STUDIES ON PHENOTYPING OF BC_3F_2 POPULATION AND MOLECULAR CHARACTERISATION OF ELITE BC_3F_3 PROGENIES FOR SORGHUM DOWNY MILDEW RESISTANCE IN MAIZE (*Zea mays* L.)

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Thesis submitted in part fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE) in PLANT BREEDING AND GENETICS** to the Tamil Nadu Agricultural University, Coimbatore.

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CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON PHENOTYPING OF BC_3F_2 POPULATION AND MOLECULAR CHARACTERISATION OF ELITE BC_3F_3 PROGENIES FOR SORGHUM DOWNY MILDEW RESISTANCE IN MAIZE (*Zea mays* L.)"** submitted in part fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE) in PLANT BREEDING AND GENETICS** to the Tamil Nadu Agricultural University, Coimbatore, is a record of bona fide research work carried out by **Miss. SRUTHY MENON V** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

Place: Coimbatore
Date:

(Dr. N. MANIVANNAN)
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Sruthy Menon V

Abstract

ABSTRACT

STUDIES ON PHENOTYPING OF BC_3F_2 POPULATION AND MOLECULAR CHARACTERISATION OF ELITE BC_3F_3 PROGENIES FOR SORGHUM DOWNY MILDEW RESISTANCE IN MAIZE (*Zea mays* L.)

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Sorghum downy mildew caused by *Peronosclerospora sorghi* is considered as the top priority biotic constraint limiting maize productivity. The cost concerns and the emerging problem of buildup of chemical resistance in the pathogen point to the use of resistant varieties as a cost-effective and environmentally safe alternative for managing the disease. Two maize inbred lines UMI 79and UMI 936(w) with varied responses to sorghum downy mildew incidence were crossed and the resultant progenies were backcrossed with the recurrent parent, UMI 79 for introgression of sorghum downy mildew resistant genes into the elite inbred. Three BC_3F_1progenies (7-2-3, 7-7-7 and 7-2-10) carrying the SDM resistant QTL selected based on the heterozygosity produced by the polymorphic SSR markers were selectively selfed to generate the BC_3F_2 population. In the current study the three progenies were phenotypically screened for sorghum downy mildew resistance in sick plot condition.Progeny 7-2-10 was found to be highly susceptible (72.38% infection) to the disease and thus eliminated from further studies. Progeny 7-7-7 (21.49% infection) and 7-2-3 (26.92% infection)showed moderate level of resistance with progeny 7-7-7 showing higher resistance to sorghum downy mildew.

In BC_3F_2generation, positively significant skewness was observed for days to maturity, cob diameter, cob weight, number of grains per row and grain yield per plant confirming complementary gene action for the traits. Negative kurtosis was observed in 100 grain weight and grain yield per plant indicating that the characters are controlled by many genes. If selection for these characters were made intensively, the gain will be faster. Plant height, days to tasseling, days to silking, cob length, cob diameter, number of rows per cob, cob weight, shelling %, 100 grain weight and grain yield per plant exhibited high to moderate heritability followed by high to moderate genetic advance. This clearly denoted that these traits are governed largely by additive gene effects.

Thirty eight phenotypically resistant individuals from 7-2-3 progeny and sixty five phenotypically resistant individuals from progeny no. 7-7-7 were selfed to generate the BC_3F_3 generation. Among these eight superior progenies were selected for genotyping with the SSR markers phi053 (chromosome 3) and nc013 (chromosome 6). One BC_3F_3progeny (7-7-7-53) was identified homozygous for UMI 936(w) allele for both the markers and two progenies (7-7-7-85 and 7-2-3-8) were identified homozygous for UMI 936(w) allele for marker nc013 alone. These three lines can be designated as NILs (Near Isogenic Lines) resistant to the sorghum downy mildew disease and can be used in further breeding programmes. FourBC_3F_3progenies (7-7-7-59, 7-7-7-84, 7-7-7-86 and 7-2-3-2) were identified heterozygous for the markers. They are to be selfed and advanced into next generation to identify more NILs differing for the resistance QTL alone.

While considering the morphological traits of the advanced progenies, the progeny 7-7-7 is found to have higher mean value than 7-2-3 progeny and also seems to have attained more uniformity with moderate variability for important biometrical traits. Also in progeny 7-7-7, the number of progenies resembling the recurrent parent was recorded to be high for biometrically important traits. Thus considering the biometric value of BC_3F_3progenies, 7-7-7-53, 7-7-7-59, 7-7-7-84, 7-7-7-85 and 7-7-7-86 may be more useful in deriving resistant NILs which can be utilized for developing sorghum downy mildew resistant single cross maize hybrids which is the need of the hour.

LIST OF ABBREVIATIONS

%	:	per cent
^{0}C	:	Degree Celsius
µg	:	micro gram
µl	:	micro litre
a.i	:	active ingredient
AFLP	:	Amplified Fragment Length Polymorphism
ARDRA	:	Amplified Ribosomal DNA Restriction Analysis
bnlg	:	Brookhaven National Laboratory
BSA	:	Bulked Segregant Analysis
bp	:	base pairs
cm	:	centi meter
cM	:	Centimorgan
cms^{-1}	:	Centi meter per second
CTAB	:	Cetyl Timethyl Ammonium Bromide
DNA	:	Deoxyribo Nucleic Acid
dNTP	:	deoxy Nucleotide Tri-Phosphate
EDTA	:	Ethylene Diamine Tetra Acetic acid
FAO	:	Food and Agricultural Organization
et al.,	:	and other workers
Fig.	:	Figure
F_1	:	First filial generation

g	:	Gram
GA	:	Genetic advance
GCV	:	Genotypic coefficient of variation
GDP	:	Gross Domestic Product
h^2	:	Heritability
ha	:	Hectare
hr	:	hours
HRGPs	:	Hydroxyproline-rich glycoproteins
ITS	:	Internal Transcribed Spacer
kg	:	Kilogram
M	:	Molar
m	:	Meter
MAS	:	Marker Assisted Selection
MAB	:	Marker Assisted Backcrossing
ml	:	millilitre
mM	:	micro Molar
mm	:	millimeter
ms^{-1}	:	meter per second
ng	:	nanogram
No.	:	Number
PCR	:	Polymerase Chain Reaction
PCV	:	Phenotypic coefficient of variation
PVP	:	Poly vinyl pyrrolidone

QTL	:	Quantitative trait loci
RAPD	:	Random Amplified Polymorphic DNA
RDM	:	Rajasthan downy mildew
RFLP	:	Restriction fragment length polymorphism
RIL	:	Recombinant inbred line
rpm	:	Revolutions per minute
Rs.	:	Rupees
SCARs	:	Sequence Characterized Amplified Regions
SDM	:	Sorghum downy mildew
SNP	:	Single Nucleotide Polymorphism
SSR	:	Simple Sequence Repeats
TBE	:	Tris Borate EDTA
TE	:	Tris EDTA
Tris. HCl	:	Tris (hydroxyl methyl) aminomethane hydro chloride
UMI	:	University Maize Inbred

CONTENTS

LIST OF TABLES

LIST OF FIGURES

	progeny 7-7-7	
19	Frequency distribution for plant height in BC_3F_2 generation for progeny 7-2-3	
20	Frequency distribution for cob length in BC_3F_2 generation for progeny 7-7-7	
21	Frequency distribution for cob length in BC_3F_2 generation for progeny 7-2-3	
22	Frequency distribution for cob diameter in BC_3F_2 generation for progeny 7-7-7	
23	Frequency distribution for cob diameter in BC_3F_2 generation for progeny 7-2-3	
24	Frequency distribution for number of rows per cob in BC_3F_2 generation for progeny 7-7-7	
25	Frequency distribution for number of rows per cob in BC_3F_2 generation for progeny 7-2-3	
26	Frequency distribution for number of grains per row in BC_3F_2 generation for progeny 7-7-7	
27	Frequency distribution for number of grains per row in BC_3F_2 generation for progeny 7-2-3	
28	Frequency distribution for cob weight in BC_3F_2 generation for progeny 7-7-7	
29	Frequency distribution for cob weight in BC_3F_2 generation for progeny 7-2-3	
30	Frequency distribution for shelling % in BC_3F_2 generation for progeny 7-7-7	
31	Frequency distribution for shelling % in BC_3F_2 generation for progeny 7-2-3	
32	Frequency distribution for 100 grain weight in BC_3F_2 generation for progeny 7-7-7	
33	Frequency distribution for 100 grain weight in BC_3F_2 generation for progeny 7-2-3	

LIST OF PLATES

Introduction

Maize (*Zea mays* L.) is one of the most versatile emerging crops having wider adaptability under varied agro-climatic conditions. Globally, it is known as queen of cereals because it has the highest genetic yield potential among the cereals. It is world's third most important crop after rice and wheat. About 50 species exist and consist of different colors, textures and grain shapes and sizes. The grains are rich in vitamins A, C and E, carbohydrates, essential minerals and contain 9% protein. They are also rich in dietary fibre and calories which are a good source of energy. It probably originated in Mexico and evolved from teosinte (*Zea mexicana*) (de Wet and Harlan, 1972). It is cultivated on nearly 150 million ha in about 160 countries having wider diversity of soil, climate, biodiversity and management practices that contributes 36% (782 million tonnes) in the global grain production. All parts of the crop can be used for food and non-food products. Maize is utilized as food for human consumption, as feed for livestock and as a raw material for industry (FAO, 1992). It has been emerging as one of the potential crops that address several issues like food and nutritional security, climate change, water scarcity, farming systems, biofuel, etc.

In India, maize is the third most important food crops after rice and wheat. It is cultivated in an area of 75.90 lakh hectares out of which Tamil Nadu contributes around 12.20 lakh hectares. Advanced national production estimate is 22.23 million tonnes with an average productivity of more than 3091 kg/ha (2012-2013) (Indiastat, 2012). It contributes nearly 9 % in the national food basket and more than Rs. 100 billion to the agricultural GDP at current prices apart from the generating employment to over 100 million man-days at the farm and downstream agricultural and industrial sectors.

Major breakthrough in the yield of corn came with the release of hybrids with high yielding potential. The pioneering work on hybrid maize was done by Shull (1952) (single crosses) and Jones (1918) (double crosses). Maize being a highly cross pollinated (allogamous) crop, it has been successfully exploited in the development of hybrids. In any crop improvement programme, the choice of the parents is an important aspect for the success of the crop improvement. Especially in heterosis breeding, the choice of good combiners plays a vital role.

Recent projections indicate that by 2020, global maize demand will increase by 50% (Pingali and Pandey, 2001). To meet the increasing demand for maize, we need to increase and stabilize the maize production while maintaining the quality of the produce. Biotic stresses are one of the most limiting factors for stable crop production worldwide. Maize suffers from 130 pests and insects and about 110 diseases caused by fungi, bacteria and viruses on a global basis. The disease spectrum varies in different agro climatic zones. Several diseases such as seed and seedling blights, foliar diseases, downy mildews, stalk rots and leaf and sheath blight occur in various parts of the country. It has been reported that about 13.20% of the economic product of maize is estimated to be lost annually due to diseases (Pingali and Pandey, 2001). Minimizing losses from biotic stresses offer tremendous opportunities for increasing and stabilizing maize productivity along with maintaining the quality of the seeds/grains. This would contribute to the stabilization of productivity and food security.

Downy mildew is considered the highest priority as a biotic constraint limiting maize productivity (Pingali, and Pandey, 2001). Globally, downy mildew affected areas with significant economic losses are reported to be as high as 30% (Jeffers *et al*., 2000). Heavy losses (as high as 100%) have been reported in maize from the downy mildew pathogens in several countries (Bonde, 1982; Rifin, 1983; Rathore and Siradhana, 1987). Downy mildews, caused by several species in the genera *Peronosclerospora* and *Sclerophthora,* represent a destructive systemic disease of major economic importance to maize. Various downy mildews such as sorghum downy mildew [*Peronosclerospora sorghi* (Weston & Uppal) C.G. Shaw], Philippine downy mildew [*Peronosclerospora philippinensis* (Weston) C.G. Shaw], sugarcane downy mildew [*Peronosclerospora sacchari* (T. Miyabe) Shirai and Hara], Java downy mildew [*Peronosclerospora maydis* (Raciborski)] and brown stripe downy mildew [*Sclerophthora rayssiae* var. *zeae* (Payak and Renfro)] have been reported from regions all over the world. The important species causing downy mildew in maize in India are the sorghum downy mildew (SDM; *Peronosclerospora sorghi*), brown stripe downy mildew (BSDM; *Sclerophthora rayssiae* var. *zeae*) and Rajasthan downy mildew (RDM; *Peronosclerospora hetropogoni*).

Sorghum downy mildew (SDM) is particularly prevalent in the peninsular of India, in the states of Karnataka, Tamilnadu and Andhra Pradesh (Krishnappa *et al*., 1995). A survey conducted by Krishnappa *et al*. (1995) in Karnataka revealed that the incidence of the disease ranged from 10 to 90% and the yield losses are as heavy as 30-40%. *P. sorghi* causes downy mildew in both maize and sorghum crops in India. The fungus can spread locally through movement of spores in the air. The disease can be seed-borne and longer distance dispersal may be possible through movement of infected seed or spore-contaminated soil. The pathogen infects the roots primarily by oospores and the leaves by conidia and finally reaches the meristem causing systemic infection.

This disease can occur at any stage of maize development from seedling to harvest, though it primarily infects its host soon after seedling emergence, until one month after planting. The disease is known by two names, “downy mildew” and “crazy top” based on two types of symptoms in maize that develop as a result of systemic infection. Infected seedlings are stunted and chlorotic (yellow), with symptoms most noticeable on the lower half of the first infected leaf. Infected young plants may die. Older leaves may exhibit alternating parallel stripes of green and yellowish-green to white tissue. Under cool, humid conditions a white downy growth is produced on the lower leaf surface. The chlorotic tissue stripes eventually die and leaves become shredded. Heads produced on these plants are fully or partially sterile. Local infection symptoms are less dramatic, with short necrotic streaks (stipples) produced on leaf blades. The most pronounced symptom was the replacement of normal tassels by leaves. Thus the name “crazy top” of this downy mildew was given due to these symptoms.

