"Morphological and Marker based Evaluation of Early Generation Sweet Corn (*Zea mays* L.) Inbred Lines for Yield and Quality Attributes"

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2021

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Thesis

Submitted to

The Faculty of Agriculture
Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir
In partial fulfilment of requirement for the award of the degree of

Master of Science in Agriculture
(Genetics and Plant Breeding)

2021

To

the ones we all owe every bit of everything, family!

Sher-e-Kashmir
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Certificate – I

This is to certify that the thesis entitled**"Morphological and Marker based Evaluation of Early Generation Sweet Corn (*Zea mays* L.) Inbred Lines for Yield and Quality Attributes"**submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science in Agriculture (Genetics and Plant Breeding),** to the **Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir** is a record of bonafide research work carried out by **Mr. Shah Mohammad Usman (Regd. No. MSA/2019/1288)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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

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We, the members of the Advisory Committee of **Mr. Shah Mohammad Usman (Regd. No. MSA/2019/1288)**, a candidate for the degree of **Master of Science in Agriculture (Genetics and Plant Breeding)** have gone through the manuscript of the thesis entitled, **"Morphological and Marker based Evaluation of Early Generation Sweet Corn (*Zea mays* L.) Inbred Lines for Yield and Quality Attributes"** and recommend that it may be submitted by the student in partial fulfillment of the requirements for the award of the degree.

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ABSTRACT

Sweet corn is gaining tremendous demand worldwide due to urbanization and changing consumer preferences. Sweet corn is different from normal corn because of high sugar levels in the endosperm of the kernels which is attributed to some recessive mutations that hinder the development of starch from the sugars in the endosperm. The present study titled "Morphological and Marker based Evaluation of Early Generation Sweet Corn (*Zea mays* L.) Inbred Lines for Yield and Quality Attributes" was carried out during *Kharif* 2020 at Mountain Research Centre for Field Crops, Khudwani, SKUAST Kashmir. Individual plants were evaluated for agro-morphological traits and maturity. The harvested cobs were subjected to evaluation for kernel characteristics and quality. During the study 30 morphological characters were recorded as per the guidelines of descriptor of Indian Institute of Maize Research ICAR, New Delhi, 2011. The cluster analysis resulted in grouping of the 80 sweet corn inbreds into two major clusters. SMC 3, a non-sugary genotype was clustered as a mono-cluster while as the rest of the inbreds were classified as a separate cluster along with the two parents. The highest amount of total carotenoids was found in the inbred S27 with a total carotenoid content of (34 μg g^{-1}) followed by the inbred S65 with a total

carotenoid content of (31.1 μg g^{-1}). The highest amount of total sugar was found in the inbred S60 which had a total sugar content of 8.54% followed by the inbred S14 that showed a total sugar content of 8.34%. S59 and S5 had a high amylose content and 6 inbreds showed a low amylose content. Except three inbreds with moderately resistant reaction all other inbreds were found to be resistant against Turcicum leaf blight and interestingly the parents were also resistant to this disease under field conditions. Out of the sixty inbreds that were used for the molecular study, seven inbreds were found to be sugary at both the loci (umc2061 and bnlg1937). The study therefore revealed the presence of some amount of variability for all the morphological and the biochemical parameters which are very imporatant in terms of the consumer acceptability, quality and nutritional values. The information generated in the present study may help in the identification of better recombinants. The identified inbred lines may be further validated and used for development of varieties for wide commercial use.

Keywords: Sweet corn, Inbreds, Carotenoids, Sugars, *su1*, *sh2*, markers, TLB.

Signature of student

Dated: _____

Signature of Major Advisor

Dated: _____

ACKNOWLEDGEMENT

When a player in any football league makes a touchdown, they very often do one of the two things. Some jump up and down, slap the hands of the fans in the stands, spike the ball and leap into the arms of their teammates. Others will simply drop to their knees and pray, thanking god for blessing them with enough strength.

First, I want to bow down to my lord, in awe of his mercy and blessings for sustaining me through the years of thick and thin, the best and the most testing times. Secondly I have a bunch of people to thank. These are the people who have been nothing less than instruments of the divine in order to get me through these years and this research. Thank you all.

Family is the fuel of every success and every milestone that has ever been accomplished in this world. Parents are the guardian angels in every home, thanks to the creator for creating such an asset. Nothing can ever be done without the family and I feel blessed to have the privilege of having a supportive and a loving family. The efforts that have been put by my parents Mr Manzoor Ahmad Shah and Ms. Hamida Akhter in bringing me up cannot be explained so briefly. Whatever I have done, and whatever will come by the Almighty's grace is all because of them and I feel blessed to dedicate this piece of work to them. My sisters Ms. Saba and Ms. Mehak have been the most amazing sisters one can dream of, their selfless love and support is unparalleled and they have been the source of my positivity. The youngest of them all is my nephew Mohammad zaid, whose smiling face brings divine energy into my soul. I could have crumbled without you all and I thank you all for making me feel so loved!

Throughout the period of completing this research I have fought, talked, laughed with my friends. So many have helped, on so many levels, that I cannot mention them all here, but if I had to name just a few, I would have to start with my advisor Dr. Farooq Ahmad Sheikh , Professor and Head, division of GPB- thank you so much for all the support and guidance. Of course, I have the best committee members one could hope for in Dr. Muhammad Ashraf Bhat, Professor, Division of GPB who has been a source of motivation and inspiration. I also want to thank the other advisory members for their timely support and assistance.

The study wouldn't have been possible without the continuous guidance, support and mentorship of Dr. Asif B. Shikari, Associate professor, MRCFC Khudwani from whom I have learned invaluable lessons about thinking,

learning and life. There has been tremedous support from Dr. Najeeb ur rehman sofi, Professor, MRCFC Khudwani and Dr. Gazala H khan, Assistant professor, MRCFC khudwani. Special thanks to Dr. Syed Zameer Hussain, Head Division of Food Science and Technology and Dr. Imtiyaz Murtaza for lending their technical support. I have been lucky enough to share the lab with the most supportive and humble Research fellows Dr. Mohammad Saleem Dar and Dr. Saba mir who have always assisted me in every part of the study. My gratitude goes to the seniors, Mr. Raheel shafeeq khan, Mr. Zakir amin, Mr. Liyaqat, Mr. Fazil Fayaz, Mr. Audil Gull, Mr. Owais ali, Mr. Ajaz Bashir, Ms. Safoora jan, Ms. Nazrana wani, Ms. Musharib , Ms. Naqeeb and Ms. Sumaira Who were always ready to help in every possible way and i thank you all for all the support you have always offered. The technical support from the technical staff of MCRS Sagam and MRCFC Khudwani has been unparalleled and I would like to thank them all for being an important part of this study. My special thanks to Mr. Farooq ahamad mir (MCRS,Sagam), Mr. Mohammad Amin (MRCFC, Khudwani) and Mr. javed ahmad (MCRS, Sagam)

I would like to thank my friends who are the backbone of strength and have been a source of emotional support especially Mr. Tabish Mushtaq, Mr. Faisal Ali, Mr. Sheikh Amjid, Mr. Furqan Farooq, Mr. Qazi zuhaib and my classmates who have been like a family throug the entire period of our study.

Shah Mohammad Usman

Place: Anantnag
Dated:

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Chapter – 1

INTRODUCTION

Maize (*Zea Mays* L.) known as the "Queen of Cereals" is the third most important cereal after wheat and rice that is grown and consumed in the form of food, feed, fodder and industrial raw material. Maize is a highly adoptable crop with tremendous versatility and it thrives quite well in different agro-ecologies and adapts well in diverse environments. Owing to its high nutritional value maize is an important staple crop all over the world and is therefore an important contributor to the global food security. Maize stands at the top in terms of genetic yield potential of the cereal crops and it is for this reason that it is titled as the 'Queen of Cereals'. During 2018-19, Maize crop was globally cultivated over an area of around 191.95 million hectares with the production of 1123.45 million metric tons (USDA, 2020). In India during 2018-19, maize was cultivated over an area of around 9.03 million hectares with the production of 27.72 million tons (USDA, 2020). In Jammu and Kashmir Maize is the most important crop in terms of acreage and is being cultivated over an area of about 0.295 million hectares with the annual production of 0.54 million tons (Directorate of Economics and Statistics, 2017).

Sweet corn (*Zea mays* L. var. *Saccharata*) is a special type of maize and is one of the popular choices worldwide, both as a fresh and processed vegetable. Sweet corn is different from the normal corn because of the increased amounts of sugar in its endosperm. It is sweeter than normal maize because of some recessive mutations that hinder the development of starch from sugars in the endosperm of the corn kernels. The total sugar content in case of sweet corn is in the range of 25 to 30% at the milky stage as compared to 2 to 5% in case of normal corn (Dagla *et al.*, 2014). The kernels of sweet corn have a thin pericarp and appear translucent when mature but wrinkle after drying. Sweet corn is consumed at an immature kernel stage, roughly 20-25 days after fertilization. The fresh and raw ears of

sweet corn can be consumed in the cooked as well as in the roasted form. The demand for fresh sweet corn is quite high in the hotels as it is used for the preparation of sweet corn soup. In addition to the use of sweet corn in the fresh form which includes the products such as baby corn. The harvested immature kernels are also dried to produce candy. The mature sweet corn kernels are also used in the beverage industry for the preparation of an alcoholic beverage 'chichi.' Sweet corn is also a source of raw material for many industrial products like starch syrup, dextrose etc. After sweet corn matures and the cobs are harvested its stalks can be put to use as livestock fodder. Owing to its numerous uses sweet corn has promising scope for export and can also be an important crop in the domestic market.

Sweet corn improvement has been done primarily by identifying and selecting for mutants with higher sugar content. Four important useful mutants are shurnken2 (*sh2*), sugary1 (*su1*), brittle1 (*bt*) and sugary enhancer (*se1*) (Lertrat and Pulam, 2007). Among them the genes brittle2 (*bt2*), shrunken1 (*sh1*) and shrunken2 (*sh2*), are present upstream of the pathway and assist in formation of glucose, whereas the enzymes which are encoded by amylose extender1 (*ae1*), sugary1 (*su1*) and waxy1 (*wx1*) assist in the production of final products of starch metabolism, amylose and amylopectin (Fisher and Boyer 1983; James *et al*., 1995). The genes affecting the starch metabolism, *su1* and *sh2* have been substantially used for sweet corn cultivar development (Hossain *et al*., 2013). The genes *sh2* and *bt* which are present at the chromosome 3 and 5 respectively are put in the class 1 mutants. The class 1 mutants are responsible for increased sugar content and a decreased starch content in the kernels. The genes su1 and se are present on chromosome 4 and 2 respectively and are classified as the class 2 mutants. The class 2 mutants are responsible for the change in the type and proportion of endosperm polysaccharides. The 'sugary' sweet corn been developed by using the *su1* allele. There is a change in the amylose and amylopectin proportion because of recessive *su1* allele. The varieties containing

the recessive *su1* allele have a sucrose content of 10.2%, water soluble polysaccharide (WSP) 22.8% at the immature milking stage(20-25 days after pollination). The grains also has a creamy texture, a sugar content thrice and a WSP 8 times than that of field corn (Creech, 1965).

The sweet corn types based on the *sh2* are commonly known as 'super sweet' or 'extra sweet corn' because of increased sugar content in the kernels. Because of elevated levels of sugars in *sh2sh2* mutant, kernels lower levels of total carbohydrates when the seed matures.

The decrease in sugar content is slower in case of the *sh2* type even without refrigeration, therefore the varieties have increased shelf life, and are suitable for prolonged storage. Besides, amalgamation of *su1* and *sh2* alleles is mostly done in commercial sweet corn development (Lertrat and Pulam 2007).

Sweet corn has tremendous marketability potential if the processing and the packing needs are taken care of. The marketability potential is not only limited to the national market but global market. The demand for normal maize has been catered by producing several high yielding maize hybrids in India but there has been quite less focus on development of sweet corn hybrids. In India only 3 sweet corn composites (Madhuri, Priya and WinOrange) have been developed by public sector organizations.

The normal maize crop is the tribal staple crop in Jammu and Kashmir. However, due to lower returns the maize cultivation is mostly restricted to the higher reaches and areas having meager or no irrigation facilities. Moreover, the lower returns in normal maize is pushing the growers towards the specialty corn 'Sweet Corn' which gives better returns and opens avenues for employment generation (Najeeb *et al*., 2011). Sweet corn can be a healthy alternative to the normal maize in terms of marketability of the crop due to the increased demand. The production and cultivation of sweet corn is also the most effective strategy when facing climate changes as it is relatively drought-tolerant and it therefore adaptable to a wide range of climates (Bray, 1997).

DNA-based markers tightly linked to the target genes, especially favourable recessive alleles, can greatly accelerate the process of introgression of targeted alleles in to desired recipients through marker-assisted backcross breeding (Prasanna *et al*. 2010; Gupta *et al*. 2013). Keeping the above things in consideration, the present study entitled "Morphological and Marker based Evaluation of Early Generation Sweet corn (*Zea mays* L.*)* Inbred Lines for Yield and Quality Attributes" was undertaken with the following objectives:

- Evaluation of sweet corn inbreds for various morphological, biochemical, yield and component traits.
- Marker based validation of inbreds for sweet kernel trait.
- Phenotypic screening of inbreds for reaction to *Exherohilum turcicum.*

Chapter 2

REVIEW OF LITERATURE

2.1 Morphological characterization

Revilla and Tracy (1995) characterized 58 sweet corn cultivars with the help of 34 DUS traits. On the basis of morphological traits, 52 cultivars were grouped into one race and were proposed to be named 'Northeastern Sweets'. Five cultivars which were from the North central U.S were thought to be representing the races of those areas. The last cultivar was an important sweet corn cultivar 'country gentleman' which was of commercial importance.

Babic *et al*. (2010) carried out a study in sweet corn inbreds in order to check whether phenotypic characterization could be helpful in grouping of inbreds into heterotic groups. The study also aimed at checking if the phenotypic distance obtained by visual estimation could be helpful in the estimation of heterosis. The study revealed that the clustering was highly corresponding to that of the pedigree.

Elezi and Ibraliu (2013) evaluated local maize populations in order to characterize and evaluate them. They evaluated different characters like plant height, tassel type, stem color. The results showed that there was a shorter plant period and the male and female flowering had a difference of 2-9 days. The cluster analysis showed the presence of 3 varietal groups.

Selvi *et al*. (2013) evaluated 17 inbreds which included 16 domestic and an exotic inbred, the inbreds were evaluated as per DUS guidelines and it was found that there was variation among the inbreds for various morphological characters. They found the two inbreds UMI 1200 and UMI 1230 different from the other inbreds. The inbred UMI551 was distinguished from other inbreds by the tassel anthocyanin coloration at the glume of the base. The study concluded that there was considerable variation among the inbreds and this variation could be helpful in selecting inbreds for future breeding programs.

Chanda *et al*. (2014) carried out a study on 18 South African maize inbred lines in order to check their variation for 25 agronomic and 12 DUS traits. They grouped the inbreds based on the quantitative or qualitative traits or both. The inbreds were grouped on the basis of Principal Component Analysis (PCA). The first PCA revealed that out of the total variation 27.8% was because of productive parameters and grain yield. The second PCA revealed that out of the total variation 13.2% was because of tassel branches and tassel length. Based on Shannon diversity, the inbreds were found to be distinct in leaf color, silking, shelling percentage, ear diameter and days to maturity. The study concluded that in order to discriminate the maize inbred effectively the DUS traits should be used along with the agro-morphological traits especially in cases were the inbreds under study are few in number.

