R (fertility restorer) lines. In total, 41 (27.7%) markers showed polymorphism with 2 to 6 (average 2.6) alleles and 0.01 to 0.81 (average 0.34) polymorphism information content (PIC) value. Of these polymorphic markers, 22 SSR markers showed polymorphism between A (ICPA 2039) and R (ICPR 2438) lines of the commercial hybrid (ICPH 2438); however, only 21 of these SSR markers showed the same profile between A (ICPA 2039) and B (ICPB 2039) lines. Finally, two SSR markers, CCB4 and CCttc006, were found most suitable for purity assessment of hybrid seeds of the ICPH 2438 hybrid. It was anticipated that molecular diversity information generated on parental lines of hybrids and identification of the two most suitable markers for testing the purity of hybrid seeds of ICPH 2438, would facilitate the pigeonpea hybrid breeding programme.

Yadav et al. (2010) evaluated a set of maize inbred lines to compare how morphological, physiological characterization and RAPD molecular marker described variety relationships. All the inbred lines were confirmed as morphologically and physiologically distinct. At morphological level the maximum genetic distance (10.8) and least genetic distance (1.6) were found. For physiological characters distance varied from 0.35 to 1.92 and results from dissimilarity matrix. RAPD based distances varied from 0.42 to 0.65. The molecular markers exposed useful genetic diversity and appeared to disperse the lines somewhat more evenly over the plot than the morphological and physiological methods.

Yang et al (2011) studied 82 SSRs and 884 SNPs with minor allele frequencies (MAF) over 0.20 were used to compare their ability to assess population structure, principal component analysis (PCA) and relative kinship in a maize association panel consisting of 154 inbred lines. Compared to SNPs, SSRs provided more information on genetic diversity. The expected heterozygosity (He) of SSRs and SNPs averaged 0.65 and 0.44, and the polymorphic information content of these two markers was 0.61 and 0.34 in this panel, respectively. Additionally, SSRs performed better at clustering all lines into groups using STRUCTURE and PCA approaches, and estimating relative kinship. For both marker systems, the same clusters were observed based on PCA and the first two eigenvectors accounted for similar percentage of genetic variations in this panel. The correlation coefficients of each eigenvector from SSRs and SNPs decreased sharply when the eigenvector varied from 1 to 3, but kept around 0 when the eigenvector were over 3. The kinship estimates based on SSRs and SNPs were moderately correlated ($r^2 = 0.69$).

Shiri (2014) on a set of maize inbreds identified 40 simple sequence repeat alleles with a mean of 3.33 alleles per locus. Polymorphism information content (PIC) of the 12 SSR loci ranged from 0.23 (Phi080) to 0.79 (UMC2359), with a mean PIC of 0.53. The analysis also led to identification of informative SSR markers, namely UMC1862 (bin 1.11), UMC1719 (bin 4.10-4.11), UMC1447 (bin 5.03), UMC2359 (bin 9.07) and UMC1432 (bin 10.02), which significantly contributed to the differentiation of the drought tolerant and susceptible genotypes analyzed in the study.

f. ***Genetic purity testing of inbreds and hybrids:***

Xin et al(2013) used nine SSR primer pairs to standardize the protocol for carrying out genetic purity analysis of four maize hybrids. Polymorphic SSR loci were found that could be applied to test the purities of these four hybrids. Authors claimed that a procedure featuring inexpensive equipment and ease of use was set up for testing maize hybrid purity

using SSR markers. The feasibility of applying SSR markers to maize hybrid purity using this procedure was discussed.

Rom *et al.* (1995) tested genetic purity of three commercial tomato hybrids and their parental lines using RAPD markers. Only four out of 50 RAPD primers tested, were found polymorphic and were useful in seed purity control and cultivar identification.

Paran *et al.* (1995) screened 160 arbitrary 10-mer oligonucleotide primers for polymorphism between the four pairs of parents. Thirteen primers amplified polymorphic bands between the pairs of parents, of which three detected bands were specific to the male parents and were useful in distinguishing a true hybrid from female parent.

Liu *et al.* (2000) assessed genetic purity of F1 rape hybrid seeds using RAPD markers. Out of 40 primers, six primers generated polymorphic products, of which a fragment at 510 bp was unique to the female line and a fragment at 260bp was unique to the male line. Both the fragments appeared in F1 hybrids.

Pendser *et al*. (2001) used RAPD markers for identifying cotton (*Gossypium hirsutum* hybrid at the seed stage. Out of 160 arbitrary primers, five random primers showed polymorphism between the inbreds. The results obtained from RAPD for different hybrid seed lots agree with the grow out test results.

Rao et al (2001) screened seven hybrids and their A, B, R lines with thirty one arbitrary primers to identify suitable primers for genetic purity assessment. A combination of four primers (OPA-4, OPA-18, OPA-12 and OPB-18) were useful in differentiating all 24 genotypes. RAPD analysis also established hybridity of the four hybrids.

Yadav *et al*. (2001) assessed genetic purity in DCH-32 cotton hybrid and their parental lines using random amplified polymorphic DNA (RAPD) analysis. The results obtained from RAPD for different hybrid seed lots of DCH-32 agreed with the field based grow out test and indicated that the RAPD method might be an alternative to the time consuming grow out test.

Srivastava *et al* (2003) studied the inter-varietal variation and maintenance of purity in the parental lines of pearl millet hybrid PHB-47 (Pb111A female parent and P1I 1234 restorer) by using RAPD markers. The DNA profile of male sterile plants and dendrogram showed homology of 70 per cent, indicating high heterozygosity, where as restorer plant and bulk indicated genetic similarity of 81 per cent.

Ilbi (2003) conducted genetic purity test in five pepper hybrids and their parents and concluded that, out of 12 primers, six primers generated 11 useful bands to determine seed purity of all tested hybrids.

Sonti *et al* (2003) assessed genetic purity in rice hybrid using 13 SSR and 50 RAPD primers. Both SSR and RAPD markers produced polymorphic bands between parental lines.

Ilbi *et al* (2004) used RAPD marker as a tool for testing hybrid purity in tomato varieties. Four F1 tomato hybrids and their parents were analyzed with eight 10-mer primers. Out of the primers tested, five primers generated useful RAPD markers to determine the hybrid seed purity of three varieties, the remaining showed monomorphic amplification across all genotypes.

Mongkolporn *et al.* (2004) determined genetic purity in three chilli hybrids using two marker techniques, RAPD and ISSR. They concluded that RAPD analysis successfully detected all three hybridity, while ISSR denoted only two hybridity.

Rana *et al* (2006) assessed genetic purity and diversity of eight cotton hybrids and their parental lines using PCR based molecular marker technique. They reported that a narrow range of genetic diversity was observed in the hybrid and parents and no polymorphic male parent specific RAPD or ISSR marker was useful for determining genetic purity of the hybrids HHH 223 and Kirti. Only two polymorphic ISSR markers were observed, one each for Hybrid 4 and Mallika.

Vamadevaiah *et al.* (2006) with the help of 120 RAPD primers , analysed the DHH-11 intra hirsutum cotton hybrid (*Gossypium hirsutum*) and its parents. Eleven markers showed polymorphism between the male (CPD-420) and female (CPD-423) parents. The primer OPC-17 produced a 700-bp amplicon in DHH-11 and was found to be reproducible marker to evaluate the genetic purity of DHH-11 in place of grow out test.

Andreoli *et al.* (2006) studied genetic contamination in hybrid corn (*Zea mays* L.) using RAPD marker. The RAPD product consisted of 456 bands, of which eight were polymorphic. Primers OPN-04 and OPR-19 were able to discriminate the contaminants among the pure seed hybrids and their parents.

Liu *et al.* (2007) determined the genetic purity of cabbage cultivars with RAPD, ISSR and SSR markers. Two RAPD, two ISSR and one SSR primer produced male and female parent specific markers. Of the 228 hybrid seeds tested eight appeared to be selfed and off type plants. The results were in high accordance with those from field grow out trials (GOT).

Liu *et al.,* 2007 used RAPD, ISSR and SSR to test seed genetic purity of two commercial hybrid tomato cultivars. Among the 321 primers, four primers for Hezuo 903 and three for Sufen No.8 hybrid which could produce both female and male parent specific markers were selected for testing the genetic purity. The combined results of the marker analysis showed that eight of 210 plants in Hezuo 903 and 13 of 210 plants in Sufen No.8 were false hybrids and overall genetic purity of the two hybrid lots was 96.2 and 93.8 per cent respectively.

Rana *et al.* (2006) assessed genetic purity in eight commercial cotton hybrids and their parental lines using RAPD, ISSR and STMS markers. No polymorphic male parent specific RAPD and ISSR marker was useful for determining genetic purity of HHH-223 hybrid. Only two polymorphic ISSR markers were observed in Hybrid 4 and Mallika. Co-dominant STMS marker produced polymorphic bands in all the hybrids, except for the diploid hybrid AAH-1.

Asif et al., 2006 conducted RAPD based genotyping for hybrid identification in maize (*Zea mays* L.). A total of 40 random primers were surveyed of which, OPR-03, OPR-11 and OPT-06 were found polymorphic and were also able to distinguish among different hybrids. These three polymorphic primers produced unique banding patterns that clearly detect the purity of the hybrid lots.

3. Materials and methods

The present investigation was carried out having three broad objectives as mentioned in the previous chapter. Brief description about the location where the work was carried out is given below:

3.1. Brief description of the experimental site

The experimental materials were planted at Experimental Farm, Division of Genetics and Plant Breeding. The trials were planted during *kharif* season of the year 2011. The marker analysis was carried out at Centre for Biotechnology, Division of Genetics and Plant Breeding, Shalimar Campus, SKUAST-Kashmir. Shalimar Campus is located on geographic coordinates of 34°E and 74°N at an altitude of 1580 m amsl.