Though the disease can be controlled by cultural practices such as the eradication of infected plants, deep ploughing, adjusting the time of planting and by the use of systemic fungicides like metalaxyl for seed treatment (Odovody and Frederiksen, 1984a) or foliar application (Odovody and Frederiksen, 1984b), their effectiveness on disease incidence is variable and in most cases, offer incomplete control. Seed treatments with fungicide, undertaken by commercial companies, makes the seeds expensive and generally beyond the financial reach of resource-poor farmers. Also, complete reliance on chemical treatment has previously proved to be an unsound practice as cases have been

reported where crops grown with treated seed still sustained losses due to downy mildew (Dalmacio, 2000), indicating that resistance in pathogens against the fungicide is developing (Raymundo, 2000). These cost concerns, and the emerging problem of a buildup of chemical resistance in the pathogen, point to the use of resistant varieties as a more cost-effective and environmentally safe alternative for managing the problem of downy mildew in maize (Rathore and Jain, 2000).

The genetic information relating to host resistance is vital for making breeding decisions. One of the major difficulties in working with downy mildew resistance in maize is the difficulty in accurate scoring of the disease reaction. Factors like the stage of the crop or maturity and the amount of the inoculum influence the severity of the disease. As a consequence, multiple evaluations made in different locations are required for the accurate assessment of genetic contributions to resistance. Identification of simple and accurately scored markers for genes that contribute to sorghum downy mildew resistance of maize could greatly benefit future efforts to incorporate the disease resistance into utilizable inbreds.

Hence, the following objectives were designed for the present investigation of BC_3F_2 and BC_3F_3 population from UMI 79 X UMI 936(w) cross:

a) Sorghum downy mildew screening in advanced backcross population of UMI 79 x UMI 936(w).
b) Identification of backcross progenies having major QTLs responsible for downy mildew resistance using SSR markers.

Review of Literature

CHAPTER II

REVIEW OF LITERATURE

Maize (*Zea mays* L.) is an important cereal crop all over the world. It belongs to the family of monocotyleds, Poaceae. The genus *Zea* belongs to the tribe *Andropogonaceae,* but it is commonly cited in literature that it belongs to the tribe *Maydeae.* About 50 species exist and consist of different colors, textures and grain shapes and sizes. The area under maize cultivation is rapidly increasing every year due to the heavy demand for human consumption, animal feed, and industrial uses. Diversified uses of maize for maize corn starch industry, corn oil production, baby corns, popcorns, etc., and potential for exports has added to the demand of maize all over world.

Abiotic and biotic constraints that have widespread yield-reducing effects should receive high priority in maize breeding which include moisture stress, acid soils, water logging, downy mildews, post-flowering stalk rot, turcicum leaf blight, banded leaf and sheath blight, stem borers and weevils (Gerpacio and Pingali, 2007). Of the different biotic stresses reported, sorghum downy mildew (SDM) occupies prime importance as far as Tamil Nadu is concerned since it causes severe yield loss (10-100%).

2.1. Significance of downy mildew in Maize

In origin, downy mildews are old world diseases that have very successfully adapted to attack maize, a new world crop species (Frederiksen and Renfro, 1977). Downy mildew diseases occurring in Asia include sorghum downy mildew [*Peronosclerospora sorghi* (Weston & Uppal) C.G. Shaw], Philippine downy mildew [*Peronosclerospora philippinensis* (Weston) C.G. Shaw], sugarcane downy mildew [*Peronosclerospora sacchari* (T. Miyabe) Shirai and Hara], Java downy mildew [*Peronosclerospora maydis* (Raciborski)] and brown stripe downy mildew [*Sclerophthora rayssiae* var. *zeae* (Payak and Renfro)].

Sorghum downy mildew can devastate the crop and result in yield losses ranging from 10 – 100% (Gowda *et al.*, 1987). In the Asian region, where yield losses of 50% or more are common; downy mildew is considered as the top priority biotic constraint

limiting maize productivity (Pingali and Pandey, 2001). Being systemic in nature, downy mildews result in substantial yield loss (Rathore *et al*., 2002).

2.2. Downy mildew pathogens of maize

Twenty one species of downy mildew pathogens have been reported to attack the graminae family (Shaw, 1978). Of these, 10 species belonging to three genera (Seven species of *Peronosclerospora*, one species of *Sclerospora* and two species of *Sclerophthora*) have been reported to cause different types of downy mildews in maize (Frederiksen and Renfro, 1977 and Siradhana *et al.*, 1980).

Downy mildews now classified as *Peronosclerospora* have been known in maize since 1897. In total, 11 species are currently validly described within *Peronosclerospora* (Shaw, 1978; Siradhana *et al.*, 1980; Ryley and Langdon, 2001), all of which are naturally restricted to hosts in the subfamily Panicoideae, although there are some reports of artificial transfer to Poideae (Kenneth, 1981). For several downy mildews on the monocotyledonous grasses, broad host ranges encompassing several genera have been confirmed in *Sclerophthora* (Telle and Thines, 2011) and have also been reported for *Peronosclerospora* (Kenneth,1981). For example, *P. sacchari* has been reported to infect 9 host species, while *P. philippinensis* and *P. sorghi* have been reported with 6 and 5 host species, respectively. For example, maize (*Zea mays*) has been reported as a host for 7 species of *Peronosclerospora*, of which 5 species occurred on sorghum (*Sorghum* spp.) and 4 species on sugarcane (*Saccharum officinarum*), as stated by Kenneth (1981).

The important species causing downy mildew in maize in India are the sorghum downy mildew (SDM; *Peronosclerospora sorghi*), brown stripe downy mildew (BSDM; *Sclerophthora rayssiae var. zeae*) and Rajasthan downy mildew (RDM; *Peronosclerospora hetropogoni).* When maize is infected by the conidial stage of the fungus it is referred to as Rajasthan downy mildew (RDM) (White, 1999; Spencer and Dick, 2002). The disease has been observed in several districts of Rajasthan (Rathore *et al.*, 2002) and has also been found to be spreading to adjacent states.

Sorghum downy mildew of maize is caused by the oomycete *Peronosclerospora sorghi* (Weston and Uppal) C.G. Shaw, an obligate pathogen that cannot be cultured in the laboratory. *P. sorghi* causes downy mildew in both maize and sorghum crops in India.

Sorghum downy mildew is particularly prevalent on the peninsular India, in the states of Karnataka, Tamil Nadu and Andhra Pradesh (Krishnappa *et al*., 1995). It is a disease with great destructive potential since systematically infected plants seldom produce an ear.

2.3. Taxonomy of *Peronosclerospora sorghi*

P. sorghi is the member of the subdivision Mastigomycotina, class Oomycetes, Perenosporales and family Peronosporaceae. The widely prevalent sorghum downy mildew was first reported by Butler (1907) in Tamil Nadu and Maharashtra in India. He considered to it to be *Sclerospora graminicola* at that time. Subsequently, Kulkarni (1913) in India observed that, in asexual phase, germination was direct by means of the germ tube from conidia (rather than through zoospores from sporangia) and primarily on this basis he recommended designation of the varietal rank as *Sclerospora graminicola var anderopogonis-sorghi*. Weston and Uppal (1932) described this fungus in English and for further investigation of the morphology and host range he named it as *Sclerospora sorghi*. In 1978, Shaw proposed the new genus *Peronosclerospora* as subgenus of *Sclerospora* based on the conidial germination by germ tube and he transferred the genus from the *Sclerospora* to *Peronosclerospora* that produce conidia which germinate directly by the germ tube. From 1978 onwards, this sorghum downy mildew pathogen has been named as *Peronosclerospora sorghi* [(Weston and Uppal) C. G. Shaw].

2.4. Sorghum downy mildew symptoms in maize

Symptoms vary depending on plant age and climatic conditions (Frederiksen and Renfro, 1977). The environment and the host genotype interact to modify symptoms. Local lesions appears on leaves as somewhat large chlorotic areas expanding parallel with the veins, elongated chlorotic spots extending from the bottom to the tip, or more narrow elongated chlorotic spots restricted between veins (Frederiksen *et al*., 1973). Plants infected at an early stage by either zoospore downy mildew infection or conidial downy mildew infection often appear stunted, may die prematurely and may be more susceptible to other diseases (Williams, 1984 and Ajala *et al.,* 2003). In the late infected plants, the chlorosis may be more noticeable on the lower half of the leaf which is often

called half leaf symptom. The chlorosis gradually covers the entire leaf surface at the later stages.

Typical symptoms of systemic downy mildew infection on maize begins within 2-3 weeks after planting and infected plants are stunted with chlorotic areas at the base of leaf (Pande *et al*., 1997; Craig, 2000), which gradually covers a greater proportion of leaves formed at later stages (Safeeulla,1976) until the entire leaf is chlorotic. Under warm humid conditions, a white downy growth is produced on the lower leaf surface and sometimes on the both the surfaces also. The surest indication of the disease is a downy covering on the underside of the leaves. (Sabry *et al.,* 2006). This growth is combination of conidia and conidiophores (Jeger *et al.,* 1998). The infected leaves are narrow and erect and cobs fail to form. Systemically infected maize plants generally do not form the cob. In cases when the cobs are formed, those are small and poorly filled (Ajala *et al.,* 2003). At the flowering stage, infected plants produce a bushy top, referred to a crazy top in place of the tassel since the floral parts will be converted into leafy like structures.

2.5. Downy mildew control

2.5.1. Cultural control

Cultural methods for controlling downy mildews are largely aimed at sanitation and manipulation of the environment to the advantage of host and to the detriment of the pathogen. Several cultural methods are available to reduce the primary innoculum of pathogen or to stimulate plant growth during the first month after planting to reduce severity of downy mildew in maize. Since the pathogens survive in the form of oospores in the host tissues, removal, destruction and burning of the infected plant debris along with weeds serves to reduce the primary inoculums (Butler, 1918 & Vasudeva, 1958).

The eradication of collateral hosts especially of *Sorghum* spp., *Saccharum spp*., and wild hosts near maize fields and roguing of infected maize plants have been recommended (Tantera, 1975). The destruction of plant debris by deep ploughing and other methods (Kriiger and Jooste, 1967 and Frederiksen and Rosenow, 1971) and planting in well-drained soils (Kriiger and Jooste, 1967; Shah, 1976 and Ullstrup, 1970) to reduce infection by oospores has also been advocated. The time of planting has often been recommended (Le Roux, 1961 and Exconde *et al.,* 1968) to avoid periods of severe

infection from both primary and secondary inoculum. Avoiding secondary inoculum by planting in fields isolated by 500-1000 m from an inoculum source to break the asexual sporulation cycle has also been advocated (Sun, 1970).

2.5.2. Chemical control

The advent of metalaxyl [methyl N - (2, 6 - dimethylphenyl) - N - (methoxy - acetyl) - DL - alaninate), a systemic fungicide, provided a real breakthrough for the control of downy mildews. The fungicide is absorbed through the leaves, stem and roots and inhibits protein synthesis in the fungus. It has various formulations, and can be applied as a seed treatment or foliar spray. Plants grown from seeds treated with metalaxyl, or any mixture containing metalaxyl, remained free from the disease and had significantly higher grain yield than plants grown from seeds treated with thiram-based fungicides or from untreated seeds. A dosage of metalaxyl as low as 0.35 g a.i./kg seed gave complete control of downy mildew (Anaso *et al*, 1989).

2.5.3. Integrated disease management

Cultural control methods have been partially successful in most cases to reduce the primary inoculum. Complete reliance on chemical treatment has previously proved to be an unsound practice as cases have been reported where crops grown with treated seed still sustained losses due to downy mildew (Dalmacio, 2000), indicating that resistance in pathogens against metalaxyl fungicide is developing (Raymundo, 2000). Development of metalaxyl-resistant strains in some downy mildew pathogens, including *P. parasitica, P. sorghi* (Isakeit *et al.,* 2003) has been reported. The cost concerns, and the emerging problem of a buildup of chemical resistance in the pathogen, point to the use of resistant varieties as a more cost-effective and environmentally safe alternative for managing the problem of downy mildew in maize.

Biological control of downy mildews has so far been limited to experiments and it will require substantial research efforts to achieve any success. Host plant resistance provides the most practical and economic method to control the disease (Shivanna and Anahosur, 1990). It offers the best opportunity to manage the downy mildews. Integrated disease management comprising of cultural, chemical, biological controls and host plant resistance is the most effective method to control the disease.

2.6. Pathological Variability

Pathogenic variability in downy mildew fungi is well known, but no systematic research had been done in India (Thakur and Mathur, 2002). As the Variability of pathogenicity in *P. sorghi* increases, the potential for damage from sorghum downy mildew also increases. So breeders should attempt to diversify their sources of downy mildew resistance as quickly as possible to reduce the vulnerability of corn to sorghum downy mildew (Craig and Frederiksen, 1980). Nair *et al*. (2004) also reported that the two inbred lines from Kasetsart University, Thailand (KUI1411 and KUI1414A), resistant to highly prevalent downy mildew in Thailand (*P. zeae*), were found to be susceptible to *P. sorghi* at Mandya, India. Similar observations on differential responses of maize inbred lines to different sorghum downy mildew pathogens have been made by others (Singburaudom and Renfro, 1982; Yen *et al*., 2001; Nair *et al*., 2001). This might be related to the difference in virulence of different isolates (Rathore *et al*., 2002).

The first report of pathogenic variability of *P. sorghi* was found on sorghum crop from USA in the late 1970s when a previously resistant and popular sorghum hybrid was infected with sorghum downy mildew (Craig and Frederiksen, 1980). Three distinct pathotypes were identified on sorghum in USA from the differential reaction of the inbred lines Tx412,Tx430,CS3541 and QL-3 (Craig and Frederiksen, 1983) and their mode of inheritance of resistance was determined by Craig and Schertz (1985) and Sifuentes and Frederiksen (1988).