Gupta *et al.* (2014) evaluated 21 maize inbreds for 29 DUS parameters. The study revealed that among the 29 traits under investigation 5 traits were found to be monomorphic while as 18 were found to be dimorphic and 16 were polymorphic. The modified joint regression analysis revealed that there was negligible interaction of the quantitative traits under study with the environment as well as within themselves. Five inbreds were found to be uniform while the other inbreds were found to be non-uniform for 1-4 traits as revealed by the combine over year analysis. VQL 1 and CM 212 were found to be non-distinct to each other as well as to the other 19 inbreds as revealed by combine over year distinct analysis.

Madhukeshwara and Sajjan (2015) studied various morphological traits in the hybrids ‘Arjun’ and ‘GH 0727’ along with five parents at UAS Dharward, Karnataka, India. The study concluded that there was variation in the 7 genotypes for various morphological characters like thousand seed weight, tassel angle, plant height and tassel density of spikelets.

Salami *et al.* (2015) carried out a study in order to investigate morphological diversity of some maize accessions. They found the accessions to be different for all the agro-morphological characters that were evaluated. The accessions were grouped into 4 groups on the basis of numerical classification. The stepwise discriminant analysis revealed that the early characters which were related to plant height and insertion were those variables that differentiated the accessions in both the zones.

Tandzi *et al.* (2015) carried out a study in order to check the genetic diversity of parental lines and to classify the lines on the basis of the performance of the lines under acidic soil. The lines under study were classified into two broad groups. In the first group there were 3 distinct sub groups which contained lines which were moderately tolerant, susceptible and the most susceptible ones. The other major group contained the lines which were tolerant.

Gull *et al*. (2020) carried a study on 35 sweet corn inbreds in order to characterize them on the basis of DUS traits. Their study showed that there was extensive variation among the inbreds. The study concluded that the 31 traits used for accessing the genotypes were helpful in characterization.

2.2 Total Sugar estimation.

Ferguson *et al*. (1978) analyzed endosperm sugars of a sweet corn inbred (Illinois 677a) which contained the sugary enhancer gene and compared it with the other corn genotypes. The inbred (Illinoi 677a) was found to have high levels of sucrose and it differed from all the other genotypes because of its high maltose content. The maltose content of the inbred IL 677a was found increased to 3.28% 40 days after fertilization and was found to remain high at the dry mature stage. The sugary hybrid ‘silver queen’ which did not possess the *se* gene did not show any such trend in maltose accumulation.

Olsen *et al*. (1990) evaluated three commercial sweet corn cultivars for their eating quality as well as their carbohydrate composition under post-harvest

conditions. The harvested cobs were stored at temperatures of 1, 4, 7 or 18 □ for up to 10 days. The sweet corn cultivar 'sucro' contained a sucrose content three times more than the other two cultivars. It was also found that the highest depletion in total percent sugar was in the cultivar 'sucro' (74%) than the other two cultivars. The study also revealed that the variety "sucro" contained more sugars after storage than the other two cultivars.

Zhu *et al.* (1992) evaluated a set of three sweet corn genotypes which included sugary (*su1*), sugary enhancer (*se*) and super sweet (*sh2*) types of sweet corn. The genotypes were evaluated for total sugar, glucose, fructose, maltose and □brix. The highest sucrose and total sugar was found in the super sweet genotype. The sugary type had the lowest sucrose and total sugar content but had the highest □brix.

Simla *et al*. (2010) carried out a study in six waxy corn varieties in order to estimate the carbohydrate compositions of the corn varieties at different harvest times. The sugar compositions were found to get increased up to a peak and thereafter declined. The other parameters like phytoglycogen, amylopectin and total starch were found to be highest in the mature kernels. The results also revealed that when the waxy corn was stored under ambient conditions there was a rapid loss of sugar content while as the contents of phytoglycogen, total starch and amylopectin were increased.

Zilic *et al.* (2011) investigated different biochemical parameters which include sugars, fibers, total proteins, starch and tryptophan. The two sweet corn hybrids namely ZP531su and ZP504su were found to have the highest nutritive value as it contained the highest proportion of sugars, total protein and dietary fibers. All the hybrids that were evaluated were grouped into three groups on the basis of their sucrose content.

Szymanek *et al*. (2015) investigated three sweet corn genotypes for their carbohydrate concentration at different stages of their maturity. The sugary (*su1*)

corn was found to have an average sugar concentration 5% higher than the sugary enhancer (*se*) corn and 53% greater than the super sweet (*sh2)* corn. The sugary corn was also found to have an average starch concentration 27% lower than the sugary enhancer corn and 66% lesser than the super sweet corn. It was also found that the sugary corn had a moisture content 3% lesser than the sugary enhancer corn and 1% lesser than the super sweet corn.

Yu *et al.* (2015) evaluated three types of maize cultivars namely super sweet (*sh2sh2*homozygous mutant), normal maize and waxy maize (*wxwx* homozygous mutant) for different physico-chemical properties and difference in their endosperm structure. The kernels were found to exhibit different quantities of soluble sugar, amylose and total starch. The normal maize kernels were found to have the highest proportion of floury endosperm after waxy corn and super sweet corn. The study also revealed that the waxy and normal maize starches were found to be larger in size than that of the super sweet corn starch.

More *et al*. (2018) carried out an investigation in which the sweet corn kernels were blanched and the effect of blanching was studied in order to find the most suitable blanching time. The samples after being blanched for various time periods were then analyzed for some physico-chemical quality parameters such as total sugar, total carotenoids, TSS, pH and firmness. The study aimed at finding the most suitable time for blanching the samples at which there would be minimal loss of the quality parameters. It was concluded that the blanching time had a significant role on the quality parameters and the samples that were blanched for three minutes showed better quality parameters.

Hemavaty and Priyadarshani (2019) conducted a study on 26 sweet corn lines in order to study the estimates of genetic advance, variability and heritability for quality traits in case of sweet corn. The study revealed that there was great diversity for the five quality traits which included total sugar, starch, sucrose, total carbohydrate and reducing sugar. It was concluded that the quality traits under

study could be improved through simple selection as the heritability was found to be high along with high genetic advance which indicated that the inheritance of the traits under study was governed by additive genes.

Ibrahim *et al.* (2019) conducted two experiments and evaluated total sugar and total soluble solid content in seventeen sweet corn hybrids. The hybrids with highest total sugar and total soluble solids were grown under different planting dates in the following season in order to evaluate the effects on the quality and growth parameters of sweet corn under different dates. Among the hybrids GZ3B x GZ15, GZ3B x GZ20 and GZ20 x GZ10 had the highest values of total soluble solids and total sugar. The study also revealed that the highest total soluble solids and total sugar was found at the sowing date of August 22nd, 20 days after pollination. The total carotenoid content was found to be increased when the harvest date was increased.

2.3 Total carotenoids estimation.

Weber (1987) developed a procedure for the estimation of tocols and carotenoids. The procedure permitted the determination of both tocols as well as the carotenoids in the same sample prepration of the maize grain. The range of the total carotenoids of the 16 samples under study was found to be 16 to 77 µg/g dry weight and the range of the total tocols was found to be from 30 to 128 µg/g dry weight. The mean loss at room temperature of the 4 inbreds was 42% in case of carotenoids and 5% in case of tocols.

Kurilch and Juvik (1999) conducted an experiment in corn in order to analyze the contents of carotenoids and tocopherols simultaneously in corn. They used a multichannel detector in which one channel was used for the monitoring of tocopherol absorption at 290 nm while the other one monitored the carotenoid absorption at 450 nm. They compared their results with the results generated from the other laboratories and found their procedure to be relatively accurate.

Berardo *et al.* (2004) conducted a study on 64 maize genotypes in order to evaluate the concentration of carotenoids in their grains. They found that the carotenoid components (carotenes, cryptoxanthin, lutein, zeaxanthin, violaxanthin and isolutein) which were examined showed wide differences. The study also showed that most prevalent carotenoids found in maize (zeaxanthin and lutein) showed a range from not detectable to 29.70 and 0.5 to 38.20 mg kg^{-1}.

Howe and Tanumihardjo (2006) conducted a study on biofortified maize in order to evaluate the method of extraction of carotenoids and to ascertain the steps that are essential for their use in the bio fortified maize growing countries. They found that in order to release the carotenoids from the bio fortified maize and to eliminate the oils which interfere with the chromatographic analysis, saponification and heat are required. They also found that the maize samples having high levels of oils may need additional base in order to achieve complete saponification so as to get better results. It was also revealed that in order to prevent the overestimation of carotenoids the addition of internal standard after heating was found to be useful.

Muzhingi *et al.* (2007) conducted a study on corn in which they evaluated the effect of saponification on the extraction of carotenoids in yellow maize. The study also involved the determination of major carotenoids in the 36 corn genotypes and to determine the cooking effect on the concentration of the carotenoids. Their study revealed that there was a significantly high yield of carotenoids without saponification. The study also showed that the concentration of carotenoids increased when the yellow maize was boiled at 100 □ for 30 minutes whereas there was a decrease in carotenoid concentration by 70% when the samples were baked at 70 □ for 25 minutes.

Fanning *et al.* (2009) conducted a study in 385 lines of a sweet corn breeding population in order to develop an analytical screening method which included the chromamameter measurement of hue angle and also optimized

extraction for HPLC. Their study revealed that there was no effect of saponification on the extraction of carotenoids. The study concluded that in order to obtain an increased efficiency for screening of germplasm with high zeaxanthin levels hue angle of 85□ may be used for liquid extraction.

Ibrahim and Juvik (2009) conducted an investigation that included the tocopherol and carotenoid estimation of 41 corn genotypes and 24 broccoli genotypes that were grown in various environments. The study aimed at partitioning the overall variation into the genetic, the environmental and the genotype by environment interaction (G x E) components in order to measure the phenotypic stability of the genotypes for carotenoids and tocopherols. The stability analysis revealed that the corn genotypes (IL451b *sh2* and IL2027-8 *sh2*) were relatively stable across all the seasons and years. They concluded that the sweet corn and broccoli germplasm which had increased carotenoid and tocopherol concentration could be further developed by conventional breeding protocols.

Luterotti and Kljak (2010) carried out a study in corn genotypes in order to determine the total carotenoids. The total carotenoid concentration in the samples ranges from 11 to 23 μg kg^{-1} in case of yellow corn while in case of white corn the range was found to be 0.7 to 0.9 μg kg^{-1}. In case of yellow corn grits the range was found to be 17 to 22 μg kg^{-1}.

Feng *et al.* (2014) carried out a study in sweet corn inbred lines in order to investigate the accumulation of tocopherols and carotenoids in the kernels of sweet corn at different days after pollination. They evaluated 10 sweet corn inbred lines at 30, 27, 24, 21, 18 and 15 days after pollination and found that the contents of carotenoids showed the following trend from high to low, zeaxanthin showed the highest concentration followed by lutein, β cryptoxanthin, β carotene and then α carotene. The concentration from high to low of various tocopherol components showed that the levels of γ tocopherol were the highest followed by α tocopherol

and then δ tocopherol. The study revealed that the tocopherol as well as the carotenoid content was found to be the highest in the sweet corn kernels 21 days after pollination. The study concluded that 21 days after pollination was the ideal time for sweet corn harvest.

O'Hare *et al*. (2014) conducted an experiment in order to bio fortify sweet corn genotypes with zeaxanthin. They were able to enhance the concentration of zeaxanthin in the sweet corn kernels to more than 2.0 mg/100g FM from 0.2 to 0.3 mg/100g FW at the early stage of the sweet corn development. They were therefore successful in decreasing the amount of sweet corn needed to provide the dosage of same dosage of zeaxanthin. They were able to increase the zeaxanthin concentration by more than double by changing the carotenoid synthesis pathway.

Rios *et al.* (2014) conducted a study on four different Brazilian maize genotypes to study the influence of grain color in the carotenoid profiles. They carried out the selection within the same genotype on the basis of a color scale, grouping the ears with the lightest yellow color in a separate group and the ears that had the darkest orange color in another group. The ears of the corn which were colored showed the highest level of total carotenoids. It was also found that the colorful ears of the genotype 'RS535' contained 300% more α and β carotene $\mu g/g^{-1}$ in comparison to the lighter colored ears of the same material. The study concluded that there was a considerable influence on the content of carotenoids because of the grain color and therefore the breeders could have the flexibility in selecting the genotypes in terms of grain color.

Song *et al.* (2015) conducted a study in order to compare the composition of carotenoids in sweet corn and waxy corn grains at the immature milk stage and the mature dough stage. They found that the grains of the waxy corn contained lower levels of total carotenoids in the range of 1.52 to 3.68 μg/g dry weight while the carotenoid content in case of the sweet corn grain were higher than that of the waxy corn grains and were in the range of 8.42 to 39.71 μg/g dry weight.

Song *et al*. (2016) carried out an investigation in order to compare the carotenoid composition of two carotenoid rich cultivars namely jingtian 5, a sweet corn cultivar and a field corn cultivar suyu 29. It was observed that during the kernel development a similar trend in the total carotenoid composition and concentration of various carotenoid components was almost the same in both of the cultivars. There was an upward trend in the contents of lutein, α cryptoxanthin, violaxanthin, zeaxanthin and β cryptoxanthin while as there was a declining trend in the contents of neoxanthin all the time. There was a significant positive correlation between violaxanthin, zeaxanthin and lutein contents with yellow coloration indicators for the suyu 29 field corn, whereas a weak correlation was found for the same in case of the sweet corn cultivar jingtian 5.

Liu *et al.* (2017) conducted a study in sweet corn genotypes during kernel development in order to evaluate the carotenoid biosynthesis, its accumulation and antioxidant activities. The study involved the analysis of carotenoid profiles, the pattern of expression of carotenogenic genes and the antioxidant activities in two sweet corn genotypes during the kernel development. Their study revealed that when the carotenogenic genes were showing high expression there was a higher accumulation of total carotenoids in the kernels. The study also revealed that three upstream genes (zmCRTISO, zmPDS and zmZDS) had a very significant role in the total carotenoid content during the kernel development. It was also found that the predominant components of carotenoids in sweet corn were β cryptoxanthin, zeaxanthin, α cryptoxanthin and lutein. The study also found two important stages for the accumulation of carotenoids in the sweet corn kernels.

2.4 Amylose estimation

Parker *et al.* (1935) carried out an investigation in order to evaluate the chemical and physical properties of soluble polysaccharides in sweet corn. The study involved isolating and purifying the water soluble polysaccharides from the sweet corn kernels and then comparing them with the α and β amylose from the

mature sweet corn starch. The study concluded that the two fractions of the water soluble polysaccharides were similar to the starch components of the sweet corn in some aspects and therefore the water soluble polysaccharides that were present in the sweet corn may be the units responsible for the starch grain formation.

Matheson (1975) conducted a study on sweet corn and normal corn in order to find a method of extraction of starch by which minimal depolymerization could be made possible. The study revealed that the amylose content showed a decline because of increase in the force of centrifugation. It was assumed that the sweet corn contained some granules which were smaller than starch and were of different sizes. These granules were believed to be made up of amylose and phytoglycogen. The study also revealed that the iodine absorption of the extract of the flour of sweet corn indicated that the ratio of the amylose fraction to that of the branched fraction (amylopectin and phytoglycogen) fraction was near the normal ratio of 1:4.

Duffus *et al.* (1978) conducted a study on sweet corn for evaluating the variation of the amyloplast size, relative number and the composition of carbohydrates in endosperm development. The study determined the total amylose content of different size classes and was expressed as the percentage of their α glucan content in each case. The concluded that the increase in the total amylose content of the total amyloplast α glucan during the maturation of the kernels might have been due to the changes in the numbers of various size classes and also due to alteration in their amylose content.

Boyer *et al.* (1981) conducted a study in sweet corn to compare the starch fractions of the normal maize with that of sweet corn. They found a very little difference between the two sugary and the two normal maize inbred lines. The normal maize starch had an amylose content of 25-30%. The sugary endosperm was extracted by two methods and both the methods revealed different

phytoglycogen and starch distribution. The phytoglycogen was assigned into a soluble and a particulate fraction which had the same amylose content in it.