The methodology adopted under each objective is described as follows:

3.2. Plant materials:

Materials comprised of six inbreds and five single cross hybrids of maize (*Zea mays* L.) listed as under:

Table 3.1. Plant materials used in present study

S No	Inbreds/Hyrids
1	KDM-361A
2	KDM-343A
3	KDM-332A
4	KDM-914A
5	KDM-362A
6	KDM-916A
7	CML-128
8	KDM-361A X KDM-343A
9	KDM-332A X CML-128
10	KDM-914A X KDM-362A
11	KDM-916A X CML-128
12	KDM-332A X KDM-916A

3.3. Description of agronomic traits recorded:

The inbreds and hybrids were evaluated for plant height, ear height, tassel length, ears per plant, days to tassel emergence, days to silk emergence, days to maturity, ear length, ear girth, number of rows per ear, number of grains per row, number of grains per ear, grain yield per plant. The description of these traits is given as follows:

Plant height: Plant height was measured from the ground level to the tip of tassel and was expressed in centimetres (cm).

Ear height: Ear height was measured from the ground level to axial node of lower borne ear and was expressed in centimetres (cm).

Ears per plant: The number of ears borne on a plant.

100-grain weight: Total of 100 uniform grains were counted from 5 plants per genotype and weighed on electronic balance. This was recorded in grams (g).

Days to 50% tassel emergence: The number of days from sowing till emergence of tassel on half of the plants. This was recorded on plot basis.

Days to 50% silk emergence: The number of days from sowing till emergence of silk on ears of half of the plants. This was recorded on plot basis.

Days to maturity: The number of days from sowing till 80% of the plants had straw husk. This was recorded on plot basis.

Grain yield: Grain harvest from a plant was dried to optimum moisture levels and weighed on electronic balance. This was expressed as grams (g) per plant.

Ear length: Length from base of an ear upto tip. Measured on single primary ear on each of five plants per genotype and was expresses in centimetres (cm).

Ear girth: Girth at middle of the primary ear and was expresses in centimetres (cm).

Number of grain rows per ear: Recorded on single primary ear on each of five plants per genotype.

Number of grains per row: Recorded on single primary ear on each of five plants per genotype.

3.4. Attribute or parametric traits: Total of thirty one morphological traits were studied as per Maize descriptor (DMR, New Delhi).

3.5. Marker analysis:

3.5.1. Isolation of plant genomic DNA: The extraction of plant DNA was carried out by CTAB (Cetyl- Tri Methyl Ammonium Bromide) method as described by Murray and Thompson (1980).

The genomic DNA was extracted from inbreds and single cross hybrids. Fresh, healthy and young leaf tissue from 50-60 day old seedlings were harvested and stored in minus 30^oC until used for DNA extraction.

1. The leaf samples were ground to fine powder in liquid nitrogen using sterile, pre-cooled mortars and pestles.
2. The powder was transferred to a 2 ml polypropylene tubes containing 1 ml of pre-warmed (65^oC) DNA extraction buffer.
3. Suspension was incubated at 65^oC for 1 hr. with intermittent shaking at 15 minutes interval.
4. The tubes were mounted on stands and left to cool down at room temperature, after which almost equal volume of chloroform: isoamyl alcohol (24:1) was added using a micro-pipette. Solution was mixed by gentle inversion of tubes for 5 minutes.
5. An equal volume of chloroform: isoamyl alcohol (24:1) was added using a micro-pippette in order to remove the phenols and other non-aqueous substances like lipids and proteins.
6. The content was then centrifuged at 10000 rpm for 10 minutes at 4^oC. The supernatant was carefully transferred to a fresh tube with wide bore tip to avoid DNA shearing.
7. Again equal volume of chloroform: isoamyl alcohol (24:1) was added to perform second wash. The previous step was repeated.
8. Afterwards, pre-chilled iso-propanol 0.6 volume of the content in tube was added followed by gentle inversion until fibrous mass was visible. Tubes were incubated at ó 20 ^0C for 1 hour.
9. Tubes were then put to centrifugation at 5000 rpm for 10 minutes at 4^oC to obtain a precipitate. The supernatant was drained by gently inverting the tubes. The tubes were left inverted with lids open on blotting paper to drain the residual iso-propanol.
10. After a while, the DNA pellet was washed twice with 70% ethanol, and kept overnight at room temperature for drying the pellet.
11. 100-200 μl of TE buffer (pH 8.0) was added to dissolve the pellet.

12. After 6-8 h RNase (10 mg/ml) was added to the DNA voil @ of 2 µl/100 ul of crude DNA. Mixture was incubated in a water-bath for 1 hour at 37°C with intermittent mixing. Sometimes it was preferred to add RNase at iso-propanol precipitation step.

13. Purification of DNA was performed by adding an equal volume of Chloroform: isoamyl alcohol 24:1 to aqueous phase, mixed gently for 5 minutes and again centrifuged at 10000 rpm.

14. Aqueous phase was collected in a fresh tube to which 1/10th volume of 3M Sodium-Acetate (pH5.2) was added, mixed well and then two volumes of chilled ethanol was added to the mixture, mixed gently and incubated at ó20°C for 2 h.

15. The tubes were centrifuged for 5 minutes at 10000 rpm. The supernatant was discarded and pellet washed twice with 70% ethanol. Pellets was dried properly (overnight air drying) and dissolved in 100µl TE buffer (pH 8.0).

16. DNA was quantified using gel quantification method. The 2 µl samples were loaded on 0.8% agarose gel in 1x TAE buffer and using diluted λ uncut genomic DNA as standard in parallel well. The intensity of individual samples was compared with a range of known amount of λ DNA (25, 50, 75 and 100 ng). According to the concentration of DNA, samples were diluted, and again loaded on 0.8% gels till all of the samples finally reached at ~25 ng/ µl in a uniform manner.

3.5.2. Polymerase Chain Reaction

PCR assay was performed for foreground selection using gene linked markers and background selection by using genome wide markers. PCR reaction mix contained 25 ng of DNA, 10x PCR buffer (10 mM Tris, pH 8.4, 50 mM KCl, 1.8 mM $MgCl_2$), 2 mM dNTPs (MBI, Fermentas, Lithuania, USA), 5 pmol each of forward and reverse primer and 3 U/ µl of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangaluru, India) in a reaction volume of 10 l (Tables 3.2, 3.3).

Table 3.2. The PCR reaction mixture

Reagent	Stock conc.	Aliquot	Final conc.
DNA	25 ng/µl	1 µl	25 ng
PCR buffer	10x	1 µl	1x
dNTP mix	2mM	1 µl	0.2mM
Forward Primer	5 pM	0.5 µl	
Reverse Primer	5 pM	0.5 µl	
Taq DNA polymerase:	3 U/µl	0.2 µl	0.6 U
Milli-Q water	-	5.8 µl	-
Total		10 µl	-

Polymerase chain reaction (PCR) was performed in a thermal cycler (Eppendorf, Hamburg, Germany) with following thermal regimes:

Table 3.3. PCR thermal regimes and conditions

Step	Reaction	Temperature	Time	Cycle
I	Initial denaturation	94°C	4	1
II	Denaturation	94°C	1	
	Annealing	55°C	1	35
	Extension	72°C	2	
III	Final extension	72°C	7	1

3.5.3. Resolution of amplified PCR products: Gel electrophoresis is a widely used technique for the analysis of nucleic acids and proteins. Here we used the technique for size separation of amplified DNA. A concentration of 3.5% (w/v) agarose gel was prepared by dissolving 7 g of weighed Agarose powder in 200 ml of 1xTAE [196 ml double distilled water + 4 ml of 50x TAE buffer (242.2g Tris base: Mwt. 121.14; 100 ml of 0.5M EDTA: PH 8.0; 57.1ml Glacial

acetic acid: Mwt. 61.83; make vol. to 1000 ml using de-ionized Milli-Q water)] in a conical flask. The suspension was heated in microwave oven for 6 minutes at 600 watt till clear solution was obtained. The solution was allowed to cool down and to this was added 8 µl (0.05 µl/ml of 1x TAE) of Ethidium Bromide stock solution (10mg/ml of double distilled water). After gentle shaking, the gel was poured onto gel casting tray. After 15-20 minutes the gel was immersed inside the gel tank filled with 1x TAE (PH 8.0). To each PCR product of 10 µl was added 1 µl 6x loading dye (0.25% bromophenol blue; 0.25% xylene cyanol FF; 40% sucrose). With the help of 10 µl pipettes samples were loaded in individual wells. In parallel, was also loaded 50 bp size reference ladder (Fermentas, Lithuania, USA). The power pack was adjusted at 5 Volts/cm of run and the total duration of electrophoresis varied from 1.5 to 2.5 hours. After optimum run of samples the gel slabs were visualized under UV trans-illuminator and documented in gel documentation system (Bio-Rad Laboratories Inc., USA). This was followed by scoring of bands with the help of 50 bp DNA size standard (Fermentas, Lithuania, USA).

3.5.4. Markers used in hybrid purity analysis:

Forty five STMS markers were used for genotyping inbred parents and hybrids. The marker sequences were retrieved from www.maizegdb.org. (Table 3.4.).