Morphological variation between strains of *P. sorghi* is limited. However, pathogenic and molecular variability among the isolates of maize and sorghum has been well documented (Payak, 1975a; Dange, 1976; Frederiksen and Renfro, 1977; Bock *et al.,* 2000 & Mathiyazhagan *et al.,* 2006). Dange (1976) demonstrated that the form of *P. sorghi* present in Rajasthan, India, was pathogenic to maize and tanglehead (*Hereropogon contortus* (L.) *Beauv.*) but not to sorghum. In contrast, Frederiksen and Renfro (1977) reported that *P. sorghi* found in Karnataka attacks maize and sorghum but not *H. contortus*. Payak (1975a) postulated that two races of *P. sorghi*, differentiated by pathogenicity to maize and sorghum occurred in India. The downy

mildew prevalent in the Udaipur district of the Rajasthan state in India, with *H. contortus* as the collateral host has been designated as Rajasthan downy mildew (Siradhana *et al.*, 1980) caused by *P. heteropogoni.* Earlier, this pathogen was considered as a variant of *P. sorghi* (Nair *et al.*, 2004). Pawar *et al.* (1985) screened 75 sorghum varieties against sixteen isolates of *P. sorghi* from different geographic regions and found that a differential reaction identified in each isolate a different pathotype. He reported that the pathotypes from Africa and India had a much wider range of virulence than did pathotypes from the Americas.

Bock *et al.* (2000) sampled nine isolates of *P. sorghi* from maize, sorghum and wild sorghum in southern and eastern Africa and they were compared for pathogenicity on different sorghum and maize cultivars which provided evidence for the existence of pathogenic variability among the geographically distant populations of *P. sorghi* in Africa. Isakeit and Jaster (2005) reported the existence of a new pathotype of *P. sorghi* in Texas which showed metalaxyl resistance. Perumal *et al.* (2006) used PCR-based methods including amplification from RAPD primers and two systems of automated AFLP analysis to detect DNA-level genetic variation among 14 *P. sorghi* isolates including metalaxyl-resistant and susceptible isolates, as well as representatives of common pathotypes 1 and 3 and a new pathotype. Approximately 25 % of the bands were polymorphic across the 14 isolates, with the majority of differences specific to the pathotype P1 isolate.

Mathiyazhagan *et al.* (2006) reported the genetic variability between the isolates of *P. sorghi* from sorghum and corn using restriction fragment length polymorphism (RFLP) analysis of the polymerase chain reaction (PCR)-amplified internal transcribed spacer (ITS) region of ribosomal DNA.

Lukman *et al*. (2013) employed two molecular marker systems, namely SSR (Simple Sequence Repeat) and ARDRA (Amplified Ribosomal DNA Restriction Analysis) markers, to study the population structure and genetic diversity of 31 isolates of *Peronosclerospora* spp. collected from hotspot production areas of maize in Indonesia. Both molecular techniques grouped the isolates into three clusters with a genetic similarity between 66-98% and 58-100% for SSR and ARDRA markers, respectively.

2.7. Epidemiology of sorghum downy mildew

A large volume of information is available on various aspects of downy mildews (Safeeulla, 1976; Ingram, 1981; Kenneth, 1981; Williams, 1984 and Lucas *et al.,* 1995), which is relevant to the Indian situation. Reproduction in downy mildew pathogen is usually both by sexual and asexual means. During the sexual phase oospores which is primary source of inoculum are formed which are thick-walled and long-lived, and these enable the pathogens to survive during crop-free, adverse periods (Payak, 1975b; Ramalingam and Rajasab, 1981; Rajasab *et al*., 1979 and Singh, 1995).

The asexual phase occurs in periods of conducive weather and asexual reproduction is through production of conidia or sporangia. Sporangia produce zoospores which are the infecting propagules, while conidia germinate directly. Several pathogens, such as *Perenosclerospora sorghi*, *Perenospora parasitica*, and *P. tabacina* produce conidia while others, such as *S. graminicola* and *Pseudoperonospora cubensis* produce sporangia and zoospores. Conidia producing pathogens are thought to be evolutionarily more advanced than those producing sporangia (Williams, 1984). However, the epidemiological advantages of conidia over sporangia have not yet been established.

2.8. Weather factors for epidemics to develop

2.8.1. Temperature

Production of conidia and sporangia in the field has a marked periodicity of release and has a close relationship with temperature and relative humidity. In sorghum, pearl millet, and maize symptoms of downy mildew infection develop at 25^0C, but incidence is reduced below 20^0C and above 33^0C. (Bonde *et al.*, 1978). Huge grain yield losses are incurred when disease development is accelerated at temperatures above 20^0C and relative humidity (RH) above 95% (Thakur and Mathur, 2002). Optimum temperature for sporulation of *P. sorghi* is 21 - 23^0 C, with a minimum and maximum of 13^0C and 30^0C respectively.

Five isolates of *P. sorghi* (two from the United States and one each from India, Brazil and Thailand) were compared for the effects of the dew point temperature on sporulation, germination, germ tube growth, and establishment of infection in maize.

Responses of all isolates except that of Thailand were similar at each air temperature. The isolate from Thailand was more tolerant to high temperature. It germinated optimally from 12 to 32^0C, whereas Indian, Texas and Brazilian isolates germinated optimally from 12 to 20^0C. All isolate sporulated optimally from 15-23^0C. The Thailand isolate was the only one that produced mature conidia at 26^0C. Each isolate following conidial inoculation caused high level of the systemic infection at temperature from 15 to 32^0C, and moderate levels at temperature as low as 10^0C during dew period (Bonde *et al.*, 1985).

2.8.2. Humidity

All *Peronoporaceae* require surface wetness for spore germination and infection and high relative humidity for spore production. Rainfall and high relative humidity are critical weather factors for epidemics to develop. Fungal infection of grain is very much dependent on the ambient humidity and is always higher after rain (Bandyopadhyay *et al.*, 2000). The pathogen *P. sorghi* produce about 10,800 conidia/cm^2 in the diseased leaves of sorghum at a relative humidity of 100% but only around 3600 conidia at a relative humidity of 85% under *in vitro* conditions. The sporulation was totally inhibited at 80% relative humidity and below (Shetty and Safeeulla, 1981).

2.8.3. Light

Shetty and Safeeulla (1981) kept infected leaves in darkness after removing the previous spore crop and they found that it did not sporulate in continuous darkness at the optimum relative humidity and temperature. This indicated that exposure to light at least for 1 hr is necessary for sporulation. The amount of sporulation on sorghum leaves increased with increase in time of pre-sporulation exposure to light up to 3-5 hr. However, no increase or decrease in sporulation was noticed when the pre-exposure light period was more than 5 hr. Hence light has no role in sporulation.

2.8.4. Wind speed

Asexual spores of the downy-mildew pathogens are ephemeral and therefore rapid dispersal and infection is essential. Wind speed, relative humidity, temperature, sunshine, and leaf wetness can influence viability and dispersal of spores (Thakur *et al.*, 2009). Under favorable climatic conditions of temperature and relative humidity for sporulation,

disease spread is limited mainly by wind speed. *P. sorghi* conidia can remain airborne from a few meters up to several kilometers (Weston, 1923).

It is not known whether release of conidia of *P. sorghi* is active or passive, but it is assumed that in almost still air conidia fall under gravity and enters air currents. The aerodynamic diameter of conidia is 19.5 mm (Bock *et al.,* 2000), resulting in a settling velocity of only $1.18cms^{-1}$. This suggests that a very low wind speed may be sufficient to disperse conidia. In the field conidia have been sampled at least 80m from infected sorghum (Rajasab *et al.,* 1979). In Thailand, disease spread of sorghum downy mildew on maize is related to prevailing wind direction (Hau *et al.,* 1995). Conidia were transported downwind 0.9-1.8m from the source plants when average wind speed was $0\text{-}0.3ms^{-1}$.

2.9. Genetic basis of downy mildew resistance

Understanding the genetic basis of downy mildew resistance could increase the efficiency of breeding for disease resistant germplasm. Studies of the genetic basis of downy mildew resistance have been complicated by the polygenic nature of the trait and by the fact that additive effects contribute to resistance (Borges, 1987; de Leon *et al*., 1993; Kaneko and Aday, 1980 and Singburaudom and Renfro, 1982). Results of studies in Texas (Frederiksen and Rosenow, 1971 and Frederiksen *et al.,* 1973) with two different sets of U.S inbred lines on the inheritance of resistance to sorghum downy mildew concluded that there are at least two genes operating in a dominant to partially dominant manner, one gene in a recessive manner, and it is probable that modifying genes were present. Two types of resistance were found, one operative against oospores and the other against both oospores and conidia.

Jinahyon (1973) using open pollinated maize varieties found resistance to sorghum downy mildew is controlled by many genes. Frederiksen and Ullstrup (1975) studied the resistance to sorghum downy mildew in Texas and their results indicated that resistance is dominant in some crosses and recessive in others. Experiments of Singburaudom and Renfro (1982) indicated that the reactions of maize to downy mildew are governed by several genes (i.e. polygenically) and that the inheritance of resistance is complex.

A diallel cross, including reciprocals, of six maize (*Zea mays* L.) inbred lines was analyzed for reaction to sorghum downy mildew [*Peronosclerospora sorghi* (Weston and Uppal) C.G. Shaw]. The crosses between the resistant lines and the susceptible '48-S-28' showed intermediate disease reaction, suggesting a polygenic system for resistance to sorghum downy mildew in maize. The combining ability analysis revealed that both additive and nonadditive gene effects were present. However, additivity was clearly more important in determining disease reaction. Significant maternal differences were found, indicating that maternal and/or cytoplasmic inheritance is involved in the reaction to *P. sorghi* (Orangel and Borges, 1987).

Resistance to both sorghum downy mildew and Rajasthan downy mildew were found to be polygenically based, with the dominance of resistance over susceptibility. Although this was predominantly partial in case of sorghum downy mildew, it was mostly complete with respect to Rajasthan downy mildew. The mode of inheritance of resistance to Rajasthan downy mildew was less complex compared to that of sorghum downy mildew. Additive genetic components play an important role in imparting resistance to both downy mildews, although non-additive gene action also contributed significantly to Rajasthan downy mildew resistance (Nair *et al.,* 2004). Sabry *et al.* (2006) found that both additive and non-additive gene actions were important in the control of resistance and it is polygenically controlled. Fato *et al*. (2012) suggested that genes with non-additive effects played a minor role in governing the downy mildew resistance in top crosses.

2.10. Biochemical basis of Resistance

In general, all the downy mildew resistant hybrids were found to possess significantly higher concentration of total phenols, soluble protein (mg/g), peroxidase and polyphenol oxidase. Hence, these biochemical substances could have a possible role in the resistance behaviour of the genotypes against downy mildew (Premalatha *et al.,* 2010). These results are in accordance with the findings of Shetty and Ahmad (1980), Vidhyasekaran (1990) and Anwar *et al.* (1995) for phenols. Setty *et al.* (2001) reported that resistant cultivars had significantly higher amount of sugars and phenols but lesser amount of grain protein than the susceptible.

Systemic acquired resistance (SAR) is a widely distributed plant defense system that confers broad-spectrum disease resistance and is accompanied by coordinate expression of the so-called SAR genes. SAR gene expression can be mimicked with chemical inducers of resistance. Morris *et al*. (1998) reported that chemical inducers of resistance are active in maize. Chemical induction increases resistance to downy mildew and activates expression of the maize PR-1 and PR-5 genes. These genes are also coordinately activated by pathogen infection and function as indicators of the defense reaction. Specifically, after pathogen infection, the PR-1 and PR-5 genes are induced more rapidly and more strongly in an incompatible than in a compatible interaction.

Hydroxyproline-rich glycoproteins (HRGPs) are important plant cell wall structural components, which are also involved in response to pathogen attack. In pearl millet, deposition and cross-linking of HRGPs in plant cell walls was shown to contribute to the formation of resistance barriers against downy mildew (Deepak *et al*., 2007).

2.11. Breeding approaches for resistance Downy mildews

Genetic resistance continues to be the most economic and effective means for the management of downy mildew disease in maize. Breeding for downy mildew resistance in Asian maize for lowland tropics has been achieved with good success (de Leon *et al*., 1993 and George *et al*., 2004). The key challenge in breeding for host-plant resistance has been the spill-over of resistance across downy mildew pathogens and even variants within species, and also its prolonged and widespread use in an environment favourable for downy mildew disease development.

The resistance to *Peronosclerospora* is found to be polygenically governed, but the phenotypic expression varies with level of infection with some kind of threshold reaction. Kaneko and Aday (1980) suggested that selection should be done under severe epiphytotic conditions, provided inbreeding depression caused by a narrowed genetic variability is kept low. Recurrent selection procedures that use additive gene action and very effective screening procedures can be used to improve upon the levels of downy mildew resistance. Recurrent selection involves the identification and intermating of superior genotype in a population to form a new and improved population from which

further selection can be practiced and is an efficient method for increasing the frequency of desirable genes.

Orangel and Borges (1987) analyzed a diallel cross, including reciprocals, of six maize inbred lines for reaction to sorghum downy mildew and suggested a polygenic system for resistance to sorghum downy mildew in maize. Additive gene action was clearly more important in determining disease reaction. They suggested that recurrent selection would be appropriate for accumulating genes for resistance to sorghum downy mildew in maize.

Efficacy of S_1 recurrent selection in improving levels of resistance to downy mildew infection was assessed in Nigeria from 1997 to 2000 in six maize populations. Improvement procedures consisted of evaluating S_1 progenies under artificial infection with downy mildew spores and in disease-free environments and using a selection index to combine selection for reduced infection with appropriate agronomic characters from more than one environment. Three to four cycles of selection were completed in each of the populations. Products from the different cycles of selection were evaluated and data collected on infection parameters and agronomic traits. Result obtained showed 3-4 cycles of selection were adequate to reduce downy mildew infection levels significantly and increase grain yield. Downy mildew infection decreased by between 58% and 100% while grain yield increases ranged from 10 to 98% for the 2-4 cycles of selection relative to the C0 (original). Selection increased grain yield with acceptable changes in plant height while maintaining maturity in disease-free environments (Ajala et al., 2003).