Yeh *et al.* (1981) characterized the starch from different maize endosperm mutants. The maize endosperm mutants used in the study were normal, high amylose, waxy and other endosperm mutants. It was observed that the amylose content was influenced by the loosely branched polymers. The types of polymers produced were influenced by the sweet corn background of few endosperm mutants.

Boyer *et al.* (1982) conducted a study in sweet corn look for any possible relationship between starch and phytoglycogen. It involved treatment of the starch granules of the sugary (*su1*) corn with the branching enzyme I with capability of branching amylopectin as well as the amylose but not the glycogens. The treatment resulted in the release of phytoglycogen like glucan but glucan was not released. They showed that the non- solubilized amylose and amylopectin fractions were branched in incubationin case of the sugary starch. The results were consistent with the plastid changes and showed the conversion of the starch granules into phytoglycogen in the cells of the sugary endosperm.

Fisher and Boyer (1983) characterized the maize starch branching enzymes. They isolated purified fractions of the three starch branching enzymes the developing maize. They found that the two branching enzymes IIA and IIB were immunologically similar whereas the branching enzyme I was found to be distinct.

Wang *et al.* (1993) studied seventeen maize endosperm mutant genotypes for the characterization of their starch structures. The study involved the examination of the genotypes with the help of scanning electron microscopy and iodine affinity. The study revealed that there was an increased concentration of amylose and intermediate fractions in the sugary (*su1*), amylose extender (*ae*) and dull (*du1*) endosperm mutants as compared to normal starch. They also revealed

that the endosperm mutants containing *ae* gene had increased chain lengths of amylopectin. There was also a lower level of branching of amylopectin in the mutants which contained the *ae* gene, a low level of branching of amylopectin in the mutants having the *su1* or the *du1* gene was observed. They concluded that there was a very significant role of the genetic background on the structure of the components of starch.

Adjeno *et al*. (2013) conducted a study in maize to establish a relationship between the α amylase degradation and the amylopectin and amylose content of the starch of maize kernels. The study involved the measurement of amylose/amylopectin content of two different starch samples of maize. The starches were then digested by α amylase and then for each starch sample the degree of hydrolysis was compared to amylose/amylopectin content. It was found that the starch samples from two maize varieties depicted variable susceptibility to *B. cereus* α amylase attack. The study concluded that amylopectin content of the starch was inversely proportional to the susceptibility by *B. cereus* α amylase attack. It was also found that the maize starches had high amylose content also had a high value of dextrose equivalent.

Ketthaisong *et al.* (2015) studied waxy corn kernels for various physicochemical and morphological properties of starch. The starches from eight waxy corn genotypes were isolated at the immature kernel stage. The study revealed that variation was present in the kernels for starch content and was in the range of 77.6-90.97%. The study also revealed significant difference for the average chain length, pasting property and the unit chain length distribution.

Mir *et al.* (2017) conducted an experiment on five corn cultivars to investigate the structural and physicochemical properties of the cultivars. It was observed that there was a variation in the amylose content of the corn starches and the amylose content was in the range of 24.74-30.32g/100g. The mean granule size of the starch granules were also found to be in the range of 2.3 to 19.5μm.

Kou *et al.* (2018) investigated amylose content of the starch in maize kernels. The study reported a novel method of determining the amylose content of starch by a polarized microscope. Their study revealed good correlation with the results of that of the ISO method and their novel method was found to have potential in different aspects besides being quite quick and inexpensive. The method was also found to be less affected by human factors.

2.5 Screening against Turcicum leaf blight

Shikari and Gul (2009) studied maize under temperate field and controlled pot conditions to screen diverse set of genotypes for resistance to Turcicum leaf blight (TLB). The study involved growing of the sixty four genotypes under artificial epiphytotic conditions. There was no relation between the geographical origin and the disease reaction of the genotypes to TLB. The genotypes that were found to show resistance to the disease include composite girija, Ht-mono-genic sources and inbred NAI-147.

Ahangar *et al*. (2016) studied maize for screening the genotypes for resistance against TLB and to check the pathogenic variability in *Exserohilum turcicum.* They screened 26 maize genotypes for resistance against TLB. Eight genotypes were found to be resistant while other 8 genotypes were moderately resistant. A wide range of pathogenic variability of the eleven isolates of *Exserohilum turcicum* was observed.

Setyawan *et al.* (2016) conducted an experiment to study the resistance of hybrid maize genotypes to TLB. They tested 11 hybrid maize genotypes for the determination of the level of TLB resistance. Out of 11 genotypes 10 were found to be significantly better than the control genotypes at LSD 5% (α=0.05%).

Bhat *et al*. (2017) conducted a study in maize to screen seventy two hybrids along with the 18 parents and three checks for resistance to TLB under artificial epiphytotic conditions. At tasseling stage, 20 hybrids had a disease score of 2, 33 had a disease score of 1 and nineteen hybrids had a score of zero. The

percent disease index was in the range of 0-28.6%. Twenty days after tasseling fifteen hybrids had a disease score of 2 while one hybrid had a disease score of 1 and were classified as resistant and highly resistant, respectively. Three hybrids were highly susceptible with a disease score of 5 and twenty four hybrids were susceptible with a disease score of 4. The percent disease index was in the range of 20.40 to 68.57%.

Yousuf *et al*. (2017) screened maize land races against TLB. They evaluated seventy maize land races against TLB and found 34 lines resistant, 18 as moderately resistant, and 05 moderately susceptible. The land race Tral 3 was found to be highly susceptible with a disease intensity of 78.91%.

Hailu *et al.* (2018) screened maize inbred lines for their reaction to TLB and common leaf rust under artificial epiphytotic conditions. They screened 178 maize inbred lines for two consecutive years. Among 73 maize inbred lines 42 were found to be resistant and 3 were susceptible to TLB while as 33 inbred lines were found to be resistant and only 04 lines were found to be susceptible for common leaf rust disease.

Thakur *et al*. (2018) screened maize inbred lines for resistance against TLB. They studied parents as well as the crosses. Five lines and 31 cross combinations were resistant to TLB under artificial epiphytotic as well as natural conditions.

Bhaskar *et al*. (2020) evaluated maize germplasm for resistance to TLB. They screened of 335 maize entries for TLB resistance during the rabi 2018-2019 and Kharif 2019 season. Eighty-four lines were resistant while five were moderately resistant and two lines were moderately susceptible. Among the 243 lines, 12 lines were found to be resistant, 129 lines were found to be moderately resistant and 28 lines were found to be susceptible.

2.5 Molecular analysis

Smith *et al*. (1997) evaluated maize for the utility of SSR loci as molecular markers in comparison to RFLPS. They used 131 SSR markers using four hybrids and 58 inbred lines. The results obtained from the SSR markers were compared to the 80 RFLP probes. The PIC values of SSRs were in the range of 0.06 to 0.91 while that of the RFLPs ranged from 0.10 to 0.84. The study concluded that the SSR markers were advantageous over the RFLPs in terms of reliability, cost effectiveness, reproducibility and discrimination.

Enoki *et al*. (2002) conducted a study in maize access the genetic diversity of the inbred lines. They involved SSR analysis of 60 loci that were distributed throughout the genome of maize. The mean value polymorphic-index content (PIC) was found to be 0.69 for the SSR loci and it provided enough discrimination-ability in order to assess the genetic diversity among the inbred lines. They found that correlation between genetic-similarity estimates and coancestry coefficient was significant ($r = 0.70$). They concluded that the SSR analysis was effective for assessing the genetic diversity between the inbred lines of maize for assigning the lines to heterotic groups.

Sharopva *et al*. (2002) conducted a study in maize for isolating, characterizing and mapping a set of SSR markers for maize. The study led to the development of 1051 new SSR markers for maize from the microsatellite-containing sequences in the private and public databases and from the microsatellite enriched libraries. Their study led to the development of a novel high resolution map source for maize.

Amorim *et al*. (2003) characterized 13 sweet corn populations with the help of microsatellite and RAPD markers. The genotypes showed genetic variability among the genotypes analyzed. Among the 50 RAPD primers evaluated only fourteen showed scorable band patterns. The microsatellite study

assessed only 7 loci. The PIC values were found to be in the range of 0.50 to 0.89 in case of the microsatellite loci.

Bered *et al*. (2005) studied three sweet corn populations to characterize them. The study also aimed at investigating the molecular genetic diversity among and within the sweet corn populations and evaluating the potentiality of six SSR and 11 RAPD primers in detecting the intra-population and the inter-population variation in the sweet corn populations. There was a high similarity among the populations than that of within the populations. It was also found that the SSRs showed higher PIC values than that of RAPD.

Sridic *et al*. (2008) characterized 6 sweet corn inbreds with the help of SSR markers. The study involved the analysis of genetic similarity using 40 SSR primers. The molecular marker data was compared with that of the SCA data. SCA results were in accordance with the genetic similarity.

Qi *et al*. (2009) constructed a linkage map in sweet corn using SSR markers. They studied 208 F2 individuals from a cross between Ji65 and Ji557. The linkage groups were found to have a density in the range of 2.2 Cm to 65.3 Cm. Eleven QTLs were found to be associated with the kernel soluble sugar content out of which two QTLs showed additive effects, three showed partial dominance effects, three showed dominant effects and three showed over-dominance effects.

Almeida *et al*. (2010) estimated genetic variability in populations of sweet corn, common corn and teosinte. They screened two populations of all the three types of corn with the help of microsatellite markers. They observed a low intra-population genetic variability in the maize populations while there was a high variability in the teosinte populations which was found to be suggestive of the fact that there may be some limitations in future breeding cycles of the maize populations.

Sridic *et al*. (2011) conducted a study in sweet corn inbred lines to determine their genetic similarity on the basis of SSR markers and comparing it with the specific combining ability data (SCA) and heterosis for the yield of fresh ears, obtained from a diallel study. All the genotypes were found to have specific genotypic pattern on the basis of the SSR marker data. The estimates of GS were found to vary from 0.422 to 0.756. They concluded that the data of SCA and heterosis were concurrent with the data of GS based upon the SSR markers.

Kashiani *et al.* (2012) studied sweet corn inbred lines by the use of microsatellite markers to demarcate informative chromosomes. The study aimed at checking the genetic variation among the ten pairs of chromosomes that were extracted from the thirteen sweet corn inbred lines by the use of 99 microsatellite markers. The study revealed a vast genetic diversity. The allelic richness was found to be in the range of 2.78 to 4.33 with the mean value of 3.62 while the effective alleles per chromosome were found to be in the range of 1.96 to 3.47 with the mean value of 2.73. The study revealed that chromosome 10 was found to be the most informative and chromosome 2 was found to be the least informative. The presence of large amount of genetic variation in the chromosome numbers 10, 9, 8, 5 and 4 revealed that these chromosomes could be used for the study of genetic diversity in the tropical sweet corn lines. The chromosome 4 was found to have the largest number of loci in linkage disequilibrium and was considered to be ideal for QTL mapping and marker phenotype association.

Kashiani *et al*. (2012) studied on thirteen sweet corn inbred lines in order to investigate the genetic variability with the help of 95 microsatellite markers. Out of the total molecular variance 92.9% was due to the variation between the inbred lines. The inbreds were grouped into six heterotic groups on the basis of their molecular characteristics by the use of Fitch-Margoliash algorithm.

Hossain *et al*. (2013) identified and validated the microsatellite markers (SSRs) linked to the *sh2* and *su1* alleles in sweet corn. The bulk segregant

analysis showed that *su1* was present on the short arm of chromosome 4 with the bin number 4.05 and *sh2* was found to be present on the long arm of chromosome 3 with the bin number 3.08. The study also revealed umc2061 to be the closest marker from *su1* while as umc2276 was found to be the nearest marker from *sh2*.

Lopes *et al*. (2014) evaluated 16 sweet corn lines for genetic diversity with the help of microsatellite markers. They tested 100 pairs of SSR primers mapped for field corn. It was found that 15% of the primers used were polymorphic which identified 39 alleles. They also revealed the presence of 2 to 4 alleles per locus and the average alleles per locus was found to be 2.60. The loci that were found to have the highest number of alleles were umc1590, bnlg1083 and umc1241. The loci umc1506 was found to be polymorphic in 7 lines and the locus bnlg1083 was found to be polymorphic in 8 lines. The lowest genetic similarity was found between the lines DN28 and DN9 while as the lines DN6 and DN19 were found to have the highest genetic similarity.

Lopes *et al.* (2015) evaluated twenty two sweet corn cultivars using SSR markers. Forty five loci were polymorphic out of which thirty were used to evaluate the genetic diversity of the sweet corn cultivars. The study revealed the presence of 86 alleles using thirty SSR primers. The mean polymorphism was found to be 82% while as the Polymorphism information content was found to be in the range of 0.19 to 0.71. They concluded that majority of the genetic variability was condensed within the sweet corn cultivars (75%) and lesser (25%) variability between the sweet corn cultivars.

Jha *et al*. (2016) studied the complementation of the alleles that helps in the formation of normal kernels in the hybrid. They investigated allelic relationship by the use of complementation of the mutant genes in VL15 and VSL1 with sixteen diverse genotypes. From the set of first sixteen test crosses with VSL1, two test crosses, HKI 1831 x VSL1 and VSL11 x VSL1 showed complementation in the F1s and segregation in the F2s. The second set of test

crosses with VL15 showed complementation and segregation in all test crosses except HKI 1831 x VL15 and VSL11 x VL15. The study concluded that the complementation of the dissimilar mutants led to the normal kernel development while the sweet corn kernels in the hybrids were due to the non-complementation of the similar mutants.

Ko *et al*. (2016) conducted a study in sweet corn inbred lines in order to compare the efficiency of sequence specific amplified polymorphism (SSAP) and simple sequence repeats (SSR) for the analysis of population structure, genetic relationship and genetic diversity of the 87 sweet corn inbred lines. The SSR markers have greater average gene diversity as well as shannon's information index than that of the SSAPs. The dendrogram was constructed with the SSR marker data showed a complex pattern with GS of 53.0% and nine clusters while the SSAP marker data showed the presence of three clusters and GS of 50.85. The combined marker data results showed the presence of six clusters and a GS of 53.5%.

Simla *et al*. (2016) conducted a study in order to introduce sweetness character into a waxy background and to look for combination of genes suitable for the commercial exploitation by the use of sensory blind test. The study involved the incorporation of three genes both singly as well as in the combination of the two sweet genes. The genes that were used were *su*, *sh2* and *bt.* The study found out that out of the six crosses which had two gene combinations, four were found to be sweeter than that of all the crosses which had only single gene combination. The study concluded that the waxy trait was negatively related to crispiness and sweetness while as the sweetness showed a positive relation with the overall liking and crispiness.

Mehta *et al*. (2017) conducted a study in sweet corn inbred lines for genetic diversity analysis. The study involved the analysis of 48 sweet corn inbred lines involving *su1su1/sh2sh2, su1su1, sh2sh2* genotypes with 56 microsatellite

markers. The study revealed the presence of two unique and twelve rare alleles. The genetic dissimilarity was found to be 0.73 and the average PIC value was found to be 0.50. The inbreds were grouped into three major clusters on the basis of cluster analysis in which each *su1 su1/sh2sh2, su1su1, sh2sh2* were widely classified together. It was also found that the genotypes had diverse origin as depicted by the principal coordinate analysis. The study also identified prospective heterotic combinations in different genetic backgrounds.

Sharma *et al*. (2017) conducted a study in maize in order to reveal the genetic diversity of the inbred lines by the use of microsatellite markers. The study involved 33 maize inbred lines that were analyzed by 40 SSR markers. It was found out that among the 25 markers that were found to be reproducible 18 markers were found to be polymorphic and 7 markers were found to be monomorphic. The PIC value of the markers was found to be in the range of 0.1 to 0.56 and the average value was 0.36. The inbreds were grouped into two clusters on the basis of cluster analysis with Cluster I containing 25 genotypes and another cluster, Cluster II contained 9 genotypes. Both the clusters were divided into 2 sub clusters. The study found SSR markers to be good assisting tools in combination with morphological markers for the genetic characterization of maize.