Table 3.4. The list of SSR markers used for identification of polymorphic markers between parents and multi-locus profiling of hybrids

S No	Marker	Forward primer sequence	Reverse primer sequence	Bin location
1	Umc2383	CATAGACGTGCCCCTTGTCATC	CTCGCAACTGCGCTTCTAGATACT	1.02-1.03
2	Umc1664	AATTGTTTACTGCGCTGAAACTCC	CCTCTTTGCCTGTACCGTGTATTC	1.06
3	Umc1147	GAGAAACCATCGACCCTTCCTAAC	TTCCTATGGTACAGTTCTCCCTCG	1.07
4	Umc2100	AAAGGCATTATGCTCACGTTGATT	TGACGTGCAAACAACCTTCATTAC	1.12
5	Umc2245	GCCCTGTTATTGGAACAGTTTACG	CGTCGTCTTCGACATGTACTTCAC	2.01
6	Umc1696	CTAGGGTTTAACCAACGGGGAG	TAAGGAGAGGGTCGATGAACACAT	2.1
7	Umc1823	AAAGCCTTACTGTTATTAGGCTAGGCA	AGAAAACCAGCCCCAGATGTTC	2.03
8	Umc1026	TCGTCGTCTCCAATCATACGTG	GCTACACGATACCATGGCGTTT	2.04
9	Umc2372	ACCCCTTGCGTTCTCTTCTGTT	CACCAGGCGTAGTGAGACAGC	2.06
10	Umc2144	CCAGCCCCTATCTATTTGCTTGT	GAATACTATATCACGGTCGGTCGG	2.08
11	Umc1594	GCCAGGGGAGAAATAAAATAAAGC	CACTGCAGGCCACACATACATA	3.09-3.1
12	Umc2071	ACTGATGGTGTTCTTGGGTGTTTT	ATACACGCAGTTACCCGAAGGTT	3.01
13	Umc2369	TTCGTCTGATGAAAGGTTCAGAGG	GATCCTCATCAAGACCAGCAGAGT	3.02-3.03
14	Umc1644	CCATAAACTGTTCCTTTGGCACAC	CTTTCACGTGTTAAGGGAGACACC	3.06
15	Bnlg1890	ACCGGAACAGACGAGCTCTA	GTCCTGCAAAGCAACCTAGC	4.11

16	Bnlg1621	CTCTTCGATCTTTAAGAGAGAGAGAG	ACACGAGGCACTGGTACTAACG	4.06
17	Umc1478	GAAGCTTCTCCTCTCGCGTCTC	CAGTCCCAGACCCTAGCTCAGTC	5.01
18	Umc1800	TTATGGGTGCTGGTGATGTGTATC	GAAAAGCAATCGCTTCTGAGAAAA	5.05
19	Umc2136	CCAGATGCGGAAGTAGACGG	GATTCGGAGGTGATCTGACCTGT	5.08
20	Umc1766	ACAAGAAGGAATCGAGAGCAAATG	CTTCGGGATGGAGTCGTAGTTC	5.01
21	Bnlg1306	CACCTTGAAAGCATCCTCGT	CAAAAACAAATGGCAGCTGA	5.07
22	Umc1918	CACAAGAACATTATGACGACCGAG	AAGCAGGAGACATCGTTTAAGTCG	6.03
23	Umc1762	CTTACTCCAGGCACTCCATACCAT	ATCCAGGTGAATGGTGTTTACGAT	6.06
24	Umc1063	AGGCCACTGAGCAGGTGAAG	GTGATGGTAGAGGAGTCCTTGGTG	6.07
25	Umc1018	GAACGGATATTGGAACCTGTGC	GTGCACGGTGTCGTACTTGAAC	6.01
26	Phi452693	CAAGTGCTCCGAGATCTTCCA	CGCGAACATATTCAGAAGTTTG	6.04
27	Umc1424	CCGGCTGCAGGGGTAGTAGTAG	ATGGTCAGGGGCTACGAGGAG	6.06
28	Phi129	GTCGCCATACAAGCAGAAGTCCA	TCCAGGATGGGTGTCTCATAAAACTC	6.05
29	Umc1002	AGCTAGCTATACACCGCCAGG	TCAGTTTGGAACAGGGAAAAGTA	6
30	Phi051	GGCGAAAGCGAACGACAACAATCTT	CGACATCGTCAGATTATATTGCAGACCA	7.05
31	Umc1036	CTGCTGCTCAAGGAGATGGAGA	GACACACATGCACGAGCAGACT	7.02
32	Umc1708	GATATGTCGAGCTTCGCTGGAG	CGCACACTAAAGCATCCTTAACCT	7.04

33	Umc2392	CAGAGACCTCGACTTCGACCAC	CTTCTGCTTCTGCTCGACCTTCT	7.01
34	Bnlg1056	ATCGTTGTTGGGTACACGGT	ACGGGTAGTGGTGAAGATGC	8.08
35	Umc1141	AGAGGAGAAAGAGACAGACAGGCA	CAGGAACTGAATGAAAGCAACTCA	8.06
36	Umc1415	GTGAGATATATCCCCGCCTTCC	AGACTTCCTGAAGCTCGGTCCTA	8.04
37	Umc1786	ACCGTGACTTCCTCCTCATAACTG	CATTTTTCGCATTTAGGAAATCCA	8.01
38	Phi067	CTGCAAAGGTAAGCACTAGGATGCT	CATCATTGATCCGGGTGTCGCTTT	9.01
39	Phi061	GACGTAAGCCTAGCTCTGCCAT	AAACAAGAACGGCGGTGCTGATTC	9.03
40	Umc1310	AACTCCGAGATCTACGACAACAGC	GAGGAAGAGTTGGCCAGGATG	9.06
41	Umc1675	GTTCTTCCTCTTCCCCATCAGTCT	ATAGCTGCGCGTAAAGCAACC	9.07-08
42	Umc1640	ACTACACGGTGTGAGATGTGATCG	GTCGTCGCAAGAACAACAAGG	10.07
43	Umc1077	CAGCCACAGTGAGGCACATC	CAGAGACTCTCCATTATCCCTCCA	10.04
44	Mmc0501	TGCTGAACACTCTAAGCAATAC	ATTACTCTACTCGCTGCCTG	10.02
45	Bmc1655	ATTAAAATCTTGCTGATGGCG	TTCTGTTCCCGCCTGTACTT	10.03

3.5.5. Statistical Analysis:

3.5.5.1.ANOVA: A set of inbreds and parents was laid in Replicated Block Design with three replications. Analysis of variance was performed following Singh and Chowdhury (1985).

Table 3.5. Model of Analysis of Variance for agro-morphological traits in parents and hybrids

Source	d.f.	MS
Replication	r-1	
Genotype	g-1	
Error	(r-1) (g-1)	

3.5.5.2. Nei and Li (1996) coefficient of Similarity: was calculated according to following empirical formula:

=

Where, S: similarity coefficient between two hybrids; x: number of traits shared; p: total number of traits studied in one hybrid ; q: total number of traits studied in second hybrid.

4.1. Evaluation for grain yield and contributing traits

The hybrids and inbreds were evaluated for various agronomic and yield related traits (Tables 4.1, 4.2, Figs. 4.1, 4.2, 4.3).

4.1.1. Plant height: The mean values for plant height were recorded between 100 and 215 cm for inbreds CML-128 and KDM-916A, respectively. Among hybrids the plant height ranged from 166 cm for H3 to 240 cm H1. Hybrid H_1 was followed by hybrid H_4, H_2 and H_5. The inbreds recorded an average plant height of 125.29 cm against 205.70 cm across the five hybrids.

4.1.2. Days to tassel emergence: The range of variability across a set of inbreds and hybrids was recorded between 59 for KDM-343A to 67 KDM-914A DAS. Average of 61.93 and 60.73 DAS were recorded for inbreds and hybrids, respectively. The coefficient of variation recorded was 4.19%.

4.1.3. Days of silk emergence: The mean values ranged from 61.5 and 67 days within inbreds and from 63.5 to 64.5 within hybrids. The early silk emergence was recorded for CML-128 with 61.50 DAS while all the hybrids recorded around 64 days to emergence of silk. Low coefficient of variation (1.40%) was recorded for the trait.

4.1.4. Days to maturity: The range of variability was recorded from 126.5 to 136 days among inbreds with inbred KDM-361A recording 126.5 days and KDM-916A recording 136 days. The early maturity was shown by inbred KDM-361A with 126.5 days. Two inbreds KDM-914A and KDM-362A followed each other closely with 131 and 131.5 days while as KDM-332A the highest number of days with 135 and 136 recorded days. Hybrid H_2 recorded early maturity with 127 DAS while as Hybrid H3 with 137 days recorded maximum days to maturity.

4.1.5. Ear length: Longest ear length was recorded for inbred KDM-332A with 10.10 cm while the minimum ear length was recorded for CML-128 with 8.50 cm. Mean ear length for inbreds was 9.41 cm. Ear length ranged from 14.8 cm to 18.5cm for H2 and H1, respectively. Mean ear length for hybrids recorded at 16.74 cm.

4.1.6. Ears per plant: Among the inbreds the range of variability was recorded from 1.0 cm to 1.70 cm with the mean of 1.29 cm. KDM-332A and KDM-914A recorded highest number of ears per plant with 1.7 ears each followed by KDM-343A and KDM-362A both having 1.20 ears per plant. Hybrids H1 was found to be prolific with 2.10 ears per plant.

4.1.7. Kernel rows per ear: Kernel rows ranged from 8 for inbred CML-128 to 16.38 for H1. Inbreds recorded the mean of 9.86 versus hybrids with average value of 14.51. Hybrid H1 recorded 16.38 kernels rows per ear.

4.1.8. Kernel per row: KDM-332A recorded highest number of kernels per row (16.50) followed by KDM-362A with 16 kernels per row. KDM-361A and CML-128 both recorded 15.40 while as KDM-914A had 12.90. Overall inbreds showed the mean of 14.46. Among hybrids the range of variability was recorded between 34.00 to 40.90 for hybrid H2 and H4, respectively. The average value for hybrids was observed at 39.38 kernel rows per ear.

4.1.9. Ear dia: KDM-343A recorded 3.55 cms dia followed by KDM-361A and KDM-914A with an average ear dia of 3.10 cms. The range of variability was between 3.55 cms to 2.30. Lowest dia was recorded for KDM-362A & CML-128 while as 2.36 dia. Mean of 2.86 was recorded among all inbreds. Among Hybrid range of variability was between 5.51 to 3.88 for hybrid H1 & Hybid H2. Hybrid also recorded 5.29 cm followed by H3 with recorded dia of 4.83 cm. Mean of 4.70cm was recorded for hybrids.

4.1.10. 100 seed weight grams: Among inbreds highest test weight of 18 gm was recorded for KDM-914 A whereas KDM-332 A recorded 17gms seed weight per 100 grains. Lowest seed weight (14.5) was recorded for inbreds KDM-361 A and KDM-916 A. One inbred namely KDM-343A recorded 100 grain weight of 15 gms. Average grain weight among the inbreds was recorded at 15.86 gm. Hybrids recorded a range of 26.5 to 29.5 gm among hybrids with the mean of the 28.40 gm.

4.1.11. Grain yield /hectare: The mean grain yield per hectare ranged from 19.71 to 25.60 q/ha for CML-128 and KDM-362A, respectively. Grain yield in hybrids ranged from 54.16 to 78.84 q/ha with a mean of 65.08 q/ha. H1 recorded highest grain yield of 78.84 q/ha.