Downy mildew resistance breakdown has been common in breeding lines and hybrids, which is largely attributed to the evolution of new virulence in the pathogen populations (Thakur *et al*., 2009). The pathogens that possess a mixed reproductive system, both sexual and asexual (such as *Perenosclerospora* species) pose the greatest risk to the breakdown of resistance genes (McDonald and Linde, 2002). Therefore, host-plant resistance breeding programs require close monitoring of virulence changes in the pathogen and identification of new resistance sources to new virulent strains.

2.12. Molecular markers

Molecular tools enhance breeding efficiency and effectiveness and have become integral to many maize research programmes worldwide. The volume of molecular data for diverse populations and breeding lines, in both public and private breeding institutions, has been accumulating at a rapid pace, particularly in the developed world. This is now enabling the use of sophisticated biometric and modelling tools to predict genotypic value and conduct genotypic selection before evaluating phenotypes in large private breeding institutions. Eathington *et al.* (2007) reported that marker assisted selection has more than doubled the rate of gain for an index including yield, moisture content and stalk strength compared to conventional breeding for maize populations.

With the use of molecular techniques it would now be possible to hasten the transfer of desirable genes among varieties and to introgress novel genes from related wild species. Polygenic characters which were previously very difficult to analyse using traditional plant breeding methods, would now be easily tagged using molecular markers. Techniques which are particularly promising in assisting selection for desirable characters involves the use of molecular markers such as random-amplified polymorphic DNAs (RAPDs), restriction fragment length polymorphisms (RFLPs), microsatellites and PCR-based DNA markers such as sequence characterized amplified regions (SCARs), sequence-tagged sites (STS) and inter-simple sequence repeat amplification (ISA), amplified fragment length polymorphic DNAs (AFLPs) and amplicon length polymorphisms (ALPs) using F_2 and back-cross populations, near-isogenic lines, doubled haploids and recombinant inbred lines. Progress has been made in mapping and tagging many agriculturally important genes with molecular markers which forms the foundation for marker assisted selection (MAS) in crop plants. Advances in genomics led to the identification of numerous DNA markers in maize during the last few decades, including thousands of mapped microsatellite or simple sequence repeat (SSR) markers, and more recently, single nucleotide polymorphisms (SNPs) and insertion-deletion (InDel) markers. In addition to the SSRs and SNPs, a large number of genes controlling various aspects of plant development, biotic and abiotic stress resistance, quality characters, etc. have been cloned and characterized in maize, which are excellent assets for molecular marker assisted breeding.

Molecular markers have several advantages over the traditional phenotypic markers that were previously available to plant breeders. They offer great scope for improving the efficiency of conventional plant breeding by carrying out selection not directly on the trait of interest but on molecular markers linked to that trait. This, of course, would require a molecular marker to be tightly linked to the trait of interest. Besides, these markers are not environmentally regulated and are, therefore, unaffected by the conditions in which the plants are grown and are detectable in all stages of plant growth.

DNA based molecular markers have been made for disease resistance genes in miaze using RFLP analysis (Bentolila *et al*., 1992; Zaitlin *et al*., 1993; Dingerdissen *et al*., 1996 and Saghai-Maroof *et al*., 1996) and RAPD assay (Bubeck *et al*., 1993 and Agrama *et al.,* 1999). Gowda *et al.* (1995) have applied RFLP and RAPD marker techniques to identify genes for resistance to downy mildew in sorghum.

Agrama *et al.*, 1999 did genetic mapping of resistance genes for sorghum downy mildew in maize revealing multiple-locus inheritance. A combination of AFLP (amplified fragment length polymorphism) technique with bulked segregant analysis (BSA) was applied to map the genes involved in the resistance to sorghum downy mildew (*P. sorghi*) in a recombinant inbred population. Three AFLP markers were identified and mapped to chromosomes 1 and 9, in regions previously associated with sorghum downy mildew resistance. One other AFLP marker was found to be associated with disease susceptibility but could not be linked to any chromosome. Sequence characterized amplified regions (SCARs) were produced and used to assess the presence of sorghum downy mildew resistance genes and characterize specific genotypes. Ladhalakshmi *et al.*, 2009 developed a DNA sequence characterized amplified region (SCAR) marker for identification of isolates of *P. sorghi* from maize.

Partial genes for PIC15, PO145579, and ZMCf5 were identified and analyzed in a panel of 60 public and private maize inbred lines using bioinformatics and statistics tools. These candidate R genes exhibited an average nucleotide diversity value of 0.015. The negative Tajima's D values for these genes suggested that purifying selection has played a role in the evolution of maize downy mildew resistance genes. In addition, linkage disequilibrium among these genes occurred across an extent of 200 bp. Using association

analysis methods, a general linear model, and a mixed linear model, five polymorphisms within the partial PIC15 and PO145579 genes were detected and associated with downy mildew disease. These are new polymorphisms that have not previously been reported in association with downy mildew disease traits. (Wongkaew *et al.*, 2010)

2.12.1. Simple sequence repeats (SSR)

Microsatellites or SSRs have been recognized as one of the most informative type of markers for molecular ecology. SSRs are tandemly repeated motifs of one to six bases which occur frequently and randomly in all eukaryotic genomes, although their frequency varies significantly among different organisms (Selkoe and Toonen, 2006). SSR markers are useful for a variety of applications in plant genetics and breeding because of their reproducibility, multiallelic nature, co-dominant inheritance, relative abundance and good genome coverage. Moreover, they are easy to be assayed by polymerase chain reaction methodology and their dissemination is straightforward among laboratories, making them very valuable genetic markers for mapping analysis (Powell *et al.,* 1996). They have been useful for the integration of the genetic, physical and sequence based physical maps in plant species, and simultaneously has provided breeders and geneticist with an efficient tool to link phenotypic and genotypic variation.

Microsatellite markers have been developed and integrated into several existing plant linkage maps, such as rice (Chen *et al.,* 1997), wheat (Roder *et al.,* 1998), potato (Milbourne *et al.,* 1998), Eucalyptus (Brondani *et al.,* 1998), soybean (Cregan *et al.,* 1999), and sorghum (Kong *et al.,* 2000). In maize, microsatellites were very useful as genetic markers for determining genetic similarities and relationships (Smith *et al.,* 1997; Senior *et al.,* 1998 and Barbosa *et al.,* 2003) and for genetic mapping (Senior and Heun, 1993; Senior *et al.,* 1996; Taramino and Tingey, 1996 and Davis *et al.,* 1999). Phumichai *et al*., 2012 selected 48 polymorphic SSR loci from all 10 maize chromosomes and performed association analysis which revealed three significant SSR trait associations ($P < 0.05$) - bnlg1057, bnlg1138 and umc1033.

2.13. QTL analysis experiment for downy mildew in Maize

As a first step towards the development of durably resistant, high-yielding maize varieties, a region-wide activity was undertaken by the Asian Maize Biotechnology

Network to locate and characterize the QTLs involved in resistance to downy mildew disease and identify associated markers for further use in marker-assisted selection.

Quantitative trait loci (QTLs) of maize involved in mediating resistance to *P. sorghi* were detected in a population of recombinant inbred lines (RILs) derived from the *Zea mays* L. cross between resistant (G62) and susceptible (G58) inbred lines. Field tests of 94 RILs were conducted over two growing seasons using artificial inoculation. The mapping population of the RILs was also scored for restriction fragment length polymorphic (RFLP) markers. Heritability of the disease reaction was high (around 70%). One hundred and six polymorphic RFLP markers were assigned to ten chromosomes covering 1648 cM. Three QTLs were detected that significantly affected resistance to sorghum downy mildew combined across seasons. Two of these mapped quite close together on chromosome 1, while the third one was on chromosome 9. The percentage of phenotypic variance explained by each QTL ranged from 12.40% to 23.80%. Collectively, the three QTLs identified in this study explained 53.60% of the phenotypic variation in susceptibility to the infection. The three resistant QTLs appeared to have additive effects. Increased susceptibility was contributed by the alleles of the susceptible parent (Agrama *et al.*, 1999)**.**

Using a set of RILs derived using Ki3 (downy mildew-resistant) and CML139 (downy mildew-susceptible) as parental lines, QTLs conferring resistance to five different downy mildews in tropical Asia, including sorghum downy mildew and Rajasthan downy mildew in India, Philippine downy mildew in the Philippines, Java downy mildew in Indonesia, and *P. zeae* in Thailand were mapped through a collaborative study within the AMBIONET project (George *et al*. 2003). The study identified QTLs with significant effects for resistance to the five important downy mildews of maize in Asian; of particular significance was a QTL on chromosome 6 (bin 6.05) that influenced resistance to all five downy mildews and accounted for nearly 20% and 31% of the phenotypic variance for *P. sorghi* (sorghum downy mildew) and *P. heteropogoni* (Rajasthan downy mildew) disease resistance, respectively. An integrated strategy of marker assisted selection in BC_2F_1 and BC_2F_2 generations, with foreground selection for the two major QTLs (using flanking markers) and background selection for the recurrent parent genome (using 54 SSR markers), followed by phenotypic selection in

BC_2F_3 at a sorghum downy mildew nursery using artificial inoculation, was used to generate sorghum downy mildew resistant CM139 and several QTL-NILs (Prasanna and Hoisington, 2003).

Two major QTLs (one each on Chr. 6 and Chr. 3) were further validated in India using a backcross population derived from CM139 (SDM-susceptible) and NAI116 (SDM-resistant) (Nair *et al*. 2005). They identified three QTLs (one each on chromosomes 2, 3 and 6) for sorghum downy mildew resistance and two QTLs (one each on chromosomes 3 and 6) for Rajasthan downy mildew resistance. The three QTLs appeared to have additive effects on resistance. Sabry *et al.,* 2006 mapped a major QTL on chromosome 2, which explained up to 70% of the phenotypic variation for sorghum downy mildew resistance in 220 F_2 populations through SSR genotyping. The other two QTLs on chromosome 3 and 9 had minor effects; each explained no more than 4% of the phenotypic variation. The three QTLs appeared to have additive effects on resistance, identifying one major gene and two minor genes that contribute to downy mildew resistance.

Jampatong *et al.*, 2013 studied 251 F2:3 families derived from a cross between the two inbreds, Nei9008 (Thailand) and CML289 (CIMMYT), resistant and susceptible, respectively. Individuals in the population were genotyped for SSR and phenotypic resistance data were evaluated as percentage disease incidence in replicated field trials at three environments by Triple Lattice design. Heritability across environments was 94.3%. Traits were analyzed within and across environment using composite interval mapping. Nine QTLs were identified for resistance to sorghum downy mildew, one QTL each on chromosome 2, 3, 4, and 6, three QTLs on chromosome 5, and two QTLs on chromosome 9. Just one QTL on chromosome bin 5.07 came from the susceptible parent, all others from the resistant parent, Nei9008. The QTLs in chromosome bins 2.09 at umc1736, 5.03 at bnlg1902, and 6.01 at bnlg1867 had major effects and were consistent over all environments.

2.14. Marker Assisted Backcrossing (MAB) strategy

Molecular markers are now increasingly being employed to trace the presence of target genes (foreground selection) as well as for accelerating the recovery of the

recurrent parent genome (background selection) in backcross programmes. MAB improves the efficiency of backcross breeding in three ways: (i) If the phenotype of the desired gene cannot be easily assayed, backcross (BC) progeny possessing a marker allele from the donor parent at a locus near/within the target gene can be selected with a good probability of carrying the gene; (ii) Markers can be used to select BC progeny with least amounts of donor parent germplasm in the genome outside the target region; and (iii) Markers can be used to select rare progeny that are the result of recombination near the target gene, thus minimizing the effects of linkage drag.

Transfer of recessive genes through conventional breeding requires additional selfing generations after every backcross, a procedure that is prohibitively slow for most commercial breeding purposes. Marker assisted foreground selection was effectively utilized for introgression of disease resistance genes (Melchinger, 1990), who presented a priori approach for calculating the minimum number of individuals and family size required in recurrent backcrossing. Flanking markers for the target allele are necessary to remove linkage drag. The optimal distance between the target gene and flanking markers governs the selection intensity that can be exerted. The equations given by Hospital, 1992 is helpful in determining the number of backcross plants that need to be generated and typed with a special set of flanking markers.

'Marker-assisted background selection', a term coined by Hospital and Charcosset, 1997, was initially proposed by Young and Tanksley, 1989. This strategy has been used extensively in commercial maize breeding programmes, particularly for selection of lines carrying transgenes conferring biotic and abiotic stress resistance. Several parameters need to be optimized in the background selection programmes.

2.15. Sorghum downy mildew - screening methods

Sorghum downy mildew is one of the destructive disease and has capacity for epiphytotics on the susceptible genotypes in the favorable conditions in the epidemic areas of the Karnataka, Maharashtra, Andhra Pradesh and Tamil Nadu states in India (Anahosur and Hegde, 1979). Breeding resistant varieties is one of the effective and cheap methods to control disease. In any breeding program for disease resistance, the first step is to screen all available materials against disease pathogen. A sound

screening technique forms the basis for identification of source of the resistance for the disease resistance breeding programme (Anahosur and Hegde, 1979). Screening for resistance to sorghum downy mildew has been carried out in the field (Anahosur and Hegde, 1979; Cardwell *et al.*, 1997; Yen *et al.*, 2001; Nair *et al.*, 2004 and Nair *et al.*, 2005) and in green house (Jones, 1970; Schmitt and Freytag, 1974; Craig, 1976 and Narayana *et al.*, 1995).

2.15.1. Field screening

Anahosur and Hegde (1979) compared the five different techniques for screening sorghum genotypes against sorghum downy mildew in the field and revealed that infector row planting was the most reliable technique for assured screening. In this technique, oospores inoculum was incorporated into the furrows and the highly susceptible genotype was sown two weeks early in continuation to these infector rows, the test lines were sown where the oospores inoculum was previously incorporated. So, the test lines were exposed to both oospores from the soil and conidia from infector rows thus minimizing escapes.