Tosun *et al.* (2017) conducted a study in sweet corn inbred lines for the determination of sugar content and screening of the lines with SSR markers related to sugar content. The study involved the evaluation of 49 sweet corn inbred lines. They evaluated the total sugar content in the kernels of the sweet corn inbred lines after harvesting. The total sugar content in the 49 sweet corn inbred lines was found to be in the range of 0.66 to 16.84. The molecular marker screening which was done by the use of 10 SSR markers which were related to the sugar content genes revealed that the primers which were found to be immensely correlated with the sugar content of the 49 inbred lines were phi44 marker for the

gene *sh1*, phi328175 for the *ea1* gene, umc1031 for the *su* gene and umc2276 for the *sh2* gene.

Ferreira *et al*. (2018) conducted a study on 12 elite sweet corn lines with 20 microsatellite markers. The study involved the use of artificial neural network with a self-organizing map algorithm. The algorithm was found to identify 3 genetically differentiated groups and was found to produce comparatively more precise results than UPGMA. The study revealed the presence of high expected heterozygosity for 90% while as the PIC was found to be high for 40% of the SSR loci.

Mahato *et al*. (2018) carried out a study in sweet corn lines using microsatellite markers and agro-morphological traits. The study involved the assessment of genetic diversity of the 39 sweet corn inbred lines with the help of 63 microsatellite markers, two quality parameters and 14 agro-morphological characters. On the basis of the quality and agro-morphological traits the inbreds were grouped into three clusters using cluster analysis. The inbreds were also classified into three clusters on the basis of the microsatellite marker analysis. The study identified the presence of 82 alleles and the allele was found in the range of 80 to 400 bp. The major allele frequency of the markers was found to be in the range of 0.42 to 0.79 and the PIC value was found to be in the range of 0.27 to 0.63 which was found to be indicative of great amount of polymorphism between the inbreds.

Yu *et al*. (2019) conducted a study in sweet corn inbred lines for genetic diversity analysis. They screened of 37 sweet corn maize inbred lines with SSR markers. The study revealed the presence of 209 alleles with the help of 37 polymorphic SSR markers. The study also found that 5.65 alleles per SSR marker were present and the polymorphism information content of the markers were in the range of 0.62-0.95. The study concluded that there was abundant genetic diversity in the sweet corn inbred lines.

Zhang *et al*. (2019) conducted a study in sweet corn a pair of near isogenic lines were developed named W822GSe and W822Gse. The study involved high-resolution genetic mapping which revealed that the wild type *se1* was a gene Zm00001d007657 present on the chromosome number 2, the deletion of which would cause the *se1* phenotype. The comparative metabolic profiling of the tissue of the seed between the two isogenic lines showed exceptional dissimilarity in the carbohydrate metabolism with the high accumulation of maltose and sucrose in the mutant. The study also revealed that the *se1* mutant showed predominant expression in the endosperm while as its expression was low in the root and leaf tissues.

Chapter 3

MATERIAL AND METHODS

The present study titled "Morphological and Marker based Evaluation of Early Generation Sweet Corn (*Zea mays* L.) Inbred Lines for Yield and Quality Attributes" was carried out during Kharif 2020-21 at Mountain Research Centre for Field Crops, Khudwani, SKUAST Kashmir. The inbreds were grown in open field conditions over a homogenous plot of land. Individual plants were evaluated for agro-morphological traits and maturity. The harvested cobs were subjected to evaluation for kernel characteristics and quality.

3.1 Plant Material

The Experimental material comprised of 80 early generation Sweet Corn inbred lines that were developed from a cross between two sweet corn hybrids (Mithas and Sugar-75). Recommended agronomic practices were followed to raise a good crop.

3.2 Morphological characterization

The observations on various morphological traits were recorded as per the guidelines of IIMR, 2011. The traits for which observations were recorded are as:-

S.No	Characteristics
1	Leaf: The angle between the blade and the stem (on the leaf above the upper ear)
2	Leaf: The attitude of the blade of the leaf (On the leaf above the upper ear)
3	Stem: The anthocyanin coloration of the brace roots (whether Present or Absent)
4	Tassel: The time of the anthesis (on the middle third of the main axis, 50 % of plants
5	Tassel: The anthocyanin coloration at the base of the glume (in middle third of the main axis)
6	Tassel: The anthocyanin coloration of the glumes without the base (in middle third of the main axis)
7	Tassel: The anthocyanin coloration of the anthers (in middle third of the main axis on the fresh anthers)
8	Tassel: The density of the spikelets (in middle third of the main axis)
9	Tassel: The angle between the main axis and the lateral branches (in lower third of the tassel)
10	Tassel: The attitude of the lateral branches (in lower third of the tassel)
11	Ear: The time of silk emergence (50% of the plants)
12	Ear: The anthocyanin coloration of the silks (on the day of emergence)
12	Leaf: The anthocyanin coloration of the sheath (below the ear)

14 Tassel: The length of the main axis above the lowest side branch

15 Plant: The length of the plant (cm)

16 Plant: The placement of the ear

17 Leaf: The width of the blade (the leaf of the upper ear)

18 Ear: The length without the husk (cm)

19 Ear: The diameter without the husk (cm)

20 Ear: The shape of the ear

22 Ear: The type of the grain (in middle third of the ear)

23 Ear: The color of the top of the grain

24 Ear: The anthocyanin coloration of the glumes of the cob

25 Kernel: The arrangement of the rows (middle of ear)

26 Kernel: sweetness

27 Kernel: waxiness

28 Kernel: opaqueness

29 Kernel: shape

30 Kernel: 1000 kernel weight

3.3 Biochemical parameters

The 80 sweet corn inbreds were evaluated for various biochemical traits which include Total Carotenoids, Total Sugar and Amylose content.

3.3.1 Total carotenoids estimation

The total carotenoid content in the methanolic and the aqueous extracts of the sweet corn genotypes was estimated by the procedure reported by Mahadevan and R. srihar (1986).The procedure involved partitioning thrice the 20 ml extract with 10 ml petroleum ether with the help of a separatory funnel. Thereafter with the help of a rotary evaporator the samples were evaporated to dryness at 35 ^{0}C. The remnant obtained was dissolved in 10 ml ethanol. After that it was kept in a shaking water bath for 30 minutes at 37 ^{0}C with alcoholic KOH cation. 15 ml of DDH_2O was added afterwards and the saponified extract was put in a separatory funnel which contained 15 ml of petroleum ether. The funnel was shaken gently in order to mix the two so that the carotenoid pigments are taken up by the petroleum ether layer. The aqueous phase was put into another separating funnel and the upper petroleum ether extract which contained the carotenoid pigments was transferred to an amber colored bottle. The lower aqueous phase which was transferred to another funnel was then subjected to extraction thrice using petroleum ether till it turned colorless. The colorless aqueous layer was then discarded. A small amount of sodium sulfate was put in the petroleum ether extract in order to remove the turbidity. The final volume of petroleum ether extract was measured and absorbance of the extract was recorded against a reagent blank at 450 nm in a spectrophotometer.

The total carotenoid content of each of the sweet corn sample was calculated by the formulae

Carotenoid (microgram) = P × 4 × V × 100 × W

P = optical density of the sample

V= volume of the sample

W= weight of the sample

3.3.2 Amylose estimation

The amylose content of the sweet corn inbred lines was evaluated by the method given by Julianio *et al*. (1971). 80 sweet corn kernels were ground to form a fine powder with the help of a mortar and a pestle and then sieved through a 0.44mm screen. Fifty milligrams of the finely ground powder was weighed and transferred into a long test tube (150 × 15 mm). To each of the test tubes was added 0-5ml of absolute ethanol followed by agitation for 5 minutes. After that, 5ml of 1N NaOH (40g NaOH pellets dissolved in 500ml of double distilled water and then the volume was made up to 1000ml) was added followed by covering with aluminum foil and kept at room temperature overnight. Next day the solution was given a thorough shaking and put in a water bath for 15 minutes. The samples were then transferred into 100ml volumetric flasks followed by addition of 44.5 ml of de-ionized distilled water. 2.5ml of extract were taken from each sample and transferred into a fresh test tube. To the fresh test tube were added 3 drops of 0.1% phenopthalein and 20ml de-ionized distilled water and the contents mixed until pinkish color appeared which indicated the alkaline pH. 0.1N HCL was added drop by drop (till the pink color disappeared) in order to neutralize the contents. 1 ml of iodine was added and the volume was made up to 50ml and then the absorbance was read at 620nm with the help of a spectrophotometer. The standard curve was drawn by dissolving 100 mg of standard potato amylose in 10ml of 1N NaOH and then the volume was made 100 ml with de-ionized distilled water. Three replicates of the standard were taken by taking 1ml, 2ml, 3ml, 4ml and 5ml volumes of the standard amylose solution and pipetting out into three volumetric flasks. 0.2ml, 0.4ml, 0.6ml, 0.8ml and 1 ml acetic acid were added to the 1ml, 2ml, 3ml, 4ml and 5ml of the standard amylose solution followed by addition of 2ml KI solution to each of the volumes. The volume was then made up to 100ml and then the flasks were then covered with the aluminium foils. The absorbance was then noted after 20 minutes with the help of a spectrophotometer. The amylose content was then approximated by the formula:

Per cent Amylose (w/w) in sweet corn flour = A × dilution factor × 2 × extract volume/1000

$= A \times 20 \times 50 \times 2/1000$

$= A \times 2$

A= amount of amylose in ug/ml based on O.D

Table 1. Scale for Amylose content

Class	Amylose content%
Waxy	1-2%
Very Low	2.1-9%
Low	9.1-20%
Intermediate	20.1-25%
High	25.1-33%

3.3.3 Total sugar estimation.

The total sugars in the sweet corn kernels were estimated by the method reported by Ranganna (1986) with certain modifications. 10g of finely ground kernels from all the 80 samples was weighed and transferred to a 100ml beaker/volumetric flask. 10 ml of distilled water was added to each of the dry samples. An aliquot of 10ml filtrated, clarified and de-leaded filtrate was pipetted out and transferred into a 100ml volumetric flask. 5ml of concentrated HCL was added to each of the flasks containing the samples and were allowed to stand at room temperature for 24 hours. The samples were next day neutralized with concentrated NAOH solution and using phenolphthalein as an end point indicator. The volume was made up to 250ml and transferred to a 50ml burette having an off-set tip. 5ml of each Fehling's A and Fehling's B solution was pipetted out into a 250ml conical flask. 25ml water was added and the flask was heated to boiling and 5 to 6 drops of methylene blue indicator was added and the titration was

continued until the blue color disappeared to a brick- red end point. The reading at the color change was recorded and the total sugars were estimated by the following formula.

Total sugars (%) =

$$\frac{0.05 \times 250}{T.V \times 10} \times 100$$

T.V= Titration value

3.4 Screening against Turcicum leaf blight

Screening of sweet corn genotypes against Turcicum leaf blight (TLB) was carried out under field conditions. Disease reaction was recorded on 1 to 9 evaluation scale of Indian Institute of Maize Research, Ludhiana (Aggarwal *et al*., 2021).

Percent Disease Intensity (PDI) was calculated as:

$$\text{PDI} = \frac{\text{Sum of individual ratings}}{\text{No. of plants observed x maximum disease grade}} \text{ x } 100$$

On the basis of the PDI, the inbreds were categorized as Resistant (R), moderately susceptible (MS) and susceptible (S).

Table. 2. Disease screening scale

Rating scale	Degree of infection (per cent DLA*)	Disease reaction
1.0	Nil to very slight infection (≤10%).	Resistant (R)
2.0	Slight infection, a few lesions scattered on two lower leaves (10.1-20%).	
3.0	Light infection, moderate number of lesions scattered on four lower leaves (20.1-30%).	
4.0	Light infection, moderate number of lesions scattered on lower leaves,a few lesions scattered on middle leaves below the cob (30.1-40%).	Moderately resistant (MR)
5.0	Moderate infection, abundant number of lesions scattered on lower leaves, moderate number of lesions scattered on middle leaves below the cob (40.1-50%).	
6.0	Heavy infection, abundant number of lesions scattered on lower leaves, moderate infection on middle leaves and a few lesions on two leaves above the cob (50.1-60%).	Moderately susceptible (MS)
7.0	Heavy infection, abundant number of lesions scattered on lower and middle leaves and moderate number of lesions on two to four leaves above the cob (60.1-70%).	
8.0	Very heavy infection, lesions abundant scattered on lower and middle leaves and spreading up to the flag leaf (70.1-80%).	Susceptible (S)
9.0	Very heavy infection, lesions abundant scattered on almost all the leaves, plant Prematurely dried and killed (>80%).	

*DLA- Diseased leaf area

3.5 DNA isolation and genotyping

3.5.1 Genomic DNA isolation.

The genomic DNA of all the sweet corn inbreds along with the parents was isolated from fresh and young leaf tissues of twenty day old seedlings following the Cetyl-Tri Methyl Ammonium Bromide method (Murray and Thompson.1980) with certain modifications. The procedure for the isolation of the genomic DNA was as follows:

1. The leaf samples were ground to a fine powder in liquid nitrogen with sterilized, mortars and pestles.
2. The powder was put in a 2ml polypropylene tube which contained 800 µl pre-warmed (65^{o}C) DNA extraction buffer.
3. The homogenate was then incubated at 60^{o}C for 60 minutes with alternate shaking at an interval of 15 minutes.
4. The tubes were left to cool at room temperature and an equal volume of chloroform: isoamyl alcohol (24:1) was added. The solution was then mixed by mild overturn of the tubes for 5 minutes. It is done for the removal of phenols and some of the other non-aqueous constituents such as lipids and proteins.
5. The contents were centrifuged at 10000 rpm for a time period of 10 minutes at 4^{o}C. The resulting supernatant was cautiously shifted to a 1.5ml new tube through a wide bore tip in order to avoid the DNA shearing.
6. An equivalent volume of chloroform: isoamyl alcohol (24:1) was added for execution of the second wash. The preceding step was repeated.
7. Pre-chilled iso-propanol, 0.6 volume of the content was added followed by gentle overtaxing until fibrous mass was observable. The Tubes were then incubated at -20^{0}C for 60 minutes.
8. The tubes were centrifuged at 5000 rpm for a period of 10 minutes at a temperature of 4^{o}C for forming a precipitate. The supernatant was then

discarded by slowly upturning the tubes. The tubes were then inverted and left open on a blotting paper for draining the remaining iso-propanol.

9. After some time, the DNA pellet was washed two times using 70% ethanol and left overnight at room temperature in order to dry the pellet.
10. Then 100-200 µl of 1X TE buffer (pH 8.0) was added for dissolving the pellet.
11. 6-8 hours later, 2-3 ul of RNase (10 mg/ml) was added to each of the 100 ul of crude DNA solution. The mixture was then incubated in a water-bath for 60 minutes at 37^{o}C with alternate mixing.
12. The purification of the DNA was done by addition of an equivalent volume of Chloroform: isoamyl alcohol 24:1 to the aqueous phase followed by mixing gently for 5 minutes and centrifuging at 10000 rpm.
13. The aqueous part was transferred to a new tube to which 1/10th volume of 3M Sodium-Acetate (pH5.2) was added, mixed and then two volumes of chilled ethanol were added to the mixture, mixed lightly and incubated at -20^{o}C for 2 hours.
14. The tubes were then centrifuged for 5 minutes at 10000 rpm. The supernatant was decanted and the pellet was washed twice using 70% ethanol. The pellet was then dried well (overnight air drying) and dissolved in 100ul TE buffer (pH 8.0).