Table 4.1. Evaluation of parental lines and hybrids for yield and yield contributing traits

Entry	Plant height (cm)	Days to tassel emergence	Days to silk emergence	Days to maturity
KDM-361A	102.50	60.50	63.00	126.50
KDM-343A	108.50	59.00	63.00	130.50
KDM-332A	102.50	63.00	64.50	133.50
KDM-914A	128.50	67.00	67.00	131.00
KDM-362A	120.00	64.50	65.50	131.50
KDM-916A	215.00	60.50	63.00	136.00
CML-128	100.00	59.00	61.50	135.00
KDM-361A X KDM-343A	240.00	59.00	63.50	131.00
KDM-332A X CML-128	206.50	59.00	63.50	127.00
KDM-914A X KDM-362A	166.00	60.00	64.00	137.00
KDM-916A X CML-128	227.50	61.00	63.50	129.50
KDM-332A X KDM-916A	188.50	63.00	64.50	132.50
Sd	54.30	2.57	1.40	3.27
Mean	158.79	61.29	63.88	131.75
Min	100.00	59.00	61.50	126.50
Max	240.00	67.00	67.00	137.00
Mean Inbreds	125.29	61.93	63.93	132.00
Mean Hybrids	205.70	60.40	63.80	131.40
CV (%)	34.19	4.19	2.19	2.48

Fig.4.1. Evaluation of parental lines and hybrids for plant height and maturity traits

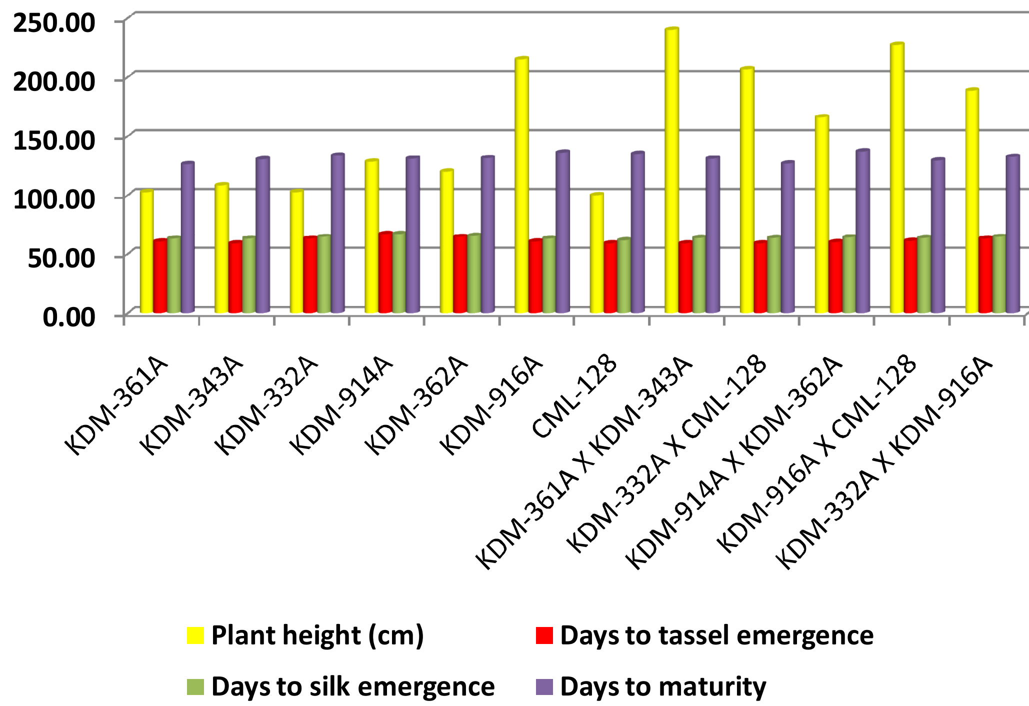


Table 4.2. Evaluation of parental lines and hybrids for ear traits and grain yield

Entry	Ear length (cm)	Ears/ plant	Kernel rows/ ear	Kernels/ row	Ear dia (cm)	100 seed weight (g)	Grain yield/ ha
KDM-361A	8.85	1.10	9.00	15.40	3.10	14.50	20.10
KDM-343A	9.70	1.20	11.00	12.50	3.55	15.00	20.63
KDM-332A	10.10	1.70	9.00	16.50	2.80	17.00	25.25
KDM-914A	9.40	1.70	11.00	12.90	3.10	18.00	25.54
KDM-362A	9.70	1.20	10.00	16.00	2.30	16.00	25.60
KDM-916A	9.60	1.10	11.00	12.50	2.85	14.50	19.94
CML-128	8.50	1.00	8.00	15.40	2.30	16.00	19.71
KDM-361A X KDM-343A	18.50	2.10	16.38	40.80	5.51	29.50	78.84
KDM-332A X CML-128	14.80	1.50	13.50	34.00	3.88	29.50	54.16
KDM-914A X KDM-362A	17.35	1.95	15.00	40.50	4.83	29.00	70.47
KDM-916A X CML-128	15.60	2.00	13.25	40.90	5.29	26.50	57.44
KDM-332A X KDM-916A	17.45	1.70	14.40	40.70	4.00	27.50	64.47
Standard deviation	3.90	0.39	2.67	13.03	1.10	6.58	22.88
Mean	12.46	1.52	11.79	24.84	3.63	21.08	40.18
Min	8.50	1.00	8.00	12.50	2.30	14.50	19.71
Max	18.50	2.10	16.38	40.90	5.51	29.50	78.84

Fig4.2. Evaluation of parental lines and hybrids for ear traits

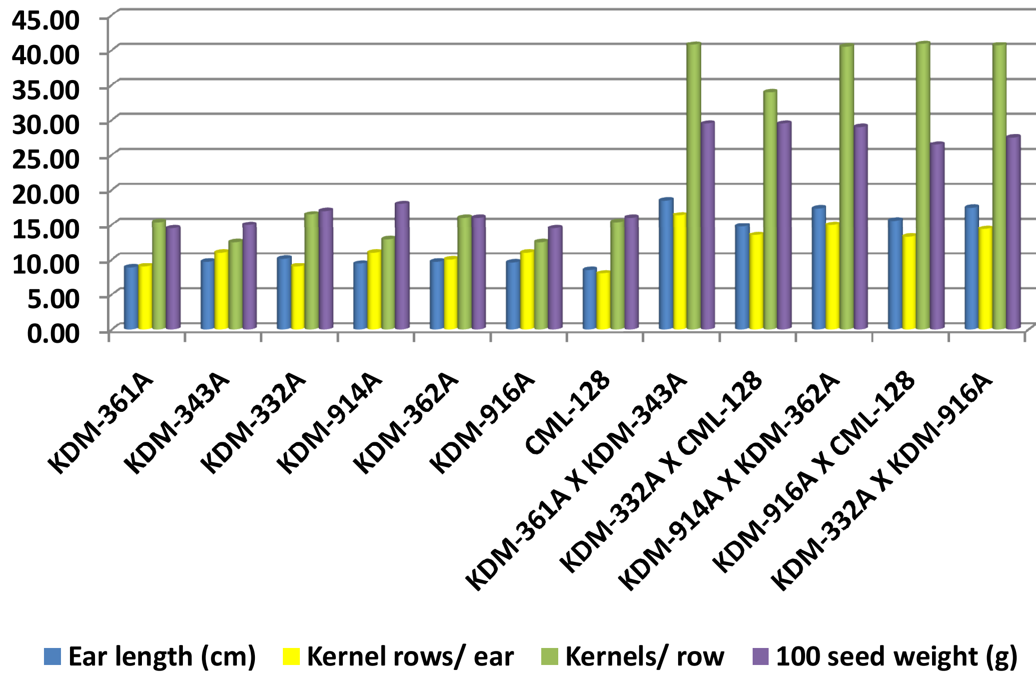
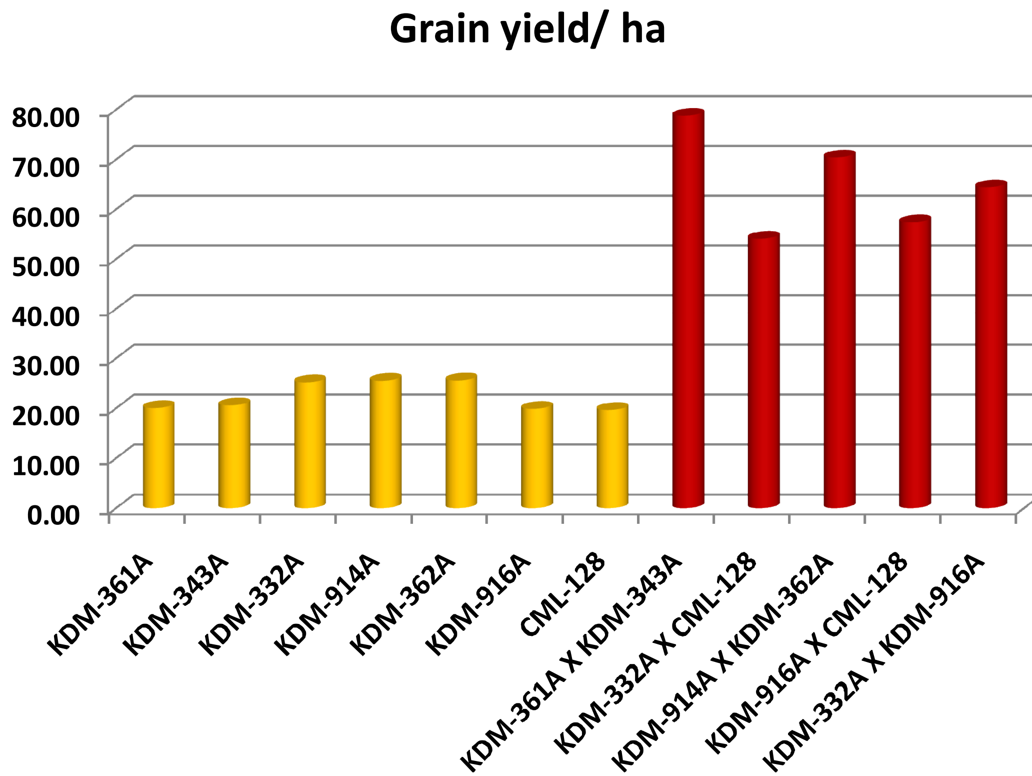


Fig.4.3. Evaluation of parental lines and hybrids for grain yield per hectare



4.2. Characterization of single cross hybrids for different morphological attributes

The five single cross hybrids were evaluated for 31 descriptive traits following (DMR, DUS descriptor) (Table 4.3). It was found that only nine out of 31 traits studied were polymorphic among hybrids and rest of the traits were completely uniform and monomorphic across all the five hybrids. Out of these nine polymorphic traits, only ÷Ear: anthocyanin colouration of glumes of cobø had three different classes, while rest eight were grouped in two contrasting phenotypes. The eight polymorphic traits included: Leaf: Altitude of blade, Tassel: Angle between main axis and lateral branches, Tassel: Altitude of lateral branches, Tassel: Length of main axis, Plant ear placement, Leaf: Width of blade, Ear: Length and Ear: color of top of grain. As many as eight out of 31 traits differed between H3 and H4 whereas, H1 and H4 differed with respect to only three traits, therefore, revealed high similarity (Table 4.4). The Nei and Liøs similarity index was devised among hybrids and ranged from 0.74 (H3 vs H4) to 0.90 (H1 vs H4) (Table 4.5).