Cardwell *et al.* (1997) have developed 'Direct seed inoculation' method for screening maize genotypes against sorghum downy mildew. They followed the same steps as in case of infector row technique but the difference is they used conidial infected pre-germinated seeds for infector sowing instead of direct sowing resulting in consistent and high incidence of downy mildew infected plants and required substantially less labour and inoculum than the spray inoculation of spreader rows. Phumichai et al. (2012) used the spreader-row technique, i.e, planting a susceptible variety in dense stands every 20 rows and in the alleyways for artificial inoculation of downy mildew in the field.

An effective and simple technique to obtain conidial inoculum of *P. sorghi* has been devised, which confers many advantages over the conventional method. Systemically infected sorghum leaves were packed into folds of paper towel. The end of folded paper-towel containing cut-ends of leaves was dipped in 3 cm deep water in a container. The container was exposed to moist field conditions at night and sporulated leaves were harvested with chilled distilled water in the morning to obtain the inoculum. Advantages of the technique include lower cost and effort, rapid harvesting and daily

production of inoculum for up to seven consecutive days from same leaves (Singh and Garampalli, 2013).

Disease nurseries planted in sick plot where sorghum downy mildew is endemic have been widely used. In India, sorghum downy mildew nursery has been maintained at the University of Agricultural Sciences - Regional Research Station (UAS-RRS) and Mandya in Karnataka state (Yen *et al.,* 2001 & Nair *et al.,* 2004 and 2005). It is considered to be the National Centre for investigations on maize downy mildew in India (Krishnappa *et al.,* 1995). However, use of these nurseries is limited to the growing season and the erratic occurrence of the disease and requires repeated test to ensure reliable identification of resistant lines (Craig and Frederiksen, 1980). To overcome such difficulty glass house techniques should be developed so that maize genotypes can be screened throughout the year.

2.15.2. Glasshouse Screening

Craig (1976) has developed seedling inoculation technique in which the maize seedlings were inoculated with conidia of *P. sorghi* in green house when the second leaf of the plant had unrolled enough for the leaf tip to flatten. A classification system was devised for leaf reactions based on the systems exhibited by the leaf, thirty days after inoculation as resistant, intermediate and susceptible (Craig and Frederiksen, 1983). Many research workers have reported corn to be more susceptible to conidia than to oospores of *P. sorghi* (Frederiksen and Renfro, 1977; Saffeulla, 1976; Frederiksen and Ullstrup, 1975 and Kenneth and Shahor, 1973). So, conidial inoculation (Craig, 1976) of corn plants is done to identify resistance to sorghum downy mildew. Craig and Frederiksen (1980) noted that corn plants become less susceptible to conidial inoculums with increased age. This suggested that conidial inoculums at early stage of plant growth would be more efficient.

Schmitt and Freytag (1974) reported that conidial spray inoculation at seedling stage was most efficient in inducing severe downy mildew infection in corn and sorghum. Narayana *et al.* (1995) compared six inoculation techniques for artificial promotion of sorghum downy mildew in green house for screening sorghum genotypes. Among the six inoculation method evaluated in the green house they obtained maximum downy

mildew incidence of 100 percent when seedlings at the first leaf stage were spray-inoculated. This system is generally used under a controlled environment. Seedlings of the test materials are spray inoculated with a suspension of mature conidia. The advantage of this system is that optimal conditions can be maintained and the amount of inoculums is regulated. It can also provide a rapid technique for screening large quantities of materials in short time.

Comparison of genotypic response under different screening methods clearly indicated that the glasshouse-based sandwich method of screening against *P. sorghi* was the best method for identifying promising genotypes with a high level of downy mildew resistance. However, the highly controlled conditions used for sandwich screening, which is most favourable for disease development, resulted in high disease pressure potentially masking the identification of genotypes with a moderate level of downy mildew resistance (Rashid *et al*., 2013).

2.16. Scoring

For comparing host reactions it is necessary to develop an accurate and precise assessment method. Normally disease assessment has been carried out as per standard procedure given by Lal and Singh (1984) worldwide in case of systemic downy mildew diseases by recording the per cent downy mildew diseased plants.

As per the modified method for maize (Craig *et al*., 1976), scoring is 0% highly resistant, 1 - 10% resistant, 11-25% moderately resistant, 26-50% moderately susceptible, 51-75% susceptible and 76-100% highly susceptible. Similar observations and scoring system were made by Sreeramasetty *et al*. (2001) while characterizing maize genotypes against downy mildew. Entries are scored as highly resistant (0 % disease incidence), resistant (0-10 % disease incidence), moderately resistant (11–25 % disease incidence), susceptible (26–50 % disease incidence) and highly susceptible (above 50 % disease incidence) as suggested by Pandurangegowda *et al.* (1986). Based on per cent downy mildew incidence, a rating scale has been developed as 0 – 10 per cent: resistant (R); >10 – 30 per cent: moderately resistant (MR); >30 – 50 per cent: moderately susceptible (MS); >50 per cent: susceptible (S) (Yen *et al.*, 2001 and Nair *et al.*, 2004 and 2005).

2.17. SELECTION OF CM 500 AS SUSCEPTIBLE CHECK

Kalpana *et al.* (2009) screened four local inbred maize genotypes CM 500, NAI-127, NAI-129 and SKV-10 in field for downy mildew and maximum incidence of downy mildew in field also was observed in CM 500, i.e., 97.2% and 99.4% respectively for Kharif and Rabi. They also subjected the genotypes to Sodium Dodecyl Sulphate (SDS)-Poly Acrylamide Gel Electrophoresis (PAGE) to characterize the total soluble seed proteins like globuline, albumin, gluteline and proline. The total soluble seed proteins were fractioned into 33 bands, which showed heterogeneity among different genotypes. The maximum numbers of bands (26) were observed in SKV-10 followed by NAI-129 (24), NAI-127 (21) and the least in CM 500 (19). The result indicated CM 500 as highly susceptible to downy mildew.

Also in experiment by Premalatha *et al*. (2010) maximum disease incidence among 100 maize inbreds screened was registered in CM 500.

2.18. Studies on variability

An insight into the magnitude of variability present in a crop species is of utmost importance as it provides the basis for effective selection. The relative magnitude of variation available in a population can be measured by coefficient of variation and is used in comparing the relative variability of two or more sets of measurements which are entirely in different units.

The phenotypic coefficient of variation is the observable variation present in a character or in a population; it includes both genotypic and environmental components of variation and as a result, its magnitude differs under different environmental conditions. The genotypic coefficient of variation on the other hand, is the component of variation which is due to genotypic differences among individuals within a population and is the main concern of plant breeders.

Fisher (1918) partitioned the total genetic variance (σ^2A) into (i) additive genetic variance (σ^2A), which is the sum of additive genetic variances contributed by individual loci (ii) Dominance variance (σ^2D) component which results from intra allelic interaction

of genes at segregating loci (iii) epistatic variance (σ^2I) results from inter allelic interaction of genes at segregating loci.

Warner (1952) has suggested different technique for estimating the degree of heritability in crop plant which is based on parent offspring regression variance component from an analysis of variance and approximation of non-heritable variance from genetically uniform population to estimate the total genetic variance.

Comstock (1955) reported that phenotype associated with a given genotype varies with the environment. This leads to complete inconsistency of genotypic value, a different value of a given genotype relative to every variance of environment major or minor.

Lonnquist (1964) reported that phenotype of a quantitative character was mainly due to the joint action of genotype and environment.

Abhirami *et al.* (2005) reported that genetic variance, heritability and genetic advance were studied in forty maize genotypes based on the data recorded on 20 quantitative traits. Genotypic and phenotypic coefficient of variance was higher for quantitative characters. Heritability estimates were high for all the characters investigated. High heritability coupled with high genetic advance was observed for number of grains per cob.

High heritability and high genetic advance have been reported for cob length, 100 grain weight and grain yield per plant (Hemavathy *et al.*, 2008).

2.18.1. Character-wise review

The review of literature pertaining to heritability with respect to quantitative characters is presented hereunder.

2.18.1.1. Days to tasseling

Days to tasseling is one of the components of maturity. Days from seedling to pollen shedding was used as a measure of maturity (Giesbrecht, 1960). Klein (1975) and Hansen *et al.* (1977) reported moderate to high range of heritability. However, Daniel and Bajtay (1976) and Rood and Major (1980) observed high heritability for this trait. Similarly, low to moderate broad sense heritability was reported by Debnath (1988) and

Alika (1994a) observed the moderate broad sense heritability for this trait. Vashishta *et al.* (2013) noted high heritability and Panwar *et al.* (2013) observed moderate heritability for the trait.

2.18.1.2. Days to silking

Days to silking is the most important attribute of maturity. Daniel and Bajtay (1976), Bonaparte (1977) and Hassaballa *et al.* (1980) reported the high heritability for days to silking. Low to moderate heritability was observed by Debnath (1988), Saadalls and Kadam (1988) and Panwar *et al.* (2013). High heritability for this trait was also noticed by Reddy and Agarwal (1992) and Vashishta *et al.* (2013).

2.18.1.3. Days to maturity

Sriani *et al.* (2003) recorded high broad sense heritability for the trait. Iqbal (2009) reported moderate while low heritability estimates was reported for the trait by Bekele and Rao (2014).

2.18.1.4. Plant height

Swamy *et al.* (1970) reported low heritability (23.42%) for plant height and he also observed the high heritability estimates of 80.40 and 88.62 per cent. Also Vashishta *et al.* (2013) and Bekele and Rao (2014) reported high estimates of heritability for plant height.

2.18.1.5. Cob length (cm)

High heritability (88.58%) was observed by Debnath (1988) and Panwar *et al.* (2013) for cob length. Vashishta *et al.* (2013) and Bekele and Rao (2014) reported moderate estimates of heritability.

2.18.1.6. Cob diameter (cm)

Satyanarayana and Sai (1995), Vashishta et al. (2013), Panwar *et al.* (2013) and Bekele and Rao (2014) observed moderate heritability for the trait.

2.18.1.7. No: of rows per cob

Satyanorayana and Sai (1995) and Vashishta et al. (2013) and Bekele and Rao (2014) observed moderate heritability for the trait.

2.18.1.8. No: of grains per row

Satyanarayana and Sai (1995), Vashishta et al. (2013) and Bekele and Rao (2014) observed high heritability for the trait while high broad sense heritability was recorded by Sultan *et al.* (2014).

2.18.1.9. Cob weight (g)

Sriani *et al.*, (2003) and Panwar *et al.* (2013) reported moderate estimates of heritability for cob weight.

2.18.1.10. Shelling %

Sriani *et al.* (2003) recorded low estimates of heritability for shelling %. Iqbal (2009) obtained moderate heritability for the same trait. High broad sense heritability was reported by Sultan *et al.* (2014).

2.18.1.11. Hundred grain weight

Radovic (1979) and Johnson and Russell (1982) reported moderate to high range of heritability of 100-grain weight. However, Beaver *et al.* (1985) observed moderate heritability. While, 52% broad sense heritability for this trait was reported by Alika (1994b). Also Vashishta *et al.* (2013), Panwar *et al.* (2013) and Bekele and Rao (2014) reported moderate to high range of heritability of 100 grain weight. High broad sense heritability was reported by Sultan *et al.* (2014).

2.18.1.12. Grain yield

Grain yield is a complex metric trait, influenced by several component characters. It is highly influenced by the environmental factors thus involving low heritability. Robinson *et al.* (1949) obtained a very low heritability of 29%. Klein (1975) and Ivanovic (1979) also observed low heritability for grain yield. Bohm and Schuster (1985) reported low heritability estimate (18%) for grain yield. High to moderate heritability with moderate estimates of genetic advance was recorded for grain yield per plant by Ling *et al.* (1996), Vashishta *et al.* (2013) and Panwar *et al.* (2013).

Material and Methods

CHAPTER III

MATERIAL AND METHODS

The present study includes 1) phenotyping the identified BC_3F_2 progenies through screening under artificial disease pressure in sick plot 2) identification of backcross progenies having major QTLs responsible for downy mildew resistance using SSR markers.

The studies on the field screening were done at Eastern Block of the Central Farm Unit, Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore. SSR marker analysis with regard to parents and progenies was done at Department of Biotechnology, TNAU, Coimbatore.

3.1. Source of Plant material

3.1.1 Study of BC_3F_2 generation

BC_3F_2 population was used in the present study. It is derived from crossing the inbred UMI 79 (Plate 1) which is susceptible for sorghum downy mildew and UMI 936(w) (Plate 2) which has resistant QTLs for sorghum downy mildew(Table 1) and backcrossing progenies with UMI79(Fig.1). Three BC_3F_2 progenies were used for screening under sick plot conditionsduring September to December by spreader row technique. The disease assessment was done at 30 days after plant emergence. Screening was carried out in the Eastern Block of the Central Farm Unit, Department of Agronomy, at Tamil Nadu Agricultural University, Coimbatore.The biometrical data was recorded for yield and its component traits. SSR marker survey was done with phi053 (chromosome 3) and nc013 (chromosome 6) as reported by Nair *et al*. (2005).

3.2. Source of primers

The SSR markers used for chromosome 3 is phi053 and for chromosome 6 is nc013. The information of SSR primers which is linked to sorghum downy mildew resistance used in the study was obtained from maize database and sequence details are given in the Table 2.