3.5.2 Assessment of quality and quantity of DNA

The quantity of DNA was assessed through Agarose gel electrophoresis. 0.8 g agarose was dissolved in 100 ml 1X TAE electrophoresis buffer (Tris base-45mM, Acetic acid- 45mM and EDTA- 1mM).The mixture was then heated until agarose dissolved completely and the solution was then cooled down to 60°C through continual stirring. Ethidium bromide was added to the final concentration of 1X TAE buffer. The agarose solution was then transferred to a previously arranged gel mold with combs and was left for 20-30 min to

solidify. The DNA samples meant for loading were prepared by adding 2 µl loading dye (6X) (0.25% w/v bromophenol blue, 50% glycerol in sterile water) to the 8 µl DNA. Then sterile water was added in order to make the volume (100ml) such that the final concentration of the loading dye was 1X. Then the DNA samples were loaded in the wells through a micropipette. Together with the DNA samples, marker of a known concentration (uncut λ DNA of 50 ng/µl concentration) was also loaded. The gel was run for 20-30 minutes at 70 V/cm. The gel was finally visualized with a UV trans-illuminator. The DNA samples were then photographed and intensity of fluorescence for each sample was compared to a standard marker and then the DNA concentration of each sample was determined. The quality of DNA samples was adjudged based on if the DNA showed a single high molecular weight band (good quality) or a smear (poor quality). The samples were diluted according to the DNA concentration and were then loaded yet again on 0.8% gels till all of the samples finally reached at ~25 ng/µl in a uniform manner.

3.5.3 Selection of primers.

The sweet corn lines were screened with the help of five SSR (Simple Sequence Repeats) markers linked to sweetness genes (*Su1, Se1* and *Sh2*). The list of primers along with their sequences and position are given in table 3

Table 3 List of primers

Locus	Location	Marker	Forward	Reverse
Su1	Chromosome 4	**bnlg1937**	AATGCTCGGTCCACAGAATC	AACTGGAGCCAAAAGTGGTG
		umc2061	GTCTGGAGAACTCCCTACCCATTC	TAGCTTGAGAGACCGGAACAGC
Se1	Chromosome 2	**UMC1736**	CCATCCACCACTAGAAAGAGAGGA	TTAATCGATCGAGAGGTGCTTTTC
Sh2	Chromosome 3	**umc1320**	TGCGAAATCTGTATACCATAGGCA	CTCTTTTAGCAGTGTGCCGAATTT
		umc2276	CTAGGTAGCCAGCTAGGTACGGGT	AGTGGAGCTTCTTCATCCTACCG

https://www.maizegdb.org

3.5.4 Polymerase Chain Reaction

The PCR reaction mix consisted of 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 the reverse primer and 3 U of Taq DNA polymerase (MBI, Fermentas, Lithuania, USA) in a reaction volume of 20 µl. The contents of reaction mixture are given in table

Table 4 PCR Reaction mixture contents

Reagent	Stock conc.	Aliquot	Final conc.
DNA	25 ng/µl	1.5 µl	25 ng
PCR buffer	10x	2 µl	1X
dNTP mix	2mM	0.8 µl	0.2mM
Forward Primer	5 pM	2 µl	
Reverse Primer	5 pM	2 µl	
Taq DNA polymerase	3 U/µl	0.2 µl	0.6 U
Sterile water	-	11.5 µl	-
Total		20 µl	-

The Polymerase chain reaction (PCR) was done in a thermal cycler (TAKARA, Japan) with the following thermal regimes:

Table 5 PCR thermal regimes

Step	Temperature (^{0}C)	Time (minutes)	No. of cycles
Initial denaturation	94	4	1
Denaturation	94	1	35
Annealing	55-59	1	
Elongation	72	2	
Final Extension	72	7	1
Hold	4		

3.5.5 Resolution of amplified PCR products.

The gel electrophoresis is an extensively used technique for the investigation of nucleic acids and proteins. We used this technique for the size separation of the amplified DNA. 3.5% (w/v) agarose gel was made by dissolving 17.5 g of Agarose powder in 500 ml of 1X TAE [490 ml double distilled water + 10 ml 50X TAE buffer (242.2g Tris base: Mwt. 121.14; 100 ml of 500mM EDTA: pH 8.0; 57.1ml Glacial acetic acid: Mwt. 61.83; volume made up to 1000 ml with de-ionized Milli-Q water)] in a conical flask. The suspension was microwaved in order to heat it for 10 minutes at 900 watt until the solution was cleared. The solution was cooled down at room temperature and to it was added 25 µl (0.05 µl/ml of 1X TAE) Ethidium Bromide stock solution (10mg/ml of double distilled water). After mild shaking, the gel was transferred onto a gel casting tray. 15-20 minutes later the gel was immersed into the gel tank which contained the 1x TAE (PH 8.0). Each of the 10 µl PCR products was added 1 µl 6X loading dye (0.25% bromophenol blue; 0.25% xylene cyanol FF; 40% sucrose). By using 10 µl pipettes the samples were then loaded into the individual wells. In parallel, was also loaded 50 bp size reference ladder (Fermentas, Lithuania, USA). The power pack was set up at 5 Volts/cm of run and the total duration of the electrophoresis was from 1.5 to 2.5 hrs. After optimal run of the samples the gel slabs were then envisaged under a UV trans-illuminator and pictures of the gel slabs were taken for evaluation and scoring.

Chapter-4

EXPERIMENTAL FINDINGS

4.1 Morphological characterization

In the present study the morphological evaluation was performed for the 80 Sweet corn **(***Zea mays* L.) inbreds according to the guidelines specified by the DUS descriptor of Indian Institute of Maize Research (IIMR), ICAR, New Delhi, 2011. Thirty DUS characters were studied according to the descriptor and state and code was given for each trait. The results of all 30 traits recorded according to the frequency distribution on the basis of state and code and are presented in the Table 6. The data revealed a wide range of variability for all the traits that were studied. Among the leaf traits, the angle between the stem and the blade was scored between small and wide with a frequency of 61.25 and 38.75, respectively. The character attitude of leaf blade showed a frequency of 60 for the drooping attitude while as a frequency of 40 was found for the straight attitude of the leaves. The anthocyanin coloration of the brace roots was found to be absent in most of the inbreds with a frequency of 87.5. The time of anthesis of the tassel was found to be late in all the inbreds with a frequency of 100. The anthocyanin coloration of the base of the glume (tassel) showed a frequency of 76.25 for absent while as a frequency of 23.75 was found for present. The anthocyanin coloration of the glumes excluding the base (tassel) was scored as absent and present which revealed a frequency of 55 and 25 respectively. The anthocyanin coloration of the anthers was found to be present in most of the inbreds with a frequency of 72.5. The density of the spikelets of the tassel was mostly dense with a frequency of 80. The angle between the main axis and the lateral branches had a frequency of 82.5 for the wide leaf angle. The attitude of the lateral branches of the tassel showed three classes of expression and most of the inbreds expressed the curved attitude of the lateral branches with a frequency of 57.5, followed by the strongly curved attitude with a frequency of 25 and the straight attitude with a

frequency of 9. The time of silk emergence was found to be late in all the inbreds with a frequency of 100.The anthocyanin coloration of the silks of all the inbreds was absent with a frequency of 100. The anthocyanin coloration of the sheath (leaf) showed a frequency of 100 for being absent. The length of the main axis of the tassel was long in almost all the inbreds with a frequency of 98.75 and medium in some of the inbreds with a frequency of 1.25.The plant length was long in all the inbreds with a frequency of 100. The placement of ears on the plant was high in most of the inbreds with a frequency of 50, followed by the medium placement of the ears with a frequency of 45 and low placement of ears was found in some inbreds with a frequency of 5. The width of the leaf blade was broad in maximum number of inbreds with a frequency of 83.75. The length of the ear without husk showed a frequency of 63.75 for medium class and the frequency of 36.25 was found long ear length. The diameter of the ear without husk showed a frequency of 71.25 for medium ear diameter while as a frequency of 28.75 was found for the large ear diameter. The shape of the ear of the inbreds was conico-cylindrical in all cases with a frequency of 100. The number of rows of the grains on the ear was many in the maximum number of inbreds and showed a frequency of 95 while as a frequency of 5 was observed for the medium number of rows of the grain. The type of the grain was dent in all the ears of the inbreds with a frequency of 100. The color of top of grain was yellow with cap in all the kernels of the ears and showed a frequency of. The anthocyanin coloration of the glumes of the cob was white with a frequency of 100. The kernel row arrangement was scored into two classes, mostly the arrangement of the rows was straight with a frequency of 80 while as a frequency of 20 was observed for the spiral state. The kernel sweetness was present in all the kernels with a frequency of 100. The kernel waxiness in the kernels of the inbreds was absent in all the cases and showed a frequency of 100. The kernel opaqueness was absent with a frequency of 100. The kernel shape was observed to be shrunken in all the cases and showed a frequency of 100. The 1000 kernel weight of the kernels was small in all the inbreds with a frequency of 100.

Table 6: Frequency distribution of morphological traits in the Sweet corn (*Zea mays* L.) inbreds

Characters	Expression	Number of inbreds	Frequency
Leaf angle	Small	49	61.25
	wide	31	38.75
Leaf attitude	Straight	32	40
	Drooping	48	60
Anthocyanin coloration of the brace roots	Absent	70	87.5
	Present	10	12.5
Tme of anthesis of the tassel	Very early	0	0
	Early	0	0
	Medium	0	0
	Late	80	100
Coloration at base of glume of tassel	Absent	61	76.25
	Present	19	23.75
Coloration of glumes excluding base of tassel	Absent	44	55
	Present	36	45
Coloration of anthers	Absent	22	27.5
	Present	58	72.5
Density of spikelets of the tassel	Sparse	64	80
	Dense	16	20
Angle between main axis and lateral branches of the tassel	Narrow	14	17.5
	Wide	66	82.5
Tassel: attitude of lateral branches	Straight	9	11.25
	Curved	46	57.5
	Strongly curved	25	31.25
Time of silk emergence	Early	0	0
	Medium	0	0
	Late	80	100
Anthocyanin coloration of silks	Absent	80	100
	Present	0	0
Anthocyanin coloration of sheath	Absent	80	100
	Present	0	0
Length of tassel	Short	0	0
	Medium	1	1.25
	Long	79	98.75
Length of plant	Short	0	0
	Medium	0	0
	Long	80	100
Ear placement	Low	4	5
	Medium	36	45

	High	40	50
Width of leaf blade	Medium	13	16.25
	Broad	67	83.75
Length of ear without husk	Short	0	0
	Medium	51	63.75
	Long	29	36.25
Diameter of ear without husk	Small	0	0
	Medium	57	71.25
	Large	23	28.75
Shape of the ear	Conical	0	0
	Conico-cylindrical	80	100
	cylindrical	0	0
Number of rows in ear	Few	0	0
	Medium	4	5
	Many	76	95
Type of grain	Flint	0	0
	Semi flint	0	0
	Dent	80	100
Color of top of grain	White	0	0
	White with cap	0	0
	Yellow	0	0
	Yellow with cap	80	100
Anthocyanin coloration of glumes of the cob	white	80	100
	Light purple	0	0
Row arrangement of kernels	Straight	64	80
	Spiral	16	20
Sweetness of the kernels	Absent	0	0
	Present	80	100
Waxiness of kernels	Absent	80	100
	Present	0	0
Opaqueness of kernels	Absent	80	100
	Present	0	0
Shape of kernels	Shrunken	80	100
	Round	0	0
1000 kernel weight	Very small	0	0
	Small	80	100
	Medium	0	0

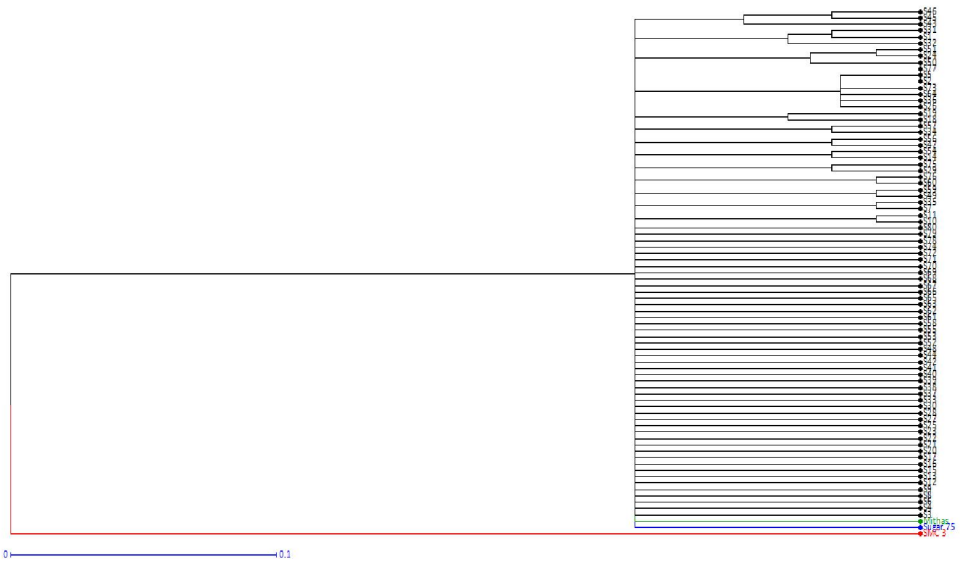

Fig. 1(a): Dendrogram showing the classification of the inbreds into clusters based on trait diversity

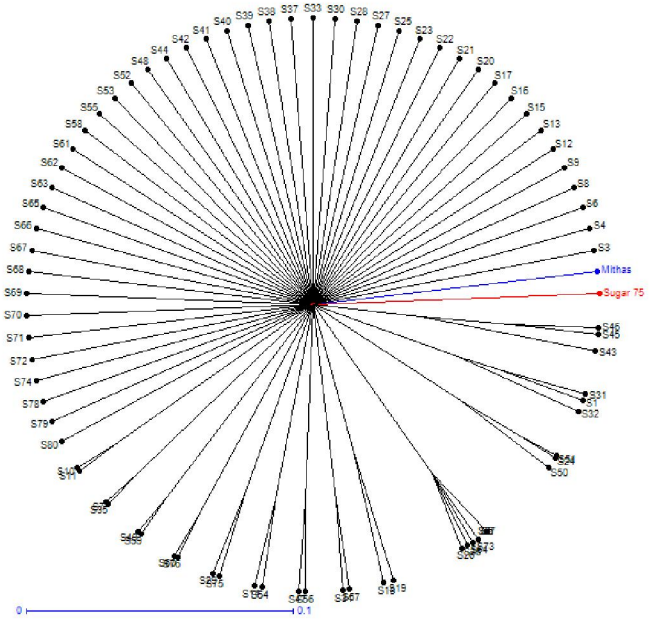

Fig. 1(b): Dendrogram showing the classification of the inbreds into clusters based on trait diversity

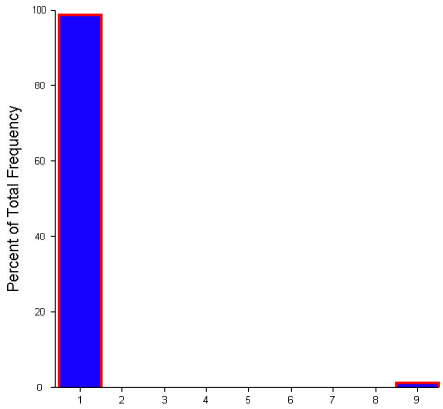

Fig. 2(a): Histogram of anthocyanin coloration of silks
Absent=1, Present=9

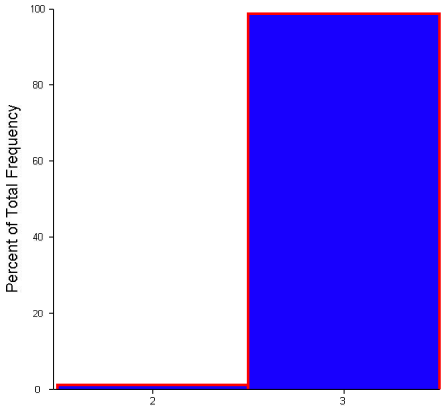

Fig.2 (b): Histogram of type of grain
Flint=1, Semi flint=2, Dent=3

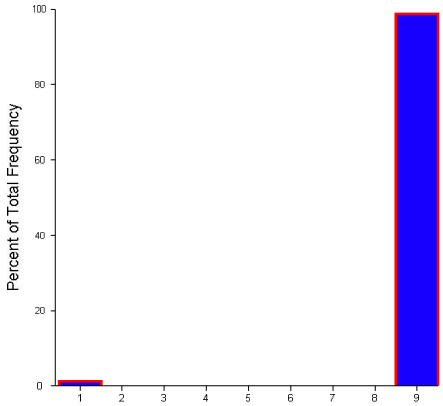

Fig. 2(c): Histogram of kernel sweetness
Absent=1, Present=9

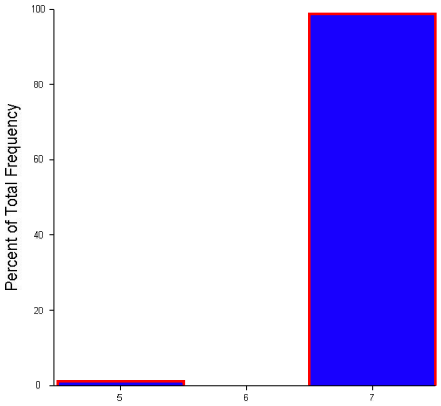

Fig.2 (d): Histogram of plant height
Medium=5, Long=7

Fig. 2: Histogram representing frequency distribution of the morphological traits

Contd…

Fig. 2: contd….