Table4.3. Characterization of single cross hybrids for different morphological attributes

S No.	Character	KDM-361A X KDM-343A	KDM-332A X CML-128	KDM-914A X KDM-362A	KDM-916A X CML-128	KDM-332A X KDM-916A
1	Leaf angle between blade and stem (on leaf just above upper ear)	Small	Small	Small	Small	Small
2	Leaf : Altitude of blade (on leaf just above upper ear)	Straight	Straight	Drooping	Straight	Drooping
3	Stem : Anthocyanin colouration of brace roots	Absent	Absent	Absent	Absent	Absent
4	Tassel : Time of anthesis	Early	Early	Early	Early	Early
5	Tassel : Anthocuanin colouration of base of glume	Absent	Absent	Absent	Absent	Absent
6	Tassel : Anthocyanin colouration of glumes excluding base	Absent	Absent	Absent	Absent	Absent
7	Tassel : Anthocyanin colouration of anthers (in middle third of main axis on fresh anthers)	Absent	Absent	Absent	Absent	Absent
8	Tassel : Density of spikelets (in middle third of main axis)	Sparse	Sparse	Sparse	Sparse	Sparse
9	Tassel : Angle between main axis and lateral branches (in lower third of tassel)	Wide	Narrow	Narrow	Wide	Narrow
10	Tassel : Altitude of lateral branches (in lower third of tassel)	Curved	Straight	Straight	Curved	Straight

S No.	Character	KDM-361A X KDM-343A	KDM-332A X CML-128	KDM-914A X KDM-362A	KDM-916A X CML-128	KDM-332A X KDM-916A
11	Ear : Time of silk emergence	Early	Early	Early	Early	Early
12	Ear : Anthocyanin colouration of silks (on day of emergence)	Absent	Absent	Absent	Absent	Absent
13	Leaf Anthocyanin colouration of sheath (below the ear)	Absent	Absent	Absent	Absent	Absent
14	Tassel : Length of main axis above lowest side branch)	Medium	Medium	Medium	Long	Long
15	Plant ear placement	Medium	Low	Medium	Low	Low
16	Leaf : Width of blade (leaf of upper ear)	Broad	Medium	Broad	Broad	Medium
17	Ear : Length without husk	Long	Long	Long	Medium	Long
18	Ear. Diameter without husk (in middle)	Large	Large	Large	Large	Large
19	Ear : Shape	Conical	Conical	Conical	Conical	Conical
20	Ear : Number of rows of grains	Many	Many	Medium	Many	Many

S No.	Character	KDM-361A X KDM-343A	KDM-332A X CML-128	KDM-914A X KDM-362A	KDM-916A X CML-128	KDM-332A X KDM-916A
22	Ear : Type of grain (in middle third of ear)	Flint	Flint	Flint	Flint	Flint
23	Ear: color of top of grain	Yellow	Yellow with cap	Yellow with cap	Yellow	Yellow
24	Ear : anthocyanin colouration of glumes of cob	White	Dark purple	Light purple	White	Light purple
25	Kernel : row arrangement (middle of ear)	Straight	Straight	Straight	Straight	Straight
26	Kernel : poppiness	Absent	Absent	Absent	Absent	Absent
27	Kernel : sweetness	Absent	Absent	Absent	Absent	Absent
28	Kernel : waxiness	Absent	Absent	Absent	Absent	Absent
29	Kernel : apaqueness	Absent	Absent	Absent	Absent	Absent
30	Kernel : Shape	Toothed	Toothed	Toothed	Toothed	Toothed
31	Kernel : 1000 kernel	Medium	Medium	Medium	Medium	Medium

Table 4.4. Paired comparison between hybrids with respect to 31 descriptive traits

Parameter	H1-H2	H1-H3	H1-H4	H1-H5	H2-H3	H2-H4	H2-H5	H3-H4	H3-H5	H4-H5
Traits shared	25	26	28	24	27	24	27	23	27	25
Polymorphic traits	6	5	3	7	4	7	4	8	4	6

Table 4.5. Nei and Li's similarity index calculated across 31 descriptive traits

	H1	**H2**	**H3**	**H4**	**H5**
H1		0.81	0.84	0.90	0.77
H2			0.87	0.77	0.87
H3				0.74	0.87
H4					0.81
H5					

Fig 4.4. Field view of Maize Hybrids at flowering stage

Fig. 4.5. Hybrid plant at maturity

Fig. 4.6. Hybrid plant at maturity

Fig. 4.7. Hybrid H_3 (KDM-914A x KDM-362A) along with inbreds

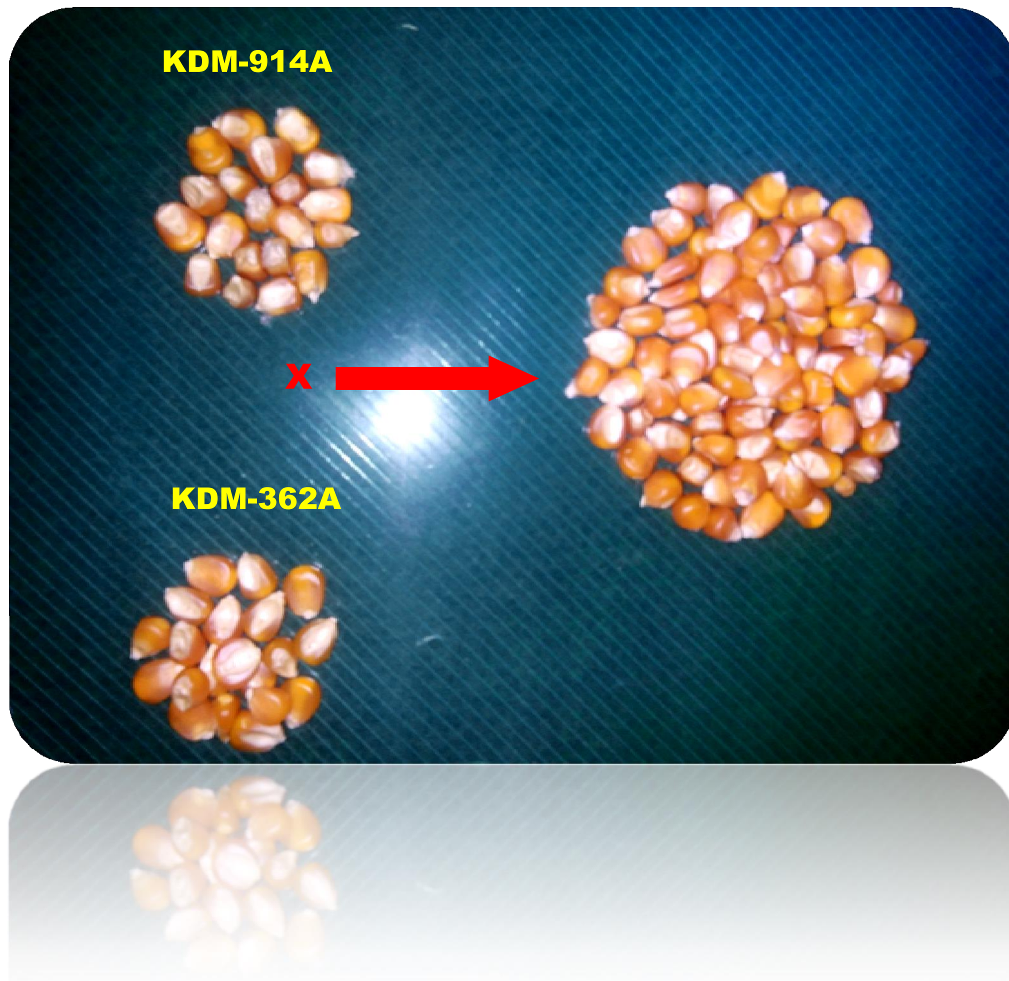


Fig. 4.8. Hybrid H_1 (KDM-361A x KDM-343A) along with inbreds

Fig.4.9. Hybrid H_2 (KDM-332A x CML-128) along with inbreds

4.3. Amplification profile of SSR markers across parents and hybrids

Umc-2383: The marker Umc-2383 amplified 135 bp allele in parent KDM-361A and KDM 343A and 150bp allele in rest of five parents. Allele size of 135 bp allele was scored in hybrids H_1 and H_2 while 150bp allele was scored in rest of three hybrids.

Umc-2100: This marker amplified 100 bp allele in parents KDM 361A and KDM 343A and 125 bp allele in rest of five parents. Fragment size of 100 bp was scored in a single hybrid H_1 while other four hybrids record 125 bp allele.

Umc-2245: The marker Umc-2245 amplified 125 bp in two parents and amplified 100 bp allele in other five. Allele size of 125 bp was scored in a single hybrid H_3 while as rest of 4 hybrids showed 100bp allele.

Umc-1696: This marker amplified 150 bp allele in most of the parents and hybrids.

Umc-2372: Marker Umc 2372 amplified 150 bp allele in parents KDM 361A and KDM 914 A. It also amplified 140 bp allele in parent CML 128 and in rest four parents 120 bp allele was scored. In hybrids this marker was polymorphic across all the five hybrids with allele size of 150/120, 140/120,150/120, 140/120, 150/120 bp in hybrids H_1, H_2,H_3 H_4 and H_5 respectively.