Plate 1. UMI 79 (Sorghum downy mildew susceptible parent)

Plate 2. UMI 936(w) (Sorghum downy mildew resistant parent)

SEASON	BACKCROSS BREEDING PROGRAMME	METHOD OF SCREENIG
Kharif' 09	UMI 79 (Susceptible) X UMI 936(w) (Resistant)	• Parental polymorphism survey using SSR markers (Kashmiri, 2010)
Kharif' 11	F_1 X UMI 79 (Recurrent parent)	• Marker assisted foreground selection in F_1 • One single true to type F_1 hybrid selected and backcrossed
Rabi' 12	BC_1F_1 X UMI 79	• Phenotypic screening for sorghum downy mildew resistance • Marker assisted foreground selection • 14 BC_1F_1s selected and backcrossed (Aarthi, 2012)
Kharif' 12	BC_2F_1 X UMI 79	• Phenotypic screening for sorghum downy mildew resistance • Marker assisted foreground selection • 5 BC_2F_1s selected and backcrossed
Rabi' 13	BC_3F_1	• Phenotypic screening for sorghum downy mildew resistance • Marker assisted foreground selection • 3 BC_3F_1s selected and selfed (Sumathi, 2013)
PRESENT STUDY		
Kharif' 13	BC_3F_2	• Phenotypic screening for sorghum downy mildew resistance • 104 resistant individual plants selfed
Rabi'14	BC_3F_3	• 8 phenotypically superior progenies identified • Marker assisted foreground selection • Three NILs identified

Fig.1. The procedure adopted to develop sorghum downy mildew resistant lines

3.3. Field trial

3.3.1. Screening Maize genotypes under sorghum downy mildew in the Natural Infection

The BC_3F_2 progenies were screened against sorghum downy mildew in sick plot under natural conditions duringkharif2013 which was conducive for the pathogen development in order to identify the resistant progeny through field screening. The parents as well as the BC_3F_2progenies were studied for their resistance or susceptible reaction in sick plot by spreader row technique. The downy mildew symptoms developed have been presented in Plate 3 and Plate 4. The procedure adopted for the above screening method is as follows.

3.3.1.1. Spreader row technique in the sick plot for screening maize genotypes against sorghum downy mildew

Screening against sorghum downy mildew was carried out during kharif2013 by taking advantage of monsoon season with low temperature which is conducive for pathogen development. Metrological observation *viz.*, minimum- maximum temperature(^{0}C), relative humidity (%) and rainfall (mm) were taken during each week as these are critical weather factors to build up the disease pressure. Most sporulation occurs when temperatures are around 20^{0}C and relative humidity more than 95% (Payak, 1975b & Shetty, 1987). The Artificial disease epiphytotic conditions were created by planting spreader rows of a susceptible maize genotype, CM 500 (Shetty and Ahmad, 1980;Krishnappa *et al*., 1995;Setty*et al*., 2001;Yen *et al*., 2001;George *et al*., 2003; Nair *et al*., 2004 and Nair *et al*., 2005), 30 days prior to sowing of test entries. Spreader row technique adapted by Craig *et al*., (1976) was followed for screening the maize genotypes against sorghum downy mildew in the field.

Table 1. Details of parents used in the study

S. No.	Genotypes	Origin	Grain colour	Grain texture	Reaction to SDM
1.	UMI 79	Selection from Pioneer 102	Orange	Flint	Susceptible
2.	UMI 936(W)	Selection from DMR pool- Taiwan 524	White	Flint	Resistant

Table 2. List of foreground markers used for SSR analysis

S. No.	SSR Marker	Chromosome Location	Sequence	Product size (bp)
1	phi053	3	CTGCCTCTCAGATTCAGAGATTGAC (F) AACCCAACGTACTCCGGCAG (R)	180-196
2	nc013	6	AATGGTTTTGAGGATGCAGCGTGG (F) CCCCGTGATTCCCTTCAACTTTC (R)	114-134

3.3.1.2 Planting of CM 500 seeds as spreader row in the sick plot

After field preparation, ridges were formed in 3m length with 60 cm between ridges. The seeds of sorghum downy mildew susceptible inbred, *i.e.*, CM500 were sown in sick plot in every 11^{th} row leaving 10 rows in between to accommodate test entries 30 days later and also on all four sides of sick plot (Plate 5). This time gap (30 days) between sowing of spreader row and test entries allowed disease development in spreader rows which forms the inoculum for infection in test entries.

3.3.1.3 Conidial inoculums preparation and spraying on spreader row entries

Being obligate parasite, conidia of *P. sorghi* were harvested from fresh, infected plants for inoculations. The method of conidial inoculums preparation used in this study was adopted from Cardwell *et al.* (1994) and by utilizing the natural spore producing cycle of the fungus, which involved spray operation in the middle of the night (Siradhana*et al*., 1975; Renfro *et al*., 1979). Conidia were obtained from three week old systematically infected maize plants. Maize leaves infected with *P. sorghi* showing visible conidial growth were collected from the infected field on the previous day early evening. Infected leaves were wiped with wet absorbent cotton to remove old and matured downy mildew conidia produced previously and they were wiped again using tissue paper to remove moisture from the leaf surface. These sorghum downy mildew infected leaves were spread in a single layer over a tray lined with moist blotting paper in such a way that abaxial leaf surface faced upwards. Another tray lined with moist blotting paper was used to close the tray containing infected leaf materials. These trays were incubated at 20^{o}C in the dark for six to seven hours for sporulation, until 3.00 AM of next day morning. At this time, conidia were harvested by washing the sporulated leaves in chilled distilled water (5^{o}C) using a camel hairbrush. The conidial suspension was filtered through a double layered muslin cloth to remove conidiophores and other leaf particles. The concentration was adjusted to 6×10^{5} per ml using a hemocytometer. The resulting spore suspension was placed into backpack sprayers and taken to the field. The spraying was taken from 3.30 to 4.30 am on ten days old spreader row (CM 500) plants. This method utilizes the natural spore producing cycle of pathogen.

Plate 3. Crazy top symptom

Plate 4. Downy growth on leaves

Plate 5. CM 500 spreader rows in field

The test entries were planted after ensuring hundred per cent disease establishment in the spreader rows. In that way test entries were exposed to infection by both oospores from the soil and conidia from spreader rows.

3.3.2 Disease assessment in maize genotypes against sorghum downy mildew

Germination count was taken one week after sowing. The disease reaction was assessed at thirty days after plant emergence of test entries in spreader row technique (under sick plot). The number of infected plants and total number of plants in each progeny were recorded. The percentage of incidence of downy mildew was calculated as per standard procedure (Lal and Singh, 1984).

$$\text{Percentage downy mildew incidence} = \frac{\text{No. of plants infected}}{\text{Total no. of plants}} \times 100$$

The rating scale was followed as below:

Percentage Downy Mildew incidence (%)	Reaction
0-10	Resistance(R)
>10-30	Moderately Resistance(MR)
>30-50	Moderately Susceptible(MS)
>50	Susceptible(S)

3.4. Molecular marker analysis

The seedlings of BC_3F_3 were allowed to grow for 20 days in order to get enough leaf material for extraction of DNA.

3.4.1. DNA extraction

DNA was extracted from the selected genotypes following the procedure of CTAB method.

3.4.1.1 Reagents required

The reagents used for DNA isolation are as follows:

a. Cetyl Trimethyl Ammonium Bromide (CTAB) Extraction Buffer

CTAB	2% w/v
Tris HCl (pH 8.0)	200 mM
Sodium Chloride	1.4 M
EDTA	20 mM
ß - mercaptoethanol	0.1% v/v(ß - mercaptoethanol was added immediately prior to use)

b. Tris EDTA (TE) Buffer

TrisHCl (pH 8.0)	10 mM
EDTA	1 mM

c.Ice-cold Isopropanol

d. Chloroform: Isoamylalcohol (24:1 v/v)

e. Phenol: chloroform (1:1)

f. Sodium acetate (3 M) pH 5.2 (pH adjusted using glacial acetic acid)

g. Ethanol (100% and 70%)

h. PVP (1%)

3.4.1.1.2 Protocol

The genomic DNA was extracted by following CTAB method.

1. The leaves were ground with 500 µl of CTAB buffer, transferred into an eppendorf tube and was incubated for 30 min.

2. PVP and ß – mercaptoethanol were addedand was incubated for 30 minutes at 65°C in water bath with occasional mixing.

3. The tubes were removed from the water bath and equal volume of chloroform: Isoamyl alcohol mixture (24:1 v/v) was added and mixed by inversion for15 minutes.

4. It was centrifuged at 10,000 rpm for 20 minutes at room temperature.

5. The clear aqueous phase was transferred to a new sterile tube.

6. Equal volumes of phenol: chloroform (1:1 v/v) was added. It was centrifuged at 4000 rpm for 20 mins and the clear aqueous phase was transferred to a new sterile tube.

7. A 2.5 volume of ice cold isopropanol and 3 M Sodium acetate was added and mixed gently by inversion and then kept in the freezer until DNA was precipitated out.

8. Then it was centrifuged at 4000 rpm for 20 min and the supernatant was discarded. The pellet was washed with 70% alcohol by centrifugation.

9. The alcohol was discarded and DNA was completely air-dried.

10. Then the DNA pellet was dissolved in 150 - 250 µl of TE and stored at 4°C.

3.4.2. DNA quality check by Agarose gel electrophoresis

3.4.2.1 Materials

- Loading Dye

  Glycerol 50% (v/v)

  Bromophenol blue 0.5% (w/v)

- 10X TBE (Tris Borate EDTA buffer)

Tris Base	107.8 g
Boric acid	55.03 g

EDTA($Na_2.2H_2O$) 8.19 g

(Dissolved in 800 ml of sterile water and made up to 1000 ml)

3.4.2.2 Protocol

1. The Pyrex gel casting plate open ends were sealed with cello tape and the comb was placed properly in casting plate kept on a perfectly horizontal platform.

2. 0.8 % (0.8 g/100 ml) agarose was added to 1x TBE, boiled until the agarose dissolved completely and then allowed to cool. Ethidium bromide (DNA intercalating agent) was added when temperature reached 55-60^0 C as a staining agent.

3. Then it was poured into the gel mould and allowed to solidify.

4. The comb and the cello tape were removed carefully after solidification of the agarose.

5. The casted gel was placed in the electrophoresis unit with wells towards the cathode and submerged with 1X TBE to a depth of about 1cm.

3.4.2.3 Loading the DNA samples

- A volume of 1 µl of DNA sample dissolved in TE was pipetted onto a parafilm and mixed well with 3 µl of 10X loading dye by pipetting up and down several times.

- The gel was run at 8 V/cm for 1-1.5 hours and bands were visualized and documented using a gel documentation system (Model Alpha Imager 1200, Alpha Innotech Corp., USA).

3.4.3 Quantification of DNA

DNA was quantified by using Nanodrop (Nanodrop Spectrophotometer ND-1000). A volume of 1 µl of genomic DNA was loaded for quantification and 1µl of TE buffer was used as blank. The absorbance for all samples was measured at 260 nm as double stranded DNA has maximal absorbance at 260 nm. If the quantified DNA shows 'x' ng/µl in Nanodrop, then dilution is done 'y' times (where, 'y' = 'x'/50). Based on the

quantification data, DNA dilutions were made in 1X TE buffer to a final concentration of 50ng/µl and stored in -20°C for further use.

3.4.4. SSR analysis

Microsatellites can be easily amplified by the Polymerase Chain Reaction using unique flanking sequence as forward and reverse primers. In general, primer pairs have been derived from sequence information obtained from DNA libraries and published sequence data. A total of two SSR primerson the two chromosomes were used for PCR amplification.

3.4.4.1 PCR amplification

Microsatellite analysis was done with the following steps:

1. PCR amplification of genomic DNA was done using forward and reverse microsatellite primers
2. Resolution of polymorphism through Agarose gel electrophoresis.
3. Staining the gel
4. Analysis of banding pattern

The cocktail for PCR amplification was prepared as follows:

Reaction mixture (15 µl) :-

DNA 50 ng/µL	2.00 µL
10X assay buffer	1.5 µL
Primer (10 µm)	1.1 µL
dNTPs (2.5 mM) (Bangalore Genei Ltd., India)	0.5µL
*Taq*polymerase (3 units/µL) (Bangalore Genei Ltd., India)	0.2 µL
Sterile distilled H_20	8.7 µL

Total 15 µL

(dNTPs, assay buffer and *Taq* DNA polymerase used were obtained from Bangalore Genei Ltd., India).

- The reaction mixture was given a momentary spin for through mixing of the cocktail components. Then 0.20 ml PCR tubes were loaded in a thermal cycler.

- The reaction in thermal cycler (PTC-100^{TM}, MJ Research Inc, Massachusetts, USA) was programmed as follows:

Profile 1:	95˚C for 5 minutes	Initial denaturation
Profile 2:	94˚C for 1 minute	Denaturation
Profile 3:	55˚C for 1 minute	Annealing
Profile 4:	72˚C for 1 minute	Extension
Profile 5:	72˚C for 5 minutes	Final extension
Profile 6:	4˚C	Hold the samples

Profiles 2, 3 and 4 were programmed to run for 35 cycles

3.4.5. Agarose gel electrophoresis of SSR-PCR products

After PCR amplification, the PCR products were separated by 3 % Agarose gel electrophoresis.

3.4.5.1.Agarose gel electrophoresis

Agarose gel electrophoresis was performed to separate amplified products.

3.4.5.1.1 Materials

- Loading Dye

  Glycerol 50% (v/v)

  Bromophenol blue 0.5% (w/v)

- 10 x TBE (Tris Borate EDTA buffer)

Tris base	107.8 g
Boric acid	55.03 g
EDTA($Na_2.2H_2O$)	8.19 g

(Dissolved in 800 ml of sterile water and made up to 1000ml)

3.4.5.1.2 Protocol

- Open ends of the gel casting plate were sealed with cello tape and placed on a horizontal perfectly leveled platform.
- The 3% agarose was added to 1X TBE buffer and boiled till the agarose dissolved completely and then cooled to 50-60°C. Ethidium bromide was used as a staining agent at the final concentration of 1 µg/ml.
- Agarose gel was poured in to the gel casting tray. The comb was placed properly and allowed to solidify.
- After solidification of the agarose, the comb and cello tape were removed.
- DNA samples were mixed well with 5 µl of loading dye and were loaded into the gel wells.
- The gel was run at 110 volts for 2.5 hours and bands were visualized and documented in gel documentation system (Model Alpha Imager 1200, Alpha Innotech Corp., USA).