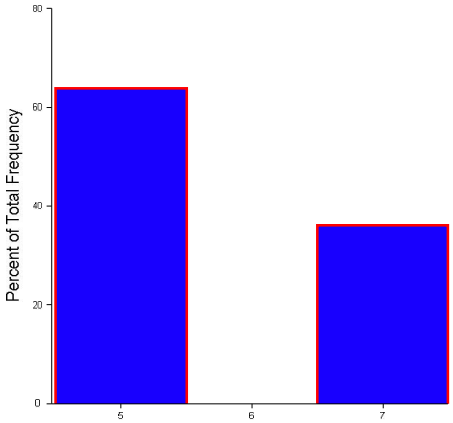

Fig.2 (e): Histogram of ear length;
Medium=5, Long=7

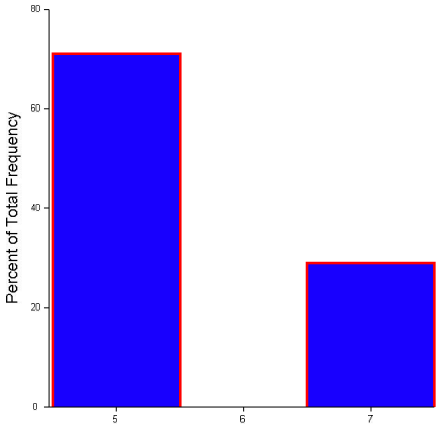

Fig.2 (f): Histogram of ear diameter
Medium=5, Large=7

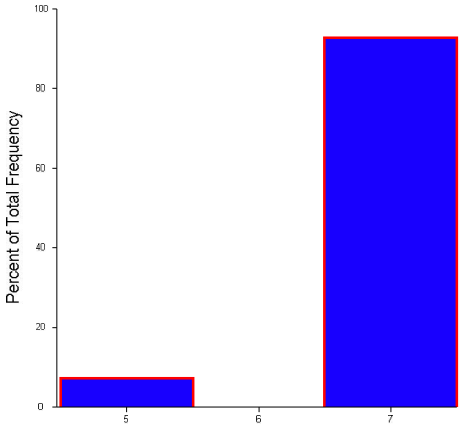

Fig.2 (g): Histogram of number of rows of grain
Medium=5, Many=7

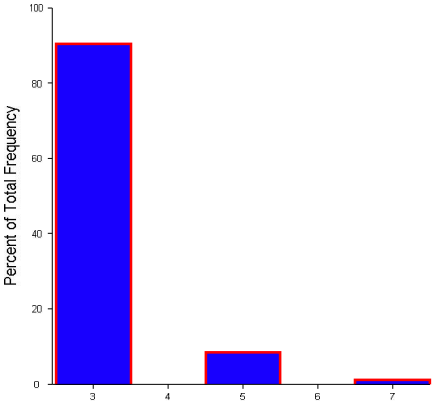

Fig.2 (h): Histogram of 1000 kernel weight
Small=2, Medium=5, Large=7

Plate 1: **Collection of the observation of agro-morphological traits and Selfing the plants**

4.1.1 Cluster analysis of a set of inbreds using morphological traits

The dendrogram obtained on the basis of the analysis of the morphological characters of all the 80 sweet corn inbreds is shown in figure 1(a) and 1(b). The cluster analysis resulted in grouping of the 80 sweet corn inbreds into two broad clusters. SMC 3, a non-sugary genotype grouped separately while as the rest of the inbreds were classified as a separate cluster along with the two parents. The cluster analysis was also performed along with the parents without the non-sugary genotype SMC 3 in order to look for further differences between the parents and the inbreds. The cluster analysis showed the inbreds clustering with the parents and some of the inbreds clustering separately (Fig.1b)

4.2 Biochemical analysis of Sweet Corn inbreds

4.2.1 Total carotenoids

The 80 sweet corn inbreds along with the two parents Sugar75 and Mithas and the two non-sugary genotypes SMC 3 and Black Pearl varied significantly with respect to the total carotenoid content and showed a range of 9-34 and a mean of 20.07 as shown in table (7) and fig (3). The highest amount of total carotenoids was found in the inbred S27 with a total carotenoid content of (34µg g^{-1}) followed by the inbred S65 with a total carotenoid content of (31.1µg g^{-1}). The lowest amount of the total carotenoids was found in the inbred S56 with a total carotenoid content of (9µg g^{-1}) followed by the inbred S60 with a total carotenoid content of (11µg g^{-1}). The estimation of biochemical attributes, significantly revealed that the total carotenoids of sweet corn inbreds showed a normal distribution pattern (Fig 4).

Table 7: Total Carotenoid content in the methanolic extracts of the sweet corn inbreds (µg g^{-1})

Genotype	Total Carotenoids(µg g^{-1})	Genotype	Total Carotenoids
Sugar-75	25	S43	19.6
Mithas	19.2	S44	22.8
SMC-3	4.2	S45	27
Black Pearl	3.4	S46	22.5
S1	14.4	S47	13.3
S2	16	S48	22
S3	14.9	S49	14
S4	21	S50	23.3
S5	20	S51	14
S6	14	S52	18
S7	15	S53	22
S8	21	S54	24.9
S9	28	S55	13.3
S10	25	S56	9
S11	23	S57	18.2
S12	21	S58	22
S13	19	S59	16.7
S14	23.2	S60	11
S15	22.5	S61	16
S16	27	S62	21.3
S17	16	S63	26.8
S18	17	S64	26.2
S19	16.1	S65	31.1
S20	18.9	S66	22.7

S21	12	S67	13.4
S22	15.2	S68	18.4
S23	31	S69	22.2
S24	25	S70	26
S25	26	S71	23.5
S26	27	S72	22
S27	34	S73	24.5
S28	15	S74	22
S29	12	S75	17.7
S30	19.8	S76	16.4
S31	17.6	S77	18
S32	26.7	S78	24
S33	21	S79	24.6
S34	24.4	S80	13
S35	25	Mean	20.07
S36	30	Standard deviation	5.81
S37	27	Standard error	0.63
S38	13.2		
S39	17.7		
S40	16		
S41	25		
S42	17.5		

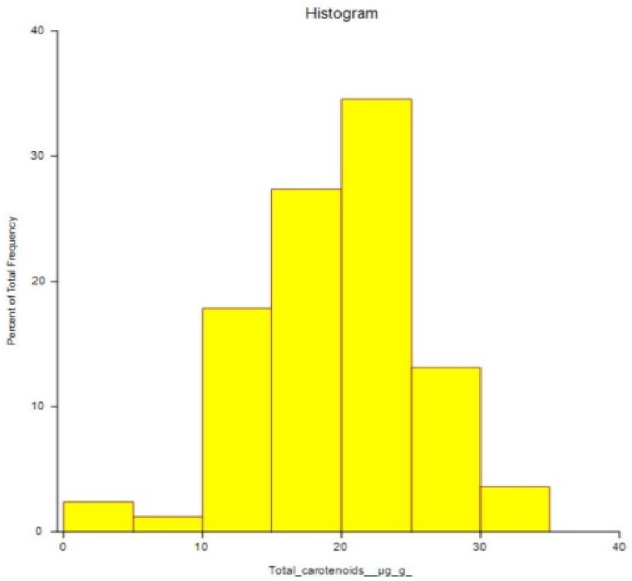

Fig. 3: Histogram of total carotenoids

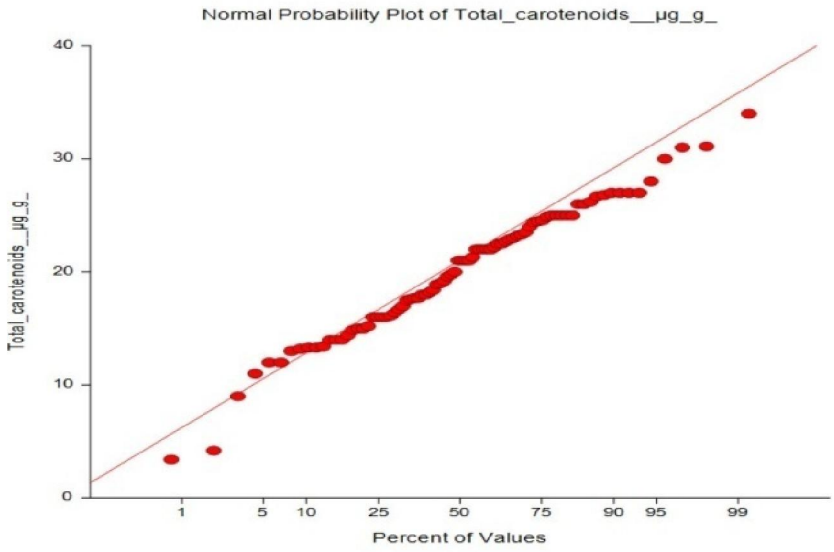

Fig. 4: Normal probability plot of total carotenoids

4.2.2 Total sugars

The total sugar of the 80 sweet corn genotypes along with the two parents Sugar75 and Mithas and the two non-sugary genotypes SMC 3 and Black Pearl was estimated and showed a range of 1.3-8.5 with a mean of 5.5 as shown in table (8) and Fig.(5). The highest amount of total sugar was found in the inbred S60 which had a total sugar content of 8.54% followed by the inbred S14 that showed a total sugar content of 8.34%. The lowest sugar content was found in the inbred S80 with a total sugar content of 1.3% followed by the inbred S71 which showed a sugar content of 2.1%. Based on biochemical analysis of 80 sweet corn inbreds along with the parents a normal probability plot was constructed for total sugars which revealed that the total sugars of sweet corn inbreds showed a normal distribution pattern (Fig. 6).

Table 8: Total sugars of the sweet corn inbreds (%)

Genotype	Total sugars (%)	Genotype	Total sugars
Sugar-75	10.87	S43	4.66
Mithas	7.34	S44	8.11
SMC-3	2.18	S45	6.28
Black Pearl	1.33	S46	3.38
S1	3.34	S47	4.45
S2	3.31	S48	5.95
S3	3.78	S49	4.55
S4	4.47	S50	7.12
S5	5.78	S51	8.10
S6	7.34	S52	3.67
S7	4.52	S53	6.21
S8	6.58	S54	4.33
S9	6.82	S55	3.58
S10	6.35	S56	5.11
S11	4.48	S57	6.66
S12	3.92	S58	3.88

S13	2.98	S59	7.77
S14	8.34	S60	8.54
S15	7.45	S61	7.75
S16	5.45	S62	5.96
S17	6.28	S63	6.66
S18	3.88	S64	4.58
S19	4.76	S66	6.67
S20	5.82	S65	3.45
S21	5.98	S67	3.88
S22	4.97	S68	8.01
S23	5.58	S69	7.06
S24	6.55	S70	5.55
S25	7.32	S71	2.10
S26	3.39	S72	7.67
S27	6.21	S73	4.35
S28	4.57	S74	6.34
S29	7.15	S75	3.88
S30	3.66	S76	4.54
S31	5.58	S77	5.34
S32	7.11	S78	3.55
S33	6.11	S79	5.55
S34	4.85	S80	1.3
S35	7.71	Mean	5.5
S36	6.61	Standard deviation	1.78
S37	5.55	Standard error	0.19
S38	7.95		
S39	4.28		
S40	4.33		
S41	7.36		
S42	5.85		

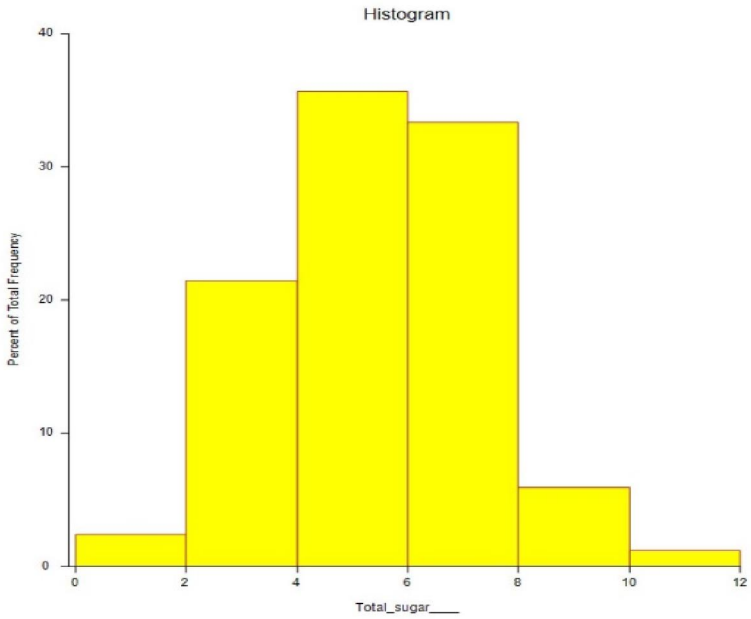

Fig. 5: Histogram of total sugars

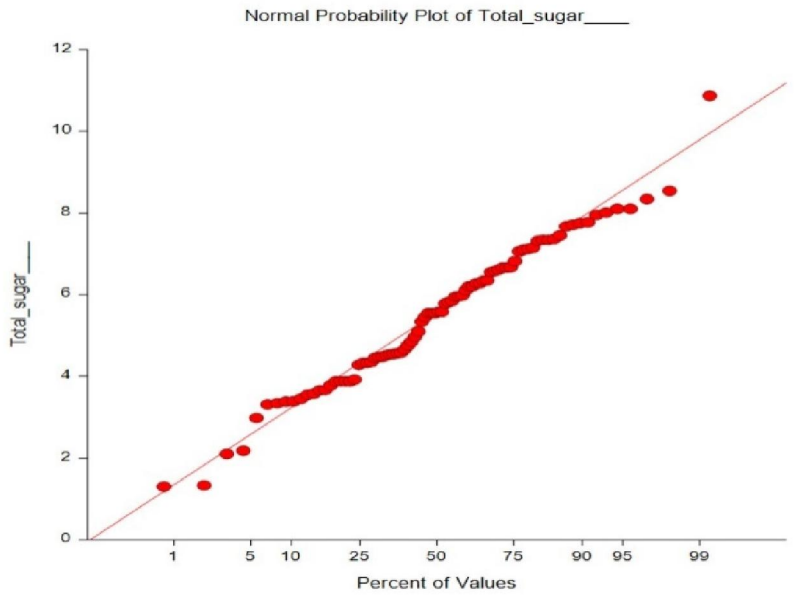

Fig. 6: Normal probablity plot of total sugars

Table 9: Analysis of variation for Biochemical traits (CRD)

Source	Degrees of freedom	Mean square (Total Carotenoids)	Mean square(Total Sugars
Genotypes	79	33.75 (P<0.05)	3.17 (P<0.05)
Error	80	1.25	0.33

4.2.3 Scatter plot of Total Carotenoids in relation to Total Sugars

The scatter plot of Total carotenoids and Total Sugars was constructed which showed that the non- sugary genotypes SMC-3 and Black pearl had low levels of total carotenoids as well as total sugars. The parents Sugar-75 and Mithas showed high levels of total carotenoids and total sugars while as the inbred S31 and S69 showed high levels of total carotenoids and total sugars as shown in Fig. (7).