Umc -1708: The marker Umc-1708 showed polymorphism between the parents of hybrid H_1 with 80 bp and 60 bp alleles. Hybrid H_2 and H_5 amplified 80 bp and 60 bp alleles, respectively.

Phi-061: Marker phi 061 was polymorphic across all hybrids with 80 bp and 60 bp size alleles. In parents KDM342A, KDM914A, KDM 916A and CML-128, 80 bp allele was amplified while in parents namely, KDM361A, KDM 332A and KDM 362A, 60 bp allele was scored.

Monomorphic markers across all the parents: The markers revealed lack of polymorphism across all parents and hybrids with fragment size of 90 bp for the marker Umc-2392; 100 bp for Phi-129, Umc-1141, Bulg-1056, Umc-1823; 140 bp for Phi-051; 195 bp for Umc-1036; 150 bp for Umc-1063, Umc-1478, Umc-1675, Umc-1800, Umc-1026 and Umc-2136; 165 bp for Umc-1766; 175 bp for Umc-1306; 200 bp for Phi-067, Umc-1415, Bnlg 1621; 300 bp with respect to markers Umc-2071 and Umc-2369 (Table 4.6; Figs. 4.10, 4.11, 4.12, 4.13, 4.14).

Table 4.6. Simple sequence repeat marker based profile of five single cross hybrids

Marker Name	KDM-361A X KDM-343A	KDM-332A X CML-128	KDM-914A X KDM-362A	KDM-916A X CML-128	KDM-332A X KDM-916A
Umc-2383	135	150	150	135	150
Umc-1664	140/120	140/120	140/120	140/120	140/120
Umc-1147	100/85	120/85	85	120/85	85
Umc-2100	100	125	125	125	125
Umc-2245	100	100	125	100	100
Umc-1696	NA	150	150	150	150
Umc-1823	100	100	100	100	100
Umc-1026	150	150	150	150	150
Umc-2372	150/120	140/120	150/120	140/120	150/120
Umc-1594	140/100	140	140	140	140
Umc-2071	150	150	150	150	150
Umc-2369	300	300	300	300	300
Bnlg-1621	200	200	200	200	200
Umc-1478	150	150	150	150	150
Umc- 1800	150	150	150	150	150
Umc-2136	150	150	150	150	150
Umc-1766	165	165	165	165	165
Bnlg-1306	175	175	175	175	175
Umc-1063	150	150	150	150	150
Umc-1036	175	175	175	175	175
Phi-129	100	100	100	100	100
Phi-051	140	140	140	140	140

Contd...

Marker Name	KDM-361A X KDM-343A	KDM-332A X CML-128	KDM-914A X KDM-362A	KDM-916A X CML-128	KDM-332A X KDM-916A
Umc-2392	90	90	90	90	90
Umc-1141	100	100	100	100	100
Phi-067	200	200	200	200	200
Umc-1675	150	150	150	150	150
Umc-1640	125	125	125	125	125
Phi-061	80/60	60/80	80/60	80/60	80/60
Umc-1002	160/140	140	150/170	140	140
Umc-1424	140	140/110	140/110	140	140
Phi-452693	125	125	125	125	105

Fig. 4.10. Allelic diversity across five single cross hybrids

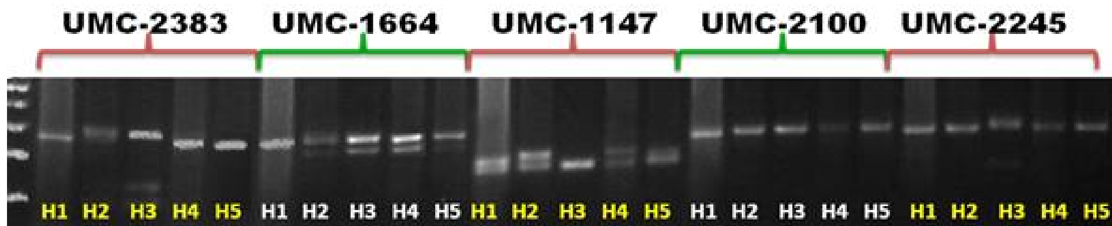
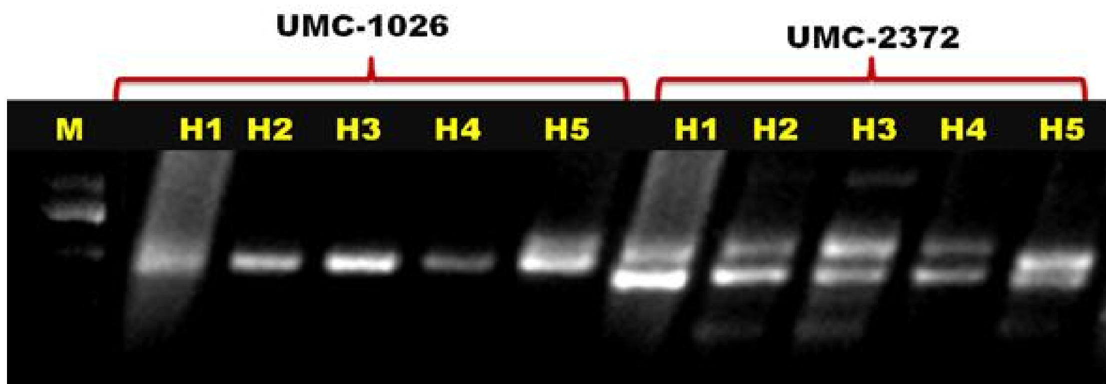


Fig. 4.11. Amplification profile of single cross hybrids against SSR marker Umc-2372



A marker UMC-2372 was polymorphic across the five hybrids.
Designation of single cross hybrids: H1: KDM-361A X KDM-343A; H2: KDM-332A X CML-128; H3: KDM-914A X KDM-362A; H4: KDM-916A X CML-128; H5: KDM-332A X KDM-916A; M: 50 bp DNA Ladder (MBI, Fermentas, U.S)

Fig.4.12. Polymorphism revealed by SSR marker Phi-061 between KDM-316A and KDM-343A

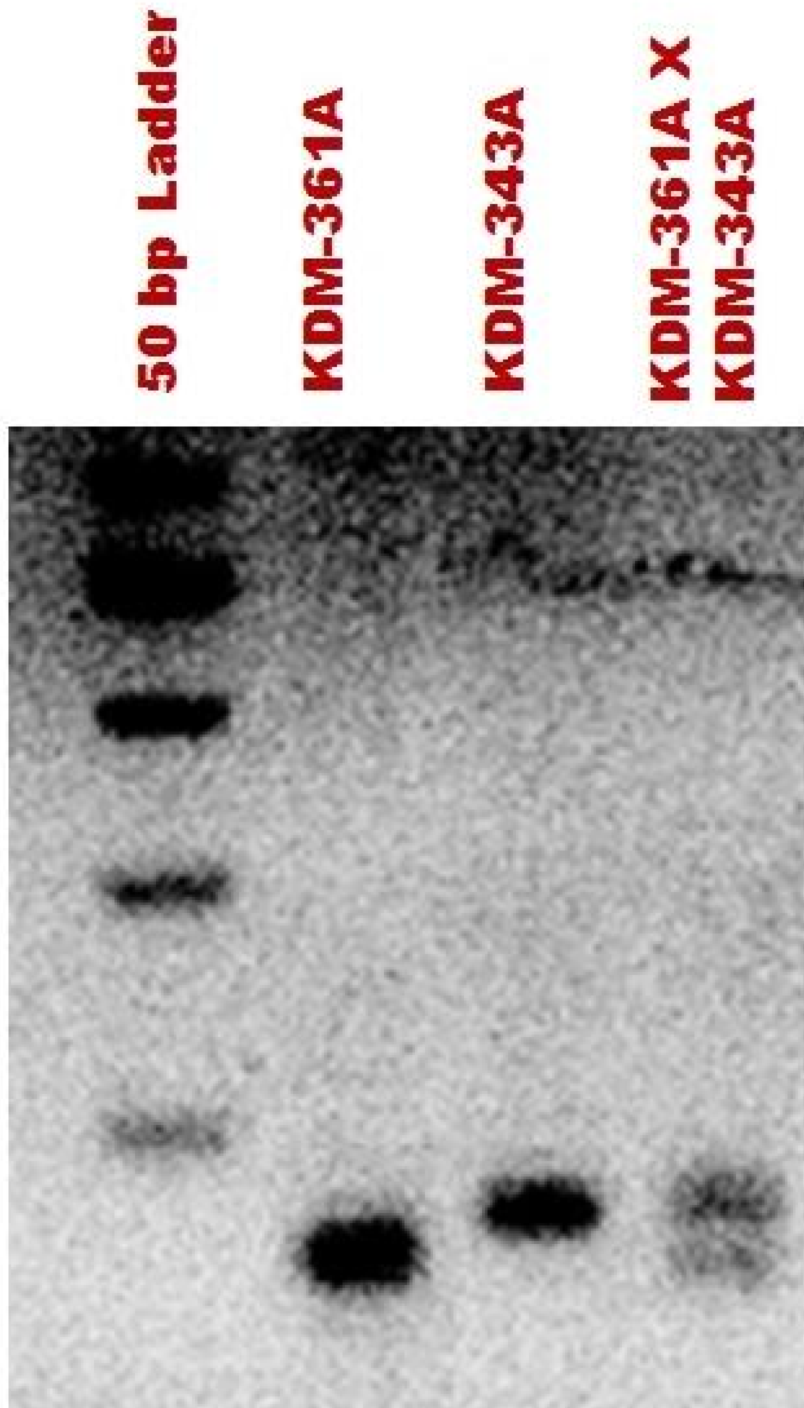


Fig.4.13. Polymorphism revealed by SSR marker Phi-061 between KDM-914A and KDM-362A