3.5. Genotyping of BC_3F_3population

Two SSR markers, one each from chromosome 3 (phi053) and chromosome 6 (nc013) were used to identify lines carrying the resistant QTL. Sixty eight BC_3F_3individuals obtained from eight cobs of the BC_3F_2 progenies were genotyped.

3.5.1. Scoring of genotypes

The genotyping data of the BC_3F_3 lines were scored against the two SSRs. The segregation pattern of SSR markers for all the lines were scored as co-dominant

fragments. The individuals showing the banding pattern similar to the parent, UMI 79 were scored as “A”, the heterozygous were scored as “H”, and the plants with the alleles similar to the parent, UMI 936(w) were scored as “B” and null bands were scored as “-”.

3.6. Field observations

The data on days to tasseling, days to silking, days to maturity, plant height, cob length, cob diameter, number of rows per cob, number of grains per row, cob weight, shelling %, hundred grain weight and grain yield per plant for BC_3F_2plants were recorded.

1. Days to tasseling

It was recorded as number of days from sowing to when plants start to shed pollen.

2. Days to silking

It was recorded as number of days from sowing to when plants haveemerged silks 2-3 cm long.

3. Days to maturity

It was recorded as number of days from sowing to when leaves start to dry.

4. Plant height (cm)

It was measured from ground level to the tip of the tassel after milk stage and expressed in cm.

5. Cob length (cm)

It was measured from one end of the cob to next end and was expressed in cm.

6. Cob diameter (cm)

The maximum diameter of the cob was measured and was expressed in cm.

7. Number of rows per cob

Number of rows on each cob was counted.

8. Number of grains per row

Number of grains in each row was counted and average was noted.

9. Cob weight (g)

Weight of each cob was recorded and was expressed in grams.

10. Shelling %

Grain yield per cob was divided by cob weight to get the shelling %.

11. 100 grain weight (g)

A total of 100 randomly selected grains per cob were weighed at 12 per cent moisture content and expressed in grams.

12.Grain yield per plant (g)

The single cob harvested per plant was shelled and grain weight was recorded as grain yield per plant and expressed in grams.

3.7. Statistical analysis

3.7.1. Estimation of genetic parameters

The various genetic parameters like variability, GCV, PCV, heritability and genetic advance as per cent mean were worked out for the BC_3F_2 progenies by adopting the formulae given by Johnson *et al.,* (1955).

3.7.1.1. Phenotypic and genotypic variance

The average variance observed in the parent UMI 79 and UMI 936(w) were considered as environmental variance. The genotypic variance of each progeny was

estimated by subtracting the estimated environmental variance from the phenotypic variance.

Environmental variance (Ve) = Average phenotypic variance of both the parents

Phenotypic variance (Vp) = Variance of population

Genotypic variance (Vg) = Vp – Ve

3.7.1.2. Phenotypic and genotypic coefficients of variability

a) Phenotypic coefficient of variation (%)

$$PCV = \frac{\sqrt{Vp}}{Mean} \quad X\ 100$$

b) Genotypic coefficient of variation (%)

$$GCV = \frac{\sqrt{Vg}}{Mean} \quad X\ 100$$

The PCV and GCV values were categorized as follows (Sivasubramanian and Menon, 1973).

PCV and GCV	Category
< 10 per cent	Low
10 – 20 per cent	Moderate
> 20 per cent	High

3.7.1.3. Heritability (h^2)

Heritability (h^2) estimate in broad sense and expected genetic advance (GA) at five per cent selection intensity were estimated by the methods devised by Lush (1940) and expressed in percentage.

$$\text{Heritability } (h^2) = \frac{Vg}{Vp} \text{ X } 100$$

The heritability % was categorized as suggested by Robinson *et al.* (1949).

Heritability (in %)	Category
< 30	Low
31 – 60	Medium
> 60	High

3.7.1.4. Genetic advance (GA)

Genetic advance was estimated by the method formulated by Johnson *et al*. (1955).

Genetic advance = $k \times h^2 \times \sigma p$

Where,

h^2 = Heritability in broad sense

σp = Phenotypic standard deviation

k = Selection differential (at 5 % selection intensity) (*i.e*.) 2.06 (Falconer, 1960)

3.7.1.5. Genetic advance as percent of mean

The genetic advance as per cent of mean was categorized as suggested by Johnson *et al*. (1955).

$$\text{GA as per cent of mean} = \frac{\text{Genetic advance}}{\text{Grand mean}} \times 100$$

GA was categorized as:

GA per cent value	Category
< 10 per cent	Low

10 - 20 per cent	Moderate
> 20 per cent	High

3.7.1.6. Skewness and Kurtosis

These were calculated using the frequency distribution (Kapur, 1980) of the characters mentioned.

β_1 = Skewness

If, $\beta_1 > 0$, then positively skewed

$\beta_1 < 0$, then negatively skewed

$\beta_1 = 0$, then symmetric distribution

β_2 = Kurtosis

If, $\beta_2 > 1$, then leptokurtic

$\beta_2 < 1$, then platykurtic

$\beta_2 = 0$, then mesokurtic

$$\beta_1 = \frac{\mu_3^2}{\mu_2^2}$$

$$\beta_2 = \frac{\mu_4^2}{\mu_2^2}$$

Where,

$$\mu_2^2 = \frac{1}{N}\sum fi(Xi-\bar{X})^2$$

$$\mu_3^2 = \frac{1}{N}\sum fi(Xi-\bar{X})^3$$

$$\mu_4^2 = \frac{1}{N}\sum fi(Xi-\bar{X})^4$$

Where,

X_i is the individual observation

X is the mean of the character under observation and

N is the number of observations

Significance

The skewness and kurtosis was divided by the respective standard errors to calculate t value. The calculated 't' value was compared with 't' table value with (n-1) degrees of freedom to assess significance.

$$SE_{\beta 1} = \frac{\sqrt{6}}{N}$$

$$SE_{\beta 2} = \frac{\sqrt{24}}{N}$$

3.7.1.7. Frequency distribution

The phenotypic data of BC_3F_2 along with the parents were utilized for studying the frequency distribution in various biometrical traits to know about the extremes in the population. It was calculated by taking minimum and maximum value of the trait. Then the difference between the maximum and minimum values is recorded as 'X' and the class interval is fixed as 10. Then the bin range was fixed by dividing the value 'X' with class interval. With the bin range, the frequency of population is obtained.

Significance

The skewness and kurtosis was divided by the respective standard errors to calculate t value. The calculated't' value was compared with 't' table value with (n-1) degrees of freedom to assess significance.

$$SE_{\beta 1} = \frac{\sqrt{6}}{N}$$

$$SE_{\beta 2} = \frac{\sqrt{24}}{N}$$

3.7.1.7. Frequency distribution

The phenotypic data of BC_3F_2 along with the parents were utilized for studying the frequency distribution in various biometrical traits to know about the extremes in the population. It was calculated by taking minimum and maximum value of the trait. Then the difference between the maximum and minimum values is recorded as 'X' and the class interval is fixed as 10. Then the bin range was fixed by dividing the value 'X' with class interval. With the bin range, the frequency of population is obtained.

Experimental Results

CHAPTER IV

EXPERIMENTAL RESULTS

The present investigation was carried out to introgress sorghum downy mildew resistance in to an elite and adapted parental line UMI 79 through marker assisted backcross breeding. Backcross populations were developed from the hybrid synthesised between susceptible adapted parent UMI 79 and sorghum downy mildew resistant UMI 936(w) as donor parent. In each generation, marker assisted screening was done to identify the introgressed progenies through foreground selection for the resistance genes. Selected backcross progenies were further used as female parents in backcross with UMI 79 as the recurrent pollen parent. The present study involved phenotypic screening of BC_3F_2 and genotypic screening of BC_3F_3 progenies developed for sorghum downy mildew resistance and selection of homozygotes for resistant gene from UMI 936(w) (donor parent) for further advancement. The BC_3F_2 population was raised under sick plot to select resistant progenies phenotypically for further selfing to generate BC_3F_3 progenies. Superior progenies from BC_3F_3 were then genotypically screened using foreground markers to identify homozygotes for resistant plants.

The experimental results are presented in this chapter under the following headings.

4.1. Marker assisted selection using SSR markers

4.2. Study of BC_3F_2 generation

4.2.1. Development of BC_3F_2 population

4.2.2. Phenotypic screening of BC_3F_2 population

4.2.3. Variability studies

4.2.4. Frequency distribution

4.3. Study of BC_3F_3 generation

4.3.1. Development of BC_3F_3 population

4.3.2. Genotyping of BC_3F_3 population

4.1. Marker assisted selection using SSR markers

Comparing the sequence information obtained from the maizegdb, and the foreground marker information obtained from Nair *et al*. (2005), two SSR loci (phi053 and bnlg420) of chromosome 3 (Fig. 2) and two (bnlg1154 and nc013) of chromosome 6 (Fig. 3) were reported to show good polymorphism by Kashmiri, (2010) for the UMI 79 x UMI 936(w) population. A higher percentage of polymorphism (42.8%) was observed in the linkage group 6 (Kashmiri, 2010). In the present study 2 SSR loci *viz.*, phi053 and nc013, one each from chromosome 3 and chromosome 6 were selected for foreground survey. Marker assisted selection has been carried out with accuracy and efficiency using these SSR markers located on the two QTLs for sorghum downy mildew resistance.

4.2. Study of BC_3F_2 generation

4.2.1. Development of BC_3F_2 population

From BC_3F_1 population developed by repeated backcrossing involving UMI 79 (susceptible recurrent parent) with single plants carrying resistant QTLs identified through phenotyping and genotyping, three progenies *viz.*, 7-2-3, 7-7-7 and 7-2-10, heterozygous for the SSR markers were selectively selfed to generate the BC_3F_2 population. The BC_3F_2 population was raised under sorghum downy mildew sick plot condition in the Eastern Block of the Central Farm Unit, Department of Agronomy, at Tamil Nadu Agricultural University, Coimbatore during September to December, 2013 field view of the progenies have been given in Plate 6 and Plate 7 and cob photos are presented in Plate 8 and Plate 9.

4.2.2. Phenotypic screening of BC_3F_2 population

The three progenies *viz.*, 7-2-3 (104 plants), 7-7-7 (121 plants) and 7-2-10 (125 plants) were phenotypically screened for sorghum downy mildew under sick plot condition by raising them in 3m rows with spacing of 60 x 25 cm. Artificial epiphytotic conditions were created by planting spreader rows of CM 500, a susceptible maize genotype, thirty days prior to sowing of test entries.

Screening against sorghum downy mildew disease was carried out during Rabi season by taking advantage of monsoon season, which is conducive for pathogen