4.2.4 Amylose content

The amylose content of 22 inbreds was determined which showed that 9 inbreds had an intermediate amylose content, 2 inbreds, S59 and S5 had a high amylose content and 6 inbreds showed a low amylose content. Five inbreds were found to have very low amylose content. Table 10 shows the amylose content of the different inbreds along with the parents Sugar75 and Mithas and the two non-sugary genotypes SMC3 and Black Pearl. The parent sugar 75 was found to contain high amount of total carotenoids, total sugars and high amylose. Among the inbreds, the inbreds S5 and S59 were found to have high level of amylose in addition to having high amounts of total carotenoids and total sugars (Fig. 8)

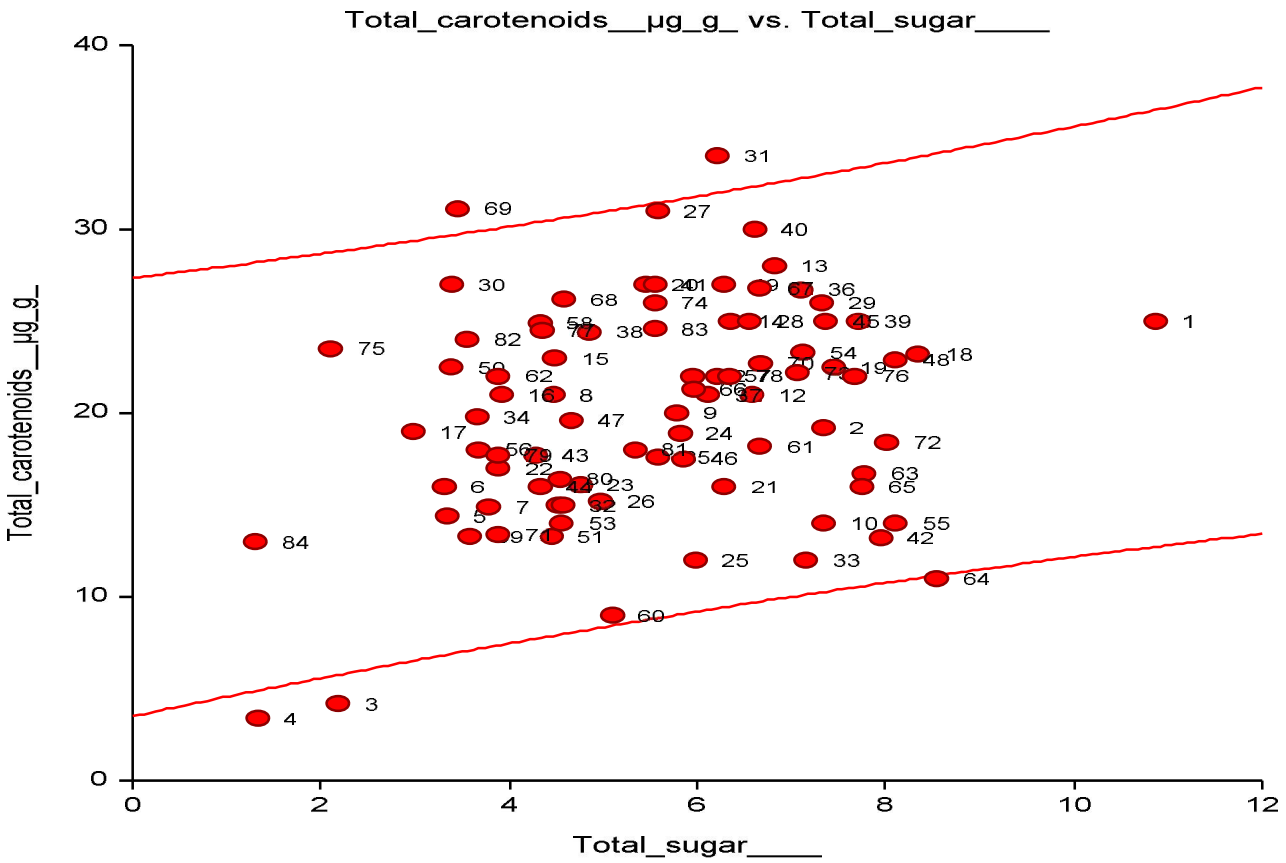

Fig. 7: Scatter plot of Total Carotenoids in relation to Total Sugar

Table 10: Amylose content of the sweet corn inbreds

Genotype	Total carotenoids (µg/g)	Total sugar (%)	Amylose content
Sugar 75	25	10.87	High
Mithas	19.2	8.66	intermediate
Black pearl	3.7	1.67	Intermediate
SMC 3	4.2	2.18	intermediate
S1	14.4	3.34	intermediate
S2	16	3.31	low
S4	21	4.47	very low
S5	20	5.78	High
S6	14	7.34	intermediate
S8	21	6.58	intermediate
S10	25	6.35	low
S16	27	5.45	low
S17	16	6.28	intermediate
S18	17	3.88	very low
S19	16.1	4.76	low
S20	18.9	5.82	very low
S22	15.2	4.97	very low
S24	25	6.55	intermediate
S26	27	3.39	intermediate
S29	12	7.15	low
S44	22.88	8.1	intermediate
S47	13.3	4.45	intermdiate
S51	14	8.1	low
S54	24.9	4.33	intermediate
S58	22	3.88	very low
S59	16.7	7.77	High

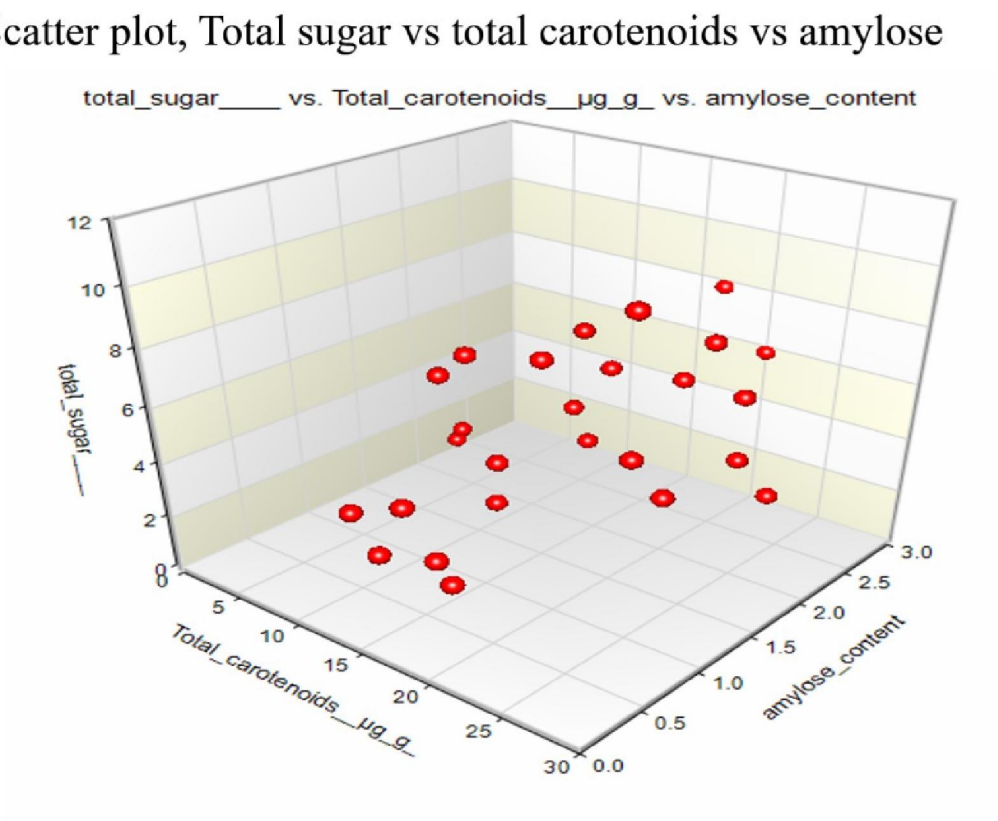

Fig.8: 3D scatter plot of amylose in relation to total carotenoids and total sugars

Plate 2: Procedure for biochemical analysis

4.3 Molecular marker based validation of sweet kernel trait

The marker based validation of the sweet kernel trait was performed with the help of five molecular markers (umc2061, bnlg1937, umc1736, umc1320, umc2276) that were linked to 3 endosperm mutant genes *su1* (sugary1), *sh2* (shrunken2) and *se1* (sugary enhancer). Polymorphism survey was done between the two sugary parents and a non-sugary parent SMC-3 using five molecular markers. Of these two were found to be polymorphic which included umc2061 and bnlg1937, both of them were linked to the sugary gene (*su1*). The list of inbreds that were found to contain the sugary gene is given in Table 11. It was found that out of the sixty inbreds, seven inbreds carried the alleles specific to sugary trait with respect to the markers umc2061 and bnlg1937. Thirty three inbreds amplified sugary specific allele of 230 bp for umc2061. Twenty inbreds carried only bnlg 1937 allele linked to the sugary gene.

Table 11: List of inbreds carrying the sugary gene

umc 2061 (sugary locus)	bnlg 1937 (sugary locus)	Number of Inbreds
Sugary	Sugary	07
Sugary	Non-Sugary	26
Non-Sugary	Sugary	13
Non-Sugary	Non-Sugary	07
Sugary	-	33
Non-Sugary	-	20
-	Sugary	20
-	Non-Sugary	32
Missing data	-	06
-	Missing data	05

Plate 3: Procedure for Molecular analysis

4.3.1 Cluster analysis based on Molecular and Biochemical traits

The cluster analysis performed on the basis of the molecular markers and the biochemical traits helped to classify the genotypes into two clusters. The parents Mithas and Sugar75 were classified into the cluster I and were present in the same subcluster. The non-sugary genotypes SMC 3 and Black pearl were also classified in the cluster I but in a different sub cluster. The dendrogram representing the cluster analysis of the inbreds is shown in figure 9.

4.3.2 PCA based on the Molecular and Biochemical traits

The principal component analysis was jointly performed on the allelic profile and the biochemical traits. The PCA 1 explained 38% of the total variation while as the PCA 2 explained 24 % of the total variation with the cumulative value of 62%. The markers umc2061 and bnlg1937 explained high variability. The two biochemical traits were comparable to one another in explaining the total variability among the inbred ppulation.

4.4 Screening against Turcicum leaf blight

Inbred lines were screened for resistance against Turcicum leaf blight (TLB) by using 1-9 disease scoring scale of IIMR (Aggarwal *et al*., 2021). The screening of the 80 sweet corn inbreds revealed that the inbreds were having moderate to high degree of resistance against the TLB. Majority of inbreds were found to be resistant against TLB under field conditions. Inbreds S3, S7 and S50 manifested a relatively higher disease intensity (>30 %) and were, therefore, categorized as moderately resistant. A susceptible check larnoo local showed a disease intensity of 73.9 and a disease score of 8 and was therefore classified as moderately susceptible. The percent disease intensity and the score of the inbreds is given in the table 12.

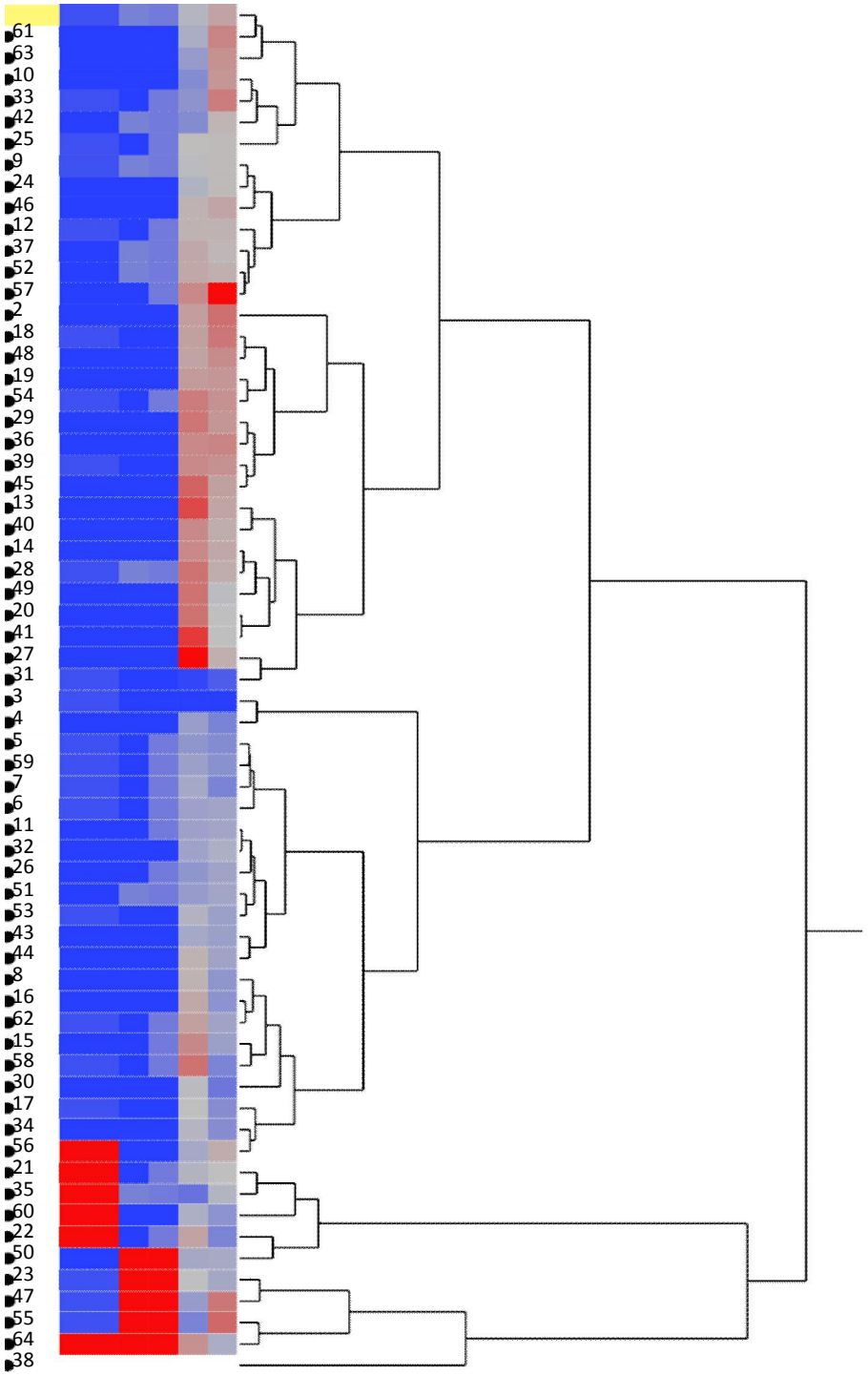

Fig. 9: Dendrogram showing the classification of the inbreds based on the biochemical traits and molecular analysis