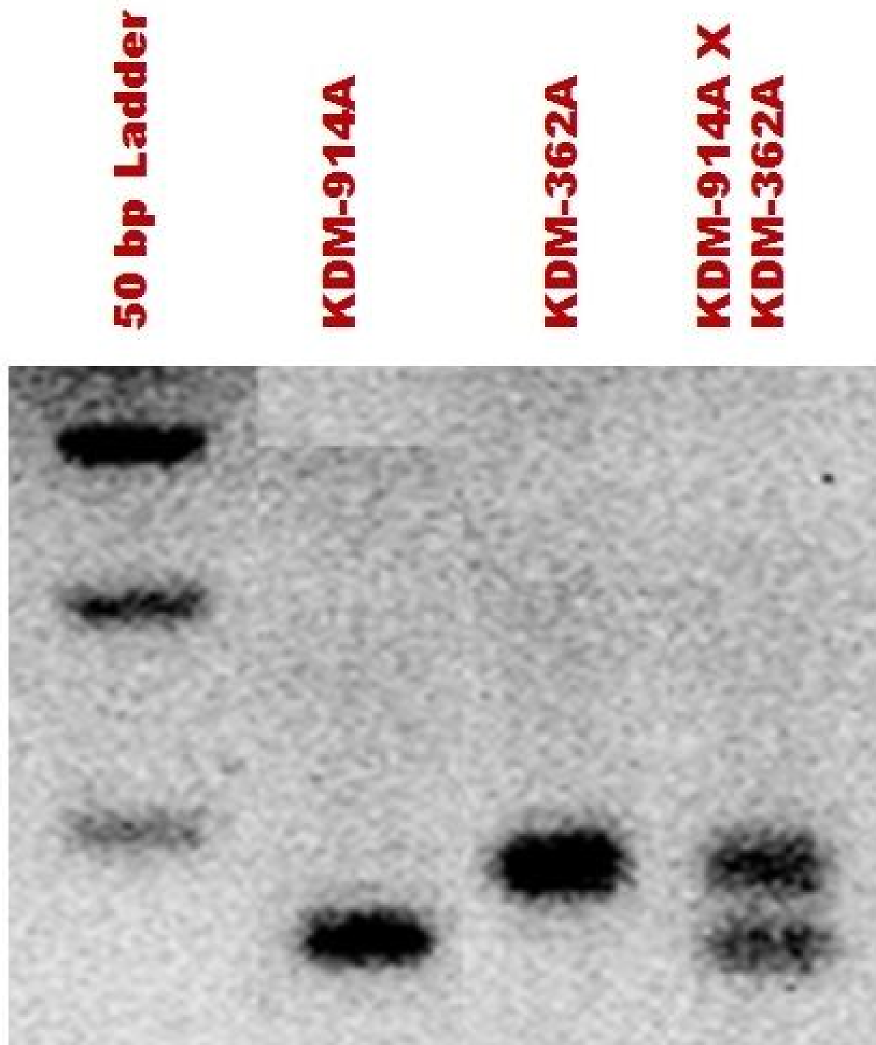


Fig.4.14. Polymorphism revealed by SSR marker Phi-061 between CML-128 and KDM-332A

4.4. SSR Bar coding of single cross hybrids

Multi-locus profiling of five single cross hybrids was carried out using 45 genome wide SSR markers. Ten most polymorphic markers were identified based on their PIC values. These included the Markers Umc-283, Umc-1664, Umc-1147, Umc-2245, Umc-2372, Umc-1594, Phi -061, Umc-1002, Umc-1424 and Phi -452693. These ten Markers were used to generate barcode or a DNA profile for five single cross hybrids. SSR Marker Umc-2383 amplified 135 bp allele in hybrids H_1, H_4 and 150 bp allele in H2, H3, and H5. Marker Umc-1664 was found to be polymorphic across all the five hybrids amplifying a fragment size of 140 and 120 bp in each of the five hybrids. Umc-1147 showed polymorphism between constitutive parents of hybrids H1, H2 and H4 and were monomorphic in H3 and H5. H1 with respect to this marker had an allele size of 100 and 85 bp .The marker amplified 120 bp and 85 bp in H2. The fragment size of 120 and 85 bp was scored in hybrid H4. The marker Umc-2245 amplied 100 bp fragment in H1, H2, H4 and H5. Umc -2375 was again polymorphic between corresponding parents of all the single cross hybrids. Umc-1594 showed fragment size of 140 bp and 100 bp in H1 and unique allele of 140 bp in rest four hybrids. Ph-061 amplified 2 bands each in hybrids H1, H4 and H5 with a size of 80 and 60 bp. Hybrid H1 and H3 were characterized having two alleles each against marker Umc-1002. Umc-1424 amplified two fragments each in H2 and H3. Phi 452693 amplified 125 bp across all the five hybrids. Taken together each hybrid revealed distinct allele profile across a set of ten markers (Fig. 4.15). Hybrids H1, and H5 recorded 16 and 13 alleles against 10 SSR markers, while H2, H3, H4 amplified 14 alleles each. Based on allelic profile generated for at 10 SSR loci, the hybrids showed maximum genetic divergence of 90% between H1 and H3. The probability of identity was highest (0.21) between hybrids H2 and H4 and lowest (0.10) between H1 and H3 (Table 4.7 and 4.8).

Fig. 4.15. Multi-locus profile and SSR Barcode of five single cross maize hybrids

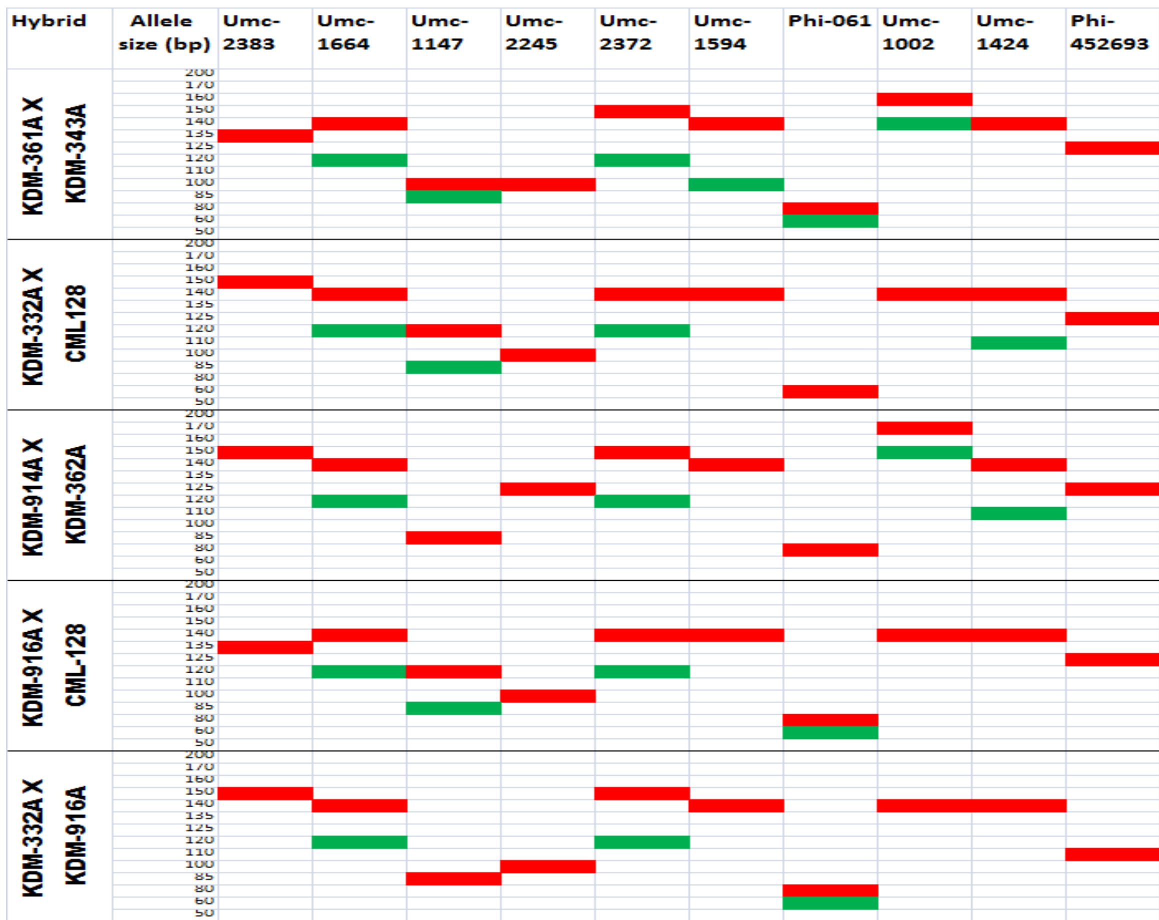


Table 4.7. The number and type of alleles amplified using ten SSR markers against five single cross hybrids

Allele size (bp)	Hybrids				
	H1	H2	H3	H4	H5
170	0	0	1	0	0
160	1	0	0	0	0
150	1	1	3	0	2
140	4	5	3	5	4
135	1	0	0	1	0
125	1	1	2	1	0
120	2	4	2	3	2
110	0	0	1	0	1
100	3	1	0	1	1
85	1	1	1	1	1
80	1		1	1	1
60	1	1	0	1	1
Total no of alleles	**16**	**14**	**14**	**14**	**13**

Table 4.8. The Probability of identity between hybrids based on amplification SSR barcode using ten SSR markers

	H1	**H2**	**H3**	**H4**	**H5**
H1		**0.16**	**0.10**	**0.15**	**0.13**
H2			**0.15**	**0.21**	**0.18**
H3				**0.13**	**0.14**
H4					**0.16**
H5					

5. Discussion

Seed production of single cross maize hybrids involves crossing two diverse homogeneous inbred lines produced through several generations of selfing. During hybrid seed production, greater emphasis is directed towards managing the process to ensure maximum kernel set and high levels of genetic purity (Fonseca *et al.,* 2003). The genetic purity standards of seed are influenced by array of factors which are environmental in nature or may depend on management of hybrid seed production plots. In the production of hybrid maize seed, main source of genetic contamination happens to be self-pollination of the female parent due to incomplete removal of its tassel. This contamination reduces the genetic and physiological quality of the seeds that consequently decreases the crop productivity (Kalinka *et al.,* 2006). It is necessary to set up a fast, economical, and effective system for testing the purity in order to prevent impure seeds from entering the market (Dou *et al.,* 2102).