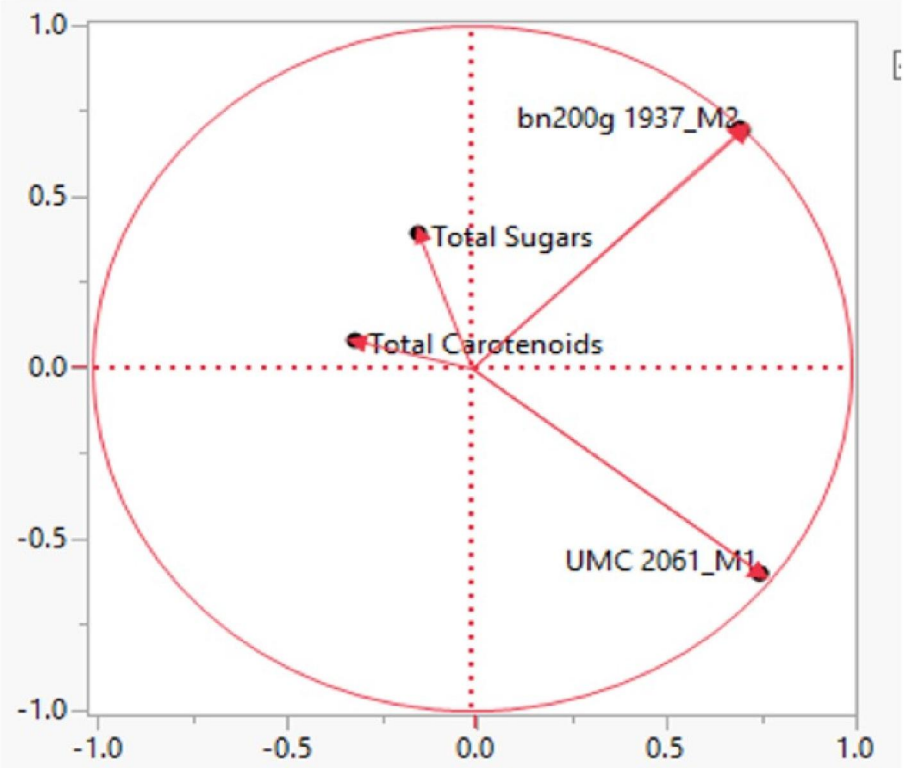

Fig. 10: PCA based on the molecular and biochemical traits

Table 12: Reaction of sweet corn inbreds against TLB under field conditions

Genotype	Percent disease intensity	Score	Reaction
Sugar 75	17.6	2	R
Mithas	24.5	3	R
Larno local	73.9	7	S
S1	13.2	2	R
S3	15.5	2	R
S4	12.2	2	R
S5	13.4	2	R
S6	13.9	2	R
S7	6.0	1	R
8	28.6	3	R
S9	16.6	2	R
S10	17.0	2	R
S11	17.3	2	R
S12	19.6	2	R
S13	30.1	4	MR
S14	20.9	3	R
S15	20.3	3	R
S16	7.2	1	R
S17	30.3	4	MR
S18	12.6	2	R
S19	13.6	2	R
S20	27.0	3	R
S21	8.4	1	R
S22	6.2	1	R
S23	28.3	3	R
S24	6.7	1	R
S25	18.3	2	R
S26	13.6	2	R
S27	19.6	2	R
S28	20.9	3	R
S29	10.2	2	R
S30	15.6	2	R
S31	16.3	2	R
S32	28.2	3	R
S33	11.6	2	R
S34	13.8	2	R
S35	12.5	2	R
S36	13.3	2	R
S37	28.4	3	R
S38	10.9	2	R

S39	15.5	2	R
S40	13.9	2	R
S41	16.3	2	R
S42	14.9	2	R
S43	12.6	2	R
S44	18.6	2	R
S45	15.4	2	R
S46	10.4	2	R
S47	15.6	2	R
S48	14.7	2	R
S49	18.5	2	R
S50	30.8	4	MR
S51	13.9	2	R
S52	20.1	3	R
S53	14.6	2	R
S54	17.4	2	R
S55	29.5	3	R
S56	18.2	2	R
S57	9..3	1	R
S58	20.1	3	R
S59	18.3	2	R
S60	17.6	2	R
S61	15.6	2	R
S62	27.0	3	R
S63	21.3	3	R
S64	28.4	3	R
S65	14.9	2	R
S66	11.3	2	R
S67	10.9	2	R
S68	14.7	2	R
S69	9.5	1	R
S70	19.6	2	R
S71	17.6	2	R
S72	28.6	3	R
S73	21.0	3	R
S74	12.3	2	R
S75	14.3	2	R
S76	17.4	2	R
S77	14.8	2	R
S78	19.5	2	R
S79	15.6	2	R
S80	28.8	3	R

Chapter 5

DISCUSSION

The present study titled "Morphological and Marker based Evaluation of Early Generation Sweet Corn (*Zea mays* L.) Inbred Lines for Yield and Quality Attributes" was carried out during *Kharif* 2020 at Mountain Research Centre for Field Crops, Khudwani, SKUAST Kashmir. The present experiment was carried out with the following three objectives.

1. Evaluation of sweet corn inbreds for various morphological, biochemical, yield and component traits.
2. Marker based validation of inbreds for sweet kernel trait.
3. Phenotypic screening of inbreds for reaction to *Exherohilum turcicum.*

The results that were obtained from the present study are briefly discussed below under appropriate headings.

5.1 Morphological charcaterization of the sweet corn inbreds (*Zea mays* L.)

The DUS (Distinctiveness, Uniformity and Stability) characterization of the sweet corn inbreds was performed for the facilitation of characterization, registration and the protection of the inbreds. In the present study 80 sweet corn inbreds were evaluated for the DUS characters as per the guidelines of the descriptor of the Indian Institute of Maize Research (IIMR), ICAR, New Delhi, 2011. Thirty traits were recorded as per the descriptor at various stages of the plant growth. An extensive range of variability was found for most of the characters in all the inbreds. The assessment of the 80 inbreds for 30 traits showed that all the traits were quite informative with respect to the trait expression cum characterization. In case of the leaf characters such as the angle between the stem and the blade the small state of expression showed a maximum frequency of 61.25 and the rest showed the state of expression as wide with a frequency of 38.75.The

anthocyanin coloration of the brace roots was found to be absent in majority of the inbreds. The time of anthesis of the tassel as well as the silk was found to be late for all the inbreds. The anthocyanin coloration of the silks was found to be absent in all the inbreds. The length of the main axis of the tassel was long in almost all the inbreds. The plant length was recorded as long in all the inbreds. The length of the ear without husk was classified as medium and long with a frequency of 63.75 and 36.25 respectively. The shape of the ears of all the inbreds under study was conico-cylindrical. The number of rows of the grains on the ear were mostly many with a frequency of 95. The type of the grain was dent in all the ears of the inbreds. The kernel sweetness was observed to be present in the kernels of all the inbreds. The kernel shape of the kernels of the inbreds was found to be shrunken in all of the 80 sweet corn inbreds. The 1000 kernel weight was measured and it was found to be small in all the inbreds. The results are closely related to the findings of Madhukeshwar and Sajjan (2015).

5.2 Total Carotenoids.

Sweet corn is an important class of specialty corn which carries a high demand because of the changing preferences of people. The demand for healthy foods is also showing the same trend as people are becoming more and more cognizant. Carotenoids are free radical quenchers and therfore their intake would lead to lesser chances of some fatal chronic diseases such as the cardiovescular diseases and cancer. Among the cereals, only maize has abundant amount of carotenoids and tocopherols (Feng *et al.,* 2015). Carotenoids are comparatively abundant in sweet corn; thus their quantification is vital from the nutritional point of view (Luterotti and Kljak, 2010). The estimation of carotenoids was therefore done in the 80 sweet corn kernels and it was found that the 80 sweet corn inbreds varied significantly with respect to the total carotenoid content and showed a range of 9-34. The highest amount of total carotenoids was found in the Inbred S27 with a total carotenoid content of (34µg/g) followed by the inbred S65 with a total carotenoid content of (31.1µg/g). The lowest amount of the total carotenoids

was found in the inbred S56 with a total carotenoid content of (9µg/g) followed by the inbred S60 with a total carotenoid content of (11µg/g). The results of the present study are in agreement with the results of Song *et al.* (2015).

5.3 Total Sugars.

Sweet corn is preferred over the normal corn because of its greater sweetness. The sweetness is generally attributed to its higher sucrose content. The presence of a blend of sugars instead of the presence of higher amounts of a single type of sugar would serve the purpose better as it would produce a flavor out of a blend of flavors of different types of sugars. Therefore the total sugars of the 80 sweet corn inbreds were determined and the results showed that the total sugars were in a range of 1.3-8.54. The highest amount of total sugars was found in the inbred S60 which had a total sugar content of 8.54% followed by the inbred S14 that showed a total sugar content of 8.34%. The lowest sugar content was found in the inbred S80 with a total sugar content of 1.3% followed by the inbred S71 which showed a sugar content of 2.1%. The total sugar content of the 80 sweet corn inbreds showed a mean of 5.5. The results of the study are in agreement with the results of Ghada and Ibrahim (2019).

5.4 Amylose estimation.

In addition to better nutritional profile the food must have good digestibility in order to have widespread acceptability**.** High amylose maize is a source of resistant starch which is a type of starch that resists digestion and therefore confers with low glycemic index thereby can prevent colon cancer in agreement with new research in food science Wu *et al*., (2008). Amylose concentration is increased in the *su1* kernels (Boyer and Liu, 1985). Therefore the sweet corn kernels have increased amounts of amylose in them. The amylose content of 22 inbreds was determined which showed that 9 inbreds had an intermediate amylose content, 2 inbreds, S59 and S5 had a high amylose content and 6 inbreds showed a low amylose content. Five inbreds were found to have

very low amylose content. The results obtained are similar to the results of that of Wang *et al*., (1993) and Mir *et al*., (2017).

5.5 Screening against Turcicum leaf blight

Turcicum leaf blight (TLB) is a widespread disease of maize which causes significant yield loss.Screening of sweet corn genotypes against TLBwas carried out under field conditions. Disease reaction was recorded on 1 (resistant) to 9 (susceptible) evaluation scale of Indian Institute of Maize Research, Ludhiana. From the screening, relatively resistant lines from various genetic backgrounds were identified. The study showed that sweet corn inbreds reaction was variable with disease intensity ranging from 6-30.8 per cent and were mostly resistant against the TLB under field conditions with only three inbreads falling in moderately resistant category. A susceptible check Larnoo local showed a disease intensity of 73.9 while as the parents also showed resistant reaction against the disease. The results are quite similar to that of the results of Yousuf *et al*., (2018).

5.6 Molecular analysis.

The increased sweetness in sweet corn is because of different endosperm mutants. Molecular marker based study is more reliable than morphological evaluation and therefore molecular marker based study would be more a helpful and a reliable tool for selecting the inbreds that conatain the sugary trait. The sweet corn lines were screened with the help of SSR (Simple Sequence Repeats) markers linked to sweetness related genes (*Su1, Se1* and *Sh2*). The molecular study was done on 60 inbreds and out of the five primers that were used for screening the inbreds only two primers were found to be polymorphic. It was found that out of the sixty inbreds that were used for the molecular study, seven inbreds were sugary at both the loci (umc2061 and bnlg1937). Thirty three inbreds were found to be sugary at the umc2061 locus. Twenty inbreds sugary at the bnlg1937 locus. Both the markers umc2061 and bnlg1937 are linked to the sugary gene (*su1*). These markers flank the sugary gene on the proximal and distal ends

of the chromosome 4. Therefore the probability of the recombination between the marker and the gene is very low. The seven lines which carry the positive sugar specific alleles for both the markers are thought to carry the sugary (*su1*) gene. The other lines which carry the sugar specific alleles for only one of the markers may undergo some recombination between the marker and the gene. Though these lines were also tested for the phenotypic appearance of the grain and with respect to the biochemical traits, the chance of these lines belonging to the non-sugary class is very low.

Chapter 6

SUMMARY AND CONCLUSION

The normal maize crop is the tribal staple crop in Jammu and Kashmir. Due to lower returns the maize cultivation is mostly restricted to the higher reaches and areas having meager or no irrigation facilities. The lower returns in normal maize is pushing the growers towards the specialty corn 'Sweet Corn' which gives better returns and opens avenues for employment generation (Najeeb *et al*., 2011). Sweet corn can be a healthy alternative to the normal maize in terms of marketability of the crop due to the increased demand. Sweet corn is different from the normal corn because of the increased amounts of sugar in its endosperm. It is sweeter than normal maize because of some recessive mutations that hinder the development of starch from sugars in the endosperm of the corn kernels. Sweet corn improvement has been done primarily by identifying and selecting for mutants with higher sugar content. Sweet corn has become quite a popular choice as a processed vegetable as well as a fresh vegetable in different countries including India. There has been a tremendous increase in the demand for sweet corn due to urbanization, changing preferences of consumers and availability of a varied range of sweet corn products.

Keeping the above things in consideration, the present study entitled "Morphological and Marker based Evaluation of Early Generation Sweet corn (*Zea mays* L.*)* Inbred Lines for Yield and Quality Attributes" was undertaken with the following objectives:

1. Evaluation of sweet corn inbreds for various morphological, biochemical, yield and component traits.
2. Marker based validation of inbreds for sweet kernel trait.
3. Phenotypic screening of inbreds for reaction to *Exherohilum turcicum.*

In the current study, 80 sweet corn inbreds were evaluated for various Morphological traits according to the guidelines specified by the DUS descriptor of Indian Institute of Maize Research (IIMR), ICAR, New Delhi,

2011 and it was found that the sweet corn inbreds showed variability for most of the morphological traits. The traits like brace root coloration, anthocyanin coloration of the silks, ear shape, anthocyanin coloration of sheath, kernel shape, color of top of grain, type of grain, kernel sweetness, kernel waxiness, kernel opaqueness and 1000 kernel weight didn't show any variability out of the 30 traits that were studied. The inbreds were also subjected to different biochemical parameters like Total carotenoids, Total sugars and Amylose content. The highest amount of total carotenoids was found in the Inbred S27 with a total carotenoid content of (34μg/g) followed by the inbred S65 with a total carotenoid content of (31.1μg/g). The highest amount of total sugar was found in the inbred S60 which had a total sugar content of 8.54% followed by the inbred S14 that showed a total sugar content of 8.34%. S59 and S5 had a high amylose content and 6 inbreds showed a low amylose content. Five inbreds were found to have very low amylose content. The inbreds were also screened for resistance against Turcicum leaf blight (TLB) which is a widespread disease in maize all over the world. Majority of the inbreds proved to be resistant against TLB with disease intensities more or less comparable with the parents which also showed resistant reaction against this disease under field conditions. Out of the sixty inbreds that were used for the molecular study seven inbreds, S21, S28, S47, S48, S49, S53, and S54 were found to be sugary at both the loci (umc2061 and bnlg1937). Thirty three inbreds were found to be sugary at the umc2061 locus. Twenty inbreds were found to be sugary at the bnlg1937 locus. Seven inbreds were found to be non-sugary at both the loci.

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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner Dr. Satish Kumar, Senior Scientists (Crop Improvement) IIWBR, Karnal during Viva-Voce examination held on 02-11-2021 have been incorporated in the manuscript entitled **"Morphological and Marker based Evaluation of Early Generation Sweet Corn (*Zea mays* L.) Inbred Lines for Yield and Quality Attributes"** submitted by **Mr. Shah Mohammad Usman (Regd. No. MSA/2019/1288)**

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