Normally, seed certification for purity and variety distinctness is based on morphological evaluation of seeds and growing plants. For an experimenter Grow Out Test is a standard technique to evaluate purity of hybrid seed by comparing seeds and plants in the same stage in identical environmental conditions. These evaluation methods often involve field inspection, which are rigorous, resource and time intensive, prone to error and have very little precision. Pertinently, thanks to developments in modern technologies, where it has been possible to evaluate the large number of plants at DNA or protein level in a much reproducible manner with greater precision and in a high throughput manner. Among these are electrophoresis (Tkachuk and Mellish, 1980) and use of molecular markers (Perry, 2004; Ali *et al.,* 2008) have made it possible to screen large number of plants with greater precision and authenticity. The DNA polymorphisms are randomly distributed in the genome without environmental effects and without influence of plant physiological development (Lanza *et al.,* 2000). DNA based markers like SSRs (Tautz, 1989) have proved to be the preferred molecular marker systems for purity identification in some crops (Yashitola *et al.,* 2002) due to their high efficiency and simplicity (Wu *et al.,* 2010). SSRs have additional attributes such as codominance and multiallelism that make them one of the best choices for the identification of genetic contamination. McDonald *et al.,* 1994 has suggested the use of SSRs as a means of genetic purity analysis to based on DNA isolation from seed. Kozhukhova and Sivolap (2004) showed that in the analysis of ten SSR loci, 40 genotypes were unique. For

every locus, SSR analysis revealed heterozygotes among simple hybrids, which made it possible to identify the parental forms with a high probability of exclusion of non-parental forms.

In the present study, the SSR markers Umc-2383, Umc-2100, Umc-2245, Umc-1696, Umc-1708 were able to discriminate between parents of one or the other hybrid. The markers Umc-1664, Umc-2372, Phi-061revealed polymorphism across parents of all the five hybrids. At least, 31 SSR markers revealed lack of polymorphism across all the parents and hybrids with fragment size ranging from 90 bp for the marker Umc-2392 to 300 bp with respect to markers Umc-2071 and Umc-2369. Among these, the markers Umc2383 and Umc2100 belonged to different bin locations on chromosome number 1. The markers Umc-2245 and Umc-1696 are located on separate bin locations in chromosome number 2. A polymorphic marker Umc-1708 is located on chromosome 7. The markers Umc-1664, Umc-2372, Phi-061which revealed polymorphism across all the hybrids under study belonged to chromosomes 1, 2 and 9, respectively.

Apart from the fact that genetic diversity of maize germplasm is important for planning breeding programmes and conservation of maize germplasm, etc, it is also important that farmers have an opportunity to choose among hybrids the one that will be a choice for highest yields and suitable for environmental stress conditions (Troyer *et al.,* 1983). In Kashmir province, several different hybrids are being pumped by private sector besides few known public bred hybrids those have occupied considerable area. Most of times it becomes difficult for a grower or even seed certification agencies to identify a given hybrid in market merely on the basis of morphological markers or descriptive traits. The hybrids with similar seed characteristics like color, indentation, size and pattern may not easily discern the identity when offered to grower. Therefore, in order to scrutinize and verify the distribution and area occupied by each of the hybrids, seed monitoring agencies, extension workers, scientists, or farmers, on demand can opt for DNA profiling of seed lots in order to differentiate a given hybrid in question from other commercially grown hybrids which may be morphologically similar but otherwise less preferable. In this context it becomes important that multi-locus profile with the help of polymorphic markers be generated and standardized which can collectively distinguish among the individual hybrids. DNA barcoding is becoming a widely applied tool for the quick and accurate toll to define species limits and genetic diversity (Kim et al., 2011). They demonstrated that DNA Barcodes can be critical factors for identifying

species boundaries and examining biogeographical patterns. Zamora et al (2011) have used ITS sequences for preliminary identification of the *Geastrum* species. The most variable marker, the ITS (Internal Transcribed Spacer) nrDNA locus, was very well suited for species discrimination and an excellent DNA barcode locus. DNA barcoding is a modern biotechnological tool which can distinguish species at molecular level (Bhagwat et al., 2011). They studied *Dalbergia* genus using 34 primer pairs specific to several chloroplast genes (matK, rpoC, rpoB, rbcL, accD, ndhJ, ycf5 and trnH-psbA) as well as the nuclear genes and ITS were tested for species discrimination.

A set of polymorphic markers in present study was thus used to generate DNA Barcode (Fig.4.15). A total of 10 ploymorphic markers were used in order to discriminate five single cross hybrids H1, H2, H3, H4 and H5. Collectively, twelve different kinds of alleles (60, 80, 85, 100, 110, 120 125, 135, 140, 150, 160 and 170 bp) were amplified with the size range of 60 bp to 170 bp. Hybrid H1, H2, H3, H4 and H5 amplified 16, 14, 14, 14 and 13 alleles, respectively. Based on SSR profile using 10 markers, the hybrids showed distinct molecular profile amongst themselves. Low probability of identity was recorded between H1 and H3 (0.10). Probablity that H2 and H4 shared alleles at 10 markers loci was only 21% that was the highest among all the paired combinations. Hence the SSR barcode standardized here holds a promise and could be used as a key to establish genetic identity of hybrids sampled form open market or farmers custody, with extreme precision and greater reliability.

Moreover, protection of Plant varieties and Farmers Right authority insists on characterization and registration of extant, farmers and new varieties as a part of national and botanical asset. Pinnisch *et al.* (2012) advocated characterizing inbred lines which serve as the seed parents to commercially grown maize hybrids. Therefore, as a set of inbreds was characterized using 31 morphological traits following Maize descriptor (DMR, New Delhi). The five single cross hybrids were evaluated for 31 descriptive traits following DUS Manual DMR, New Delhi. Only nine out of 31 traits studied were polymorphic among hybrids Among ear characters only traits ÷ear lengthø and ÷grain colorø were found polymorphic. The Nei and Liøs similarity coefficients were devised to compare genetic distance between hybrids. Hybrids H3 and H4 showed similarity coefficient of 0.74 while H1 and H4 revealed similarity value of 0.90. As many as eight out of 31 traits differed between H3 and H4 whereas, H1 and H4 differed with respect to only three traits, therefore, revealed high

similarity. Ni and Liøs index is appropriate for dominant markers. The marker analysis based on SSRs revealed 4-5 fold more genetic divergence among hybrids when compared to that based on morphological traits. In fact only 10 SSR markers revealed 80-90% divergence among hybrids that was much higher than what was found based on phenotypic trait variation among the five hybrids. SSRs have been known and several occasions advocated to be markers of choice for estimation traits diversity among hybrids and also for establishing identify and genetic purity of hybrids in crop like maize (Natalya et al., 2002; Asif et al., 2006; Wang et al., 2011). Therefore, low probability of identity based on marker information suggests that a set of markers identified in present study can be efficiently used in establishing distinctness, identity and purity of hybrids in open markets after their release.

6. Summary and Conclusion

Maize is one of the most important cereal crops of the world and contributes to food security in many of the developing countries. The increasing use of maize as feed, growing interest of the consumers in nutritionally enriched products and rising demand for maize seed are the core driving forces behind emerging importance of maize crop in India. In India, maize is emerging as third most important crop after rice and wheat. Its importance lies in the fact that it is not only used for human food and animal feed but at the same time it is also widely used for corn starch industry, corn oil production, baby corns etc. Corn production has nearly doubled from around 12.0 million tons in the early 2000s to around 22 million tons today. This remarkable production growth has been largely driven by adoption of single cross hybrids in the late 1980ǿs and continuous demand in domestic and export market. The hybrid corn enters market through various agencies, in major part, the private seed companies play an important role. There always remains a possibility of intentional or accidental loss in purity of hybrid seed that is a major detrimental factor affecting realizable hybrid yields. If goes undetected self seeds in hybrid lots result in lowering of grain yield and quality. The focus of present study was to identify microsatellite markers which may be used for estimation of the purity of hybrids in commercial seed lots. In the present study 45 SSR markers were validated against five single cross hybrids and their parents. The markers Umc-1664, Umc-2372, Phi-061 were found polymorphic across corresponding parents of all the five hybrids. Besides, few other markers were identified for one or the other parental combinations. The markers could be utilized for judgment of hybrid purity in commercial seed lots. Apart from yield and yield contributing traits, thirty one morphological characteristics were studied to discriminate among hybrids H1, H2, H3, H4 and H5. Only nine of these traits differentiated the hybrids and were polymorphic and a single trait was grouped into three phenotypic classes. Nei and Liǿs (1979) similarity coefficient of 0.74 was recorded between hybrids H1 and H3 based on all the thirty traits. Contrary to the morphological markers, SSRs revealed greater degree of polymorphism among hybrids. Maximum probability of identity was recorded for H1 vs H4 which was as low as 0.21. Clearly, SSRs revealed an advantage in discriminating hybrids amongst each other based on multi-locus profiling. With the help of ten SSR markers SSR barcode was developed which is expected to help in characterization and identification of any given hybrid in open seed

market or farmers fields. Moreover, it would serve authentic tool for seed growers, seed certifying agencies and researchers.

From the present investigations it was concluded that hybrids showed better performance with hybrid KDM-361A X KDM-343A recording a grain yield of 78 q/ha. Not much of the variability existed for descriptor traits among a set of five hybrids. Out of 31 traits studied, only eight traits could be categorized into two distinct classes each, whereas, a single trait i.e. ÷Ear anthocyanin colourationø had three phenotypes across five hybrids. Hybrids showed a similarity coefficient which varied from 0.74 (H3 vs H4) to 0.90 (H1 vs H4) indicating considerable uniformity in phenotypic attributes. However, based on marker analysis, it was found that ten SSR markers discriminated among hybrids at genotyipic level. Probability of identity stood as low as 0.21 to 0.10 between hybrids H2 and H4 and pair H1 and H3, respectively. In present study the SSR markers namely, Umc-1664, Umc-1147, Umc-2372, Umc-1594, Phi-061, Umc-1002 and Umc-1424 were found polymorphic between parents of one or more hybrids. These markers could be utilized for testing of hybrid purity in commercially available seed lots and also serve as handy protocols to establish hybrid identity and genetic purity by researchers, seed certifying agencies and farmers.

7. References

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