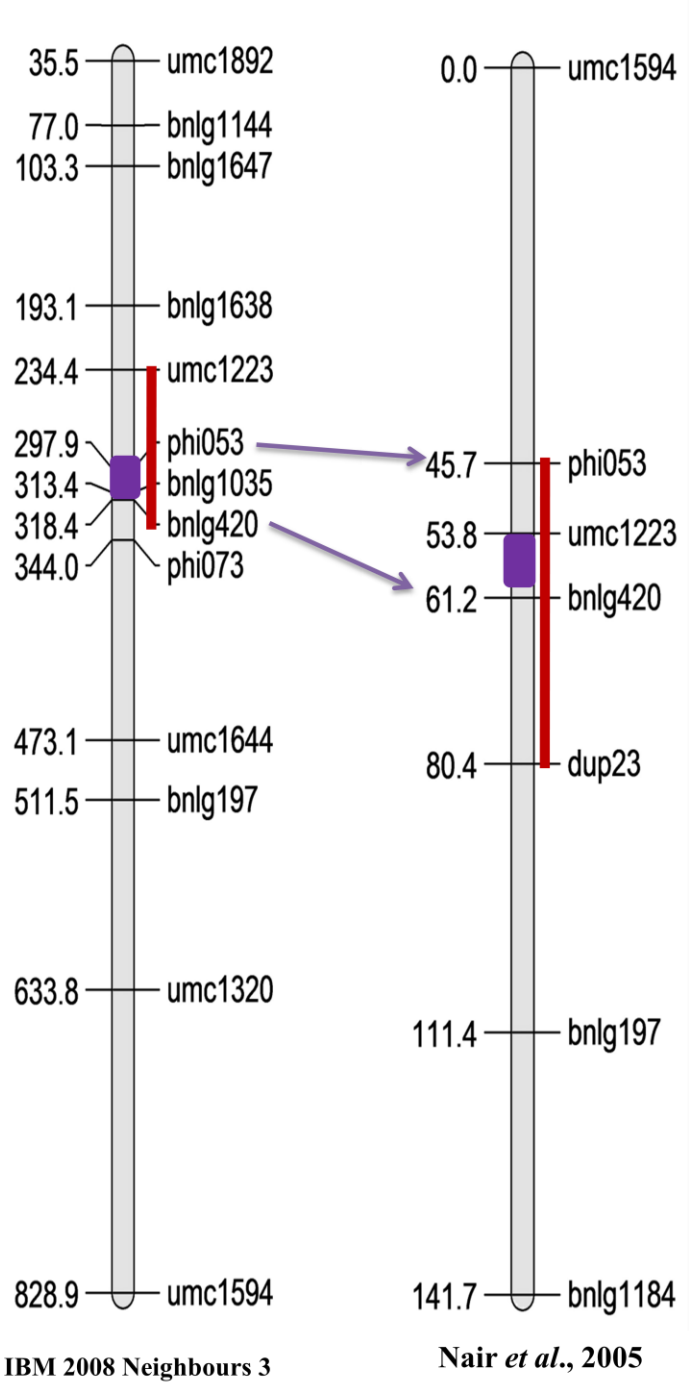

Fig. 2. Genetic linkage map showing location of SDM QTL on chromosome 3

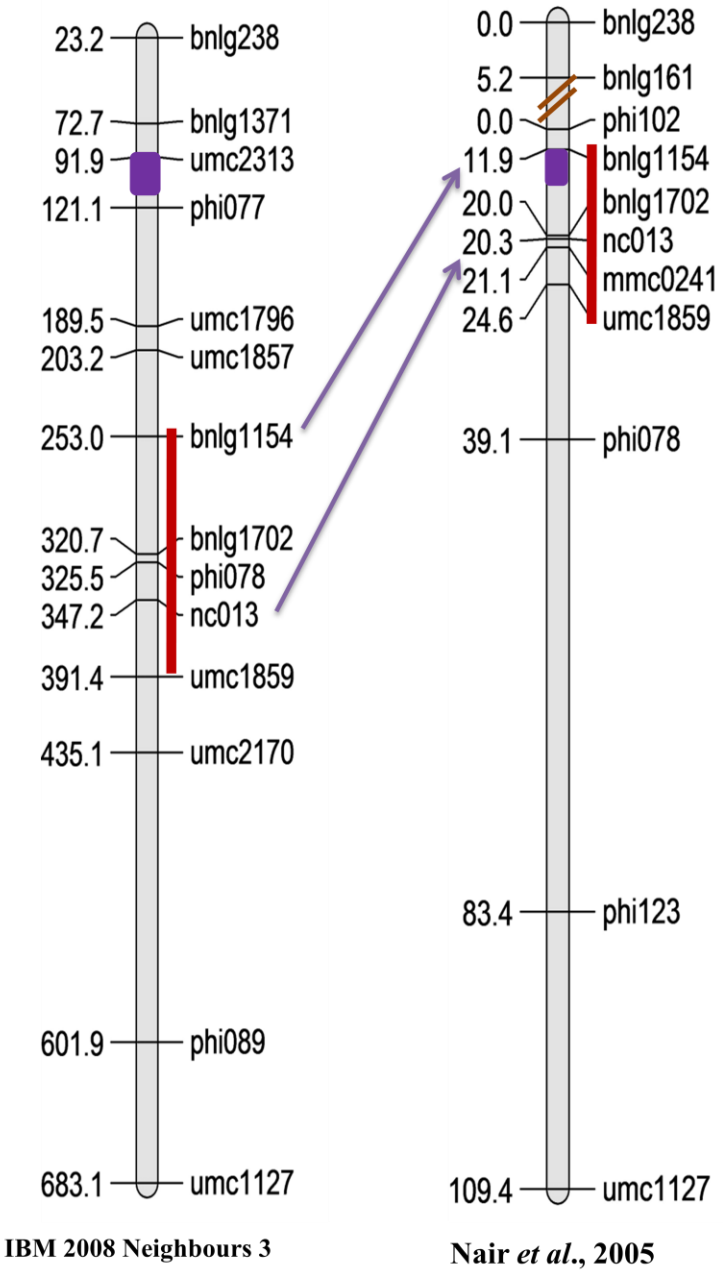

Fig. 3. Genetic linkage map showing location of SDM QTL on chromosome 6

Plate 6. Field view of 7-7-7 progeny

Plate 7. Field view of 7-2-3 progeny

Plate 8. Cob of 7-7-7 progeny

Plate 9. Cob of 7-2-3 progeny

development. Metrological observations *viz.*, minimum- maximum temperature (^{0}C), relative humidity (%) and rainfall (mm) recorded during each week is given in Fig. 4-6. The downy mildew disease scoring was done after 30 days of sowing and thus first four weeks were most important for the development of disease. In the first four weeks average minimum and maximum temperature was recorded as 21.7^{0}C and 32.1^{0}C respectively, average relative humidity was 86.25% and average rainfall was recorded as 25.9 mm.

The germination percentage for each progeny was recorded one week after sowing. The progenies 7-2-3, 7-2-10 and 7-7-7 have showed 81.89%, 84.28% and 90.29% germination respectively. The percentage of incidence of downy mildew was recorded thirty days after plant emergence as per procedure developed by Lal and Singh, 1984. Progeny 7-2-10 (72.38% infection) was found to be highly susceptible to the disease and thus eliminated from further studies. Progeny 7-7-7 (21.49% infection) and 7-2-3 (26.92% infection) showed moderate level of resistance with progeny 7-7-7 exhibiting higher resistance to sorghum downy mildew (Fig. 7).

4.2.2. Study of mean performance of BC_3F_2 progenies

Mean values for various traits were computed for resistant progenies 7-7-7 and 7-2-3. Among the progenies, 7-7-7 was showing higher mean values for traits like plant height, cob length, cob diameter, number of rows per cob, number of grains per row, cob weight, 100 grain weight, shelling % and grain yield per plant. The mean value and the range of different biometrical traits are presented in the Table 3 for progeny 7-7-7 and Table 4 for 7-2-3 progeny.

4.2.4. Variability studies in BC_3F_2 progenies

BC_3F_2 progenies showing the resistant reaction *viz.*, 7-7-7 and 7-2-3 were selected for the study of mean and variability. Comparison between the two progenies *viz.*, 7-7-7 and 7-2-3 for PCV, GCV, heritability and genetic advance as per cent of mean for the different traits are represented graphically in Fig. 8-11.

4.2.4.1. Days to tasseling

The flowering of BC_3F_2 progeny no. 7-7-7 ranged from 52 to 65 with a mean of 58.12 almost similar to the recurrent parent, UMI 79 (59.40 days). The trait exhibited low

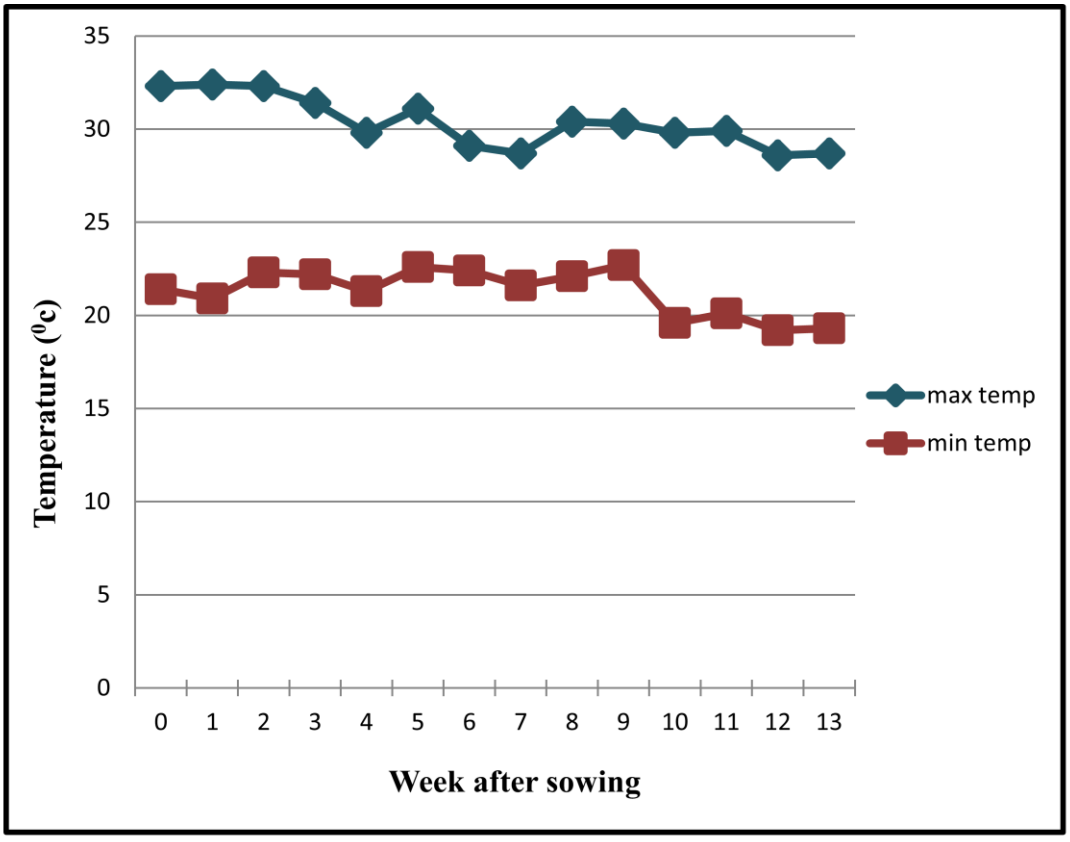

Fig. 4. Weekly temperature variation recorded during maize growing season

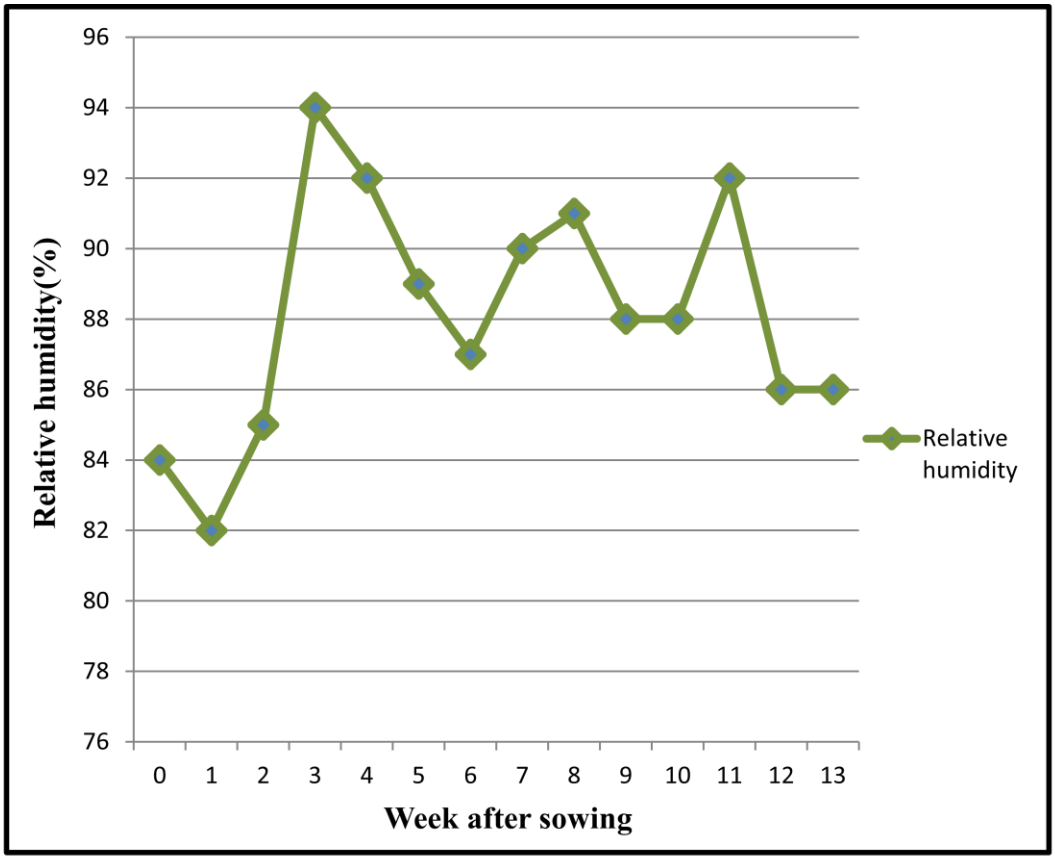

Fig. 5. Weekly relative humidity variation recorded during maize growing season

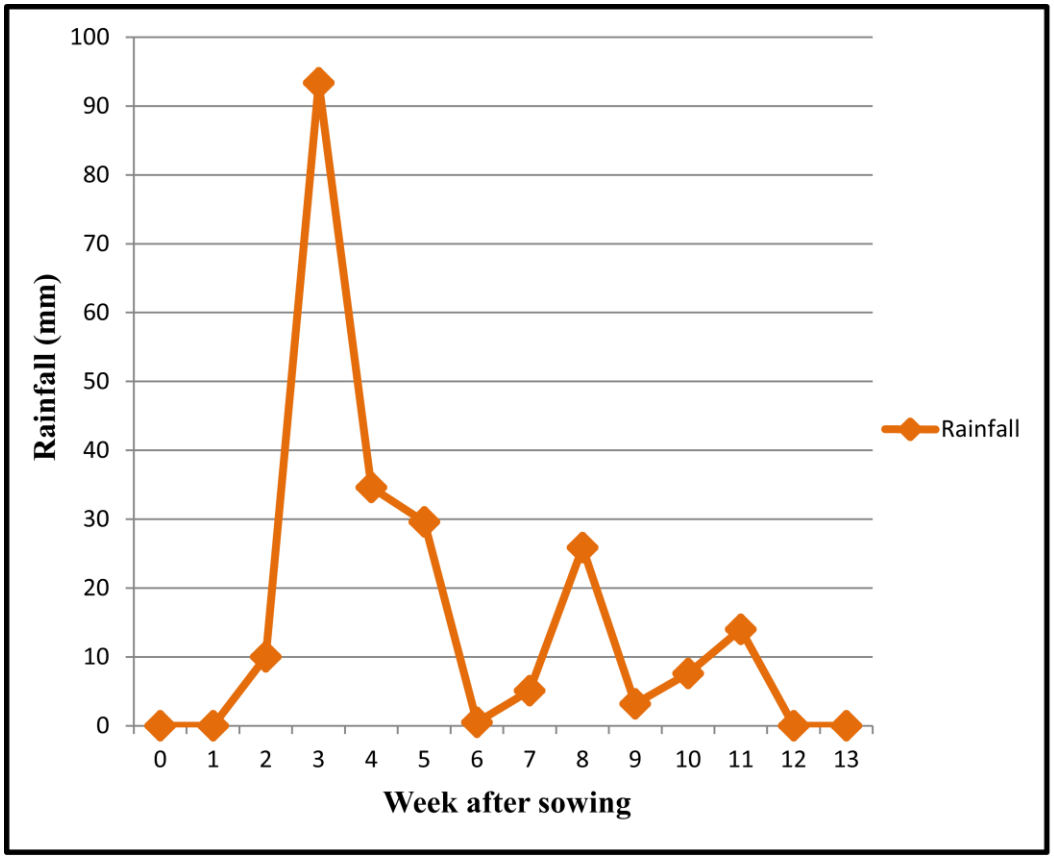

Fig. 6. Weekly rainfall variation recorded during maize growing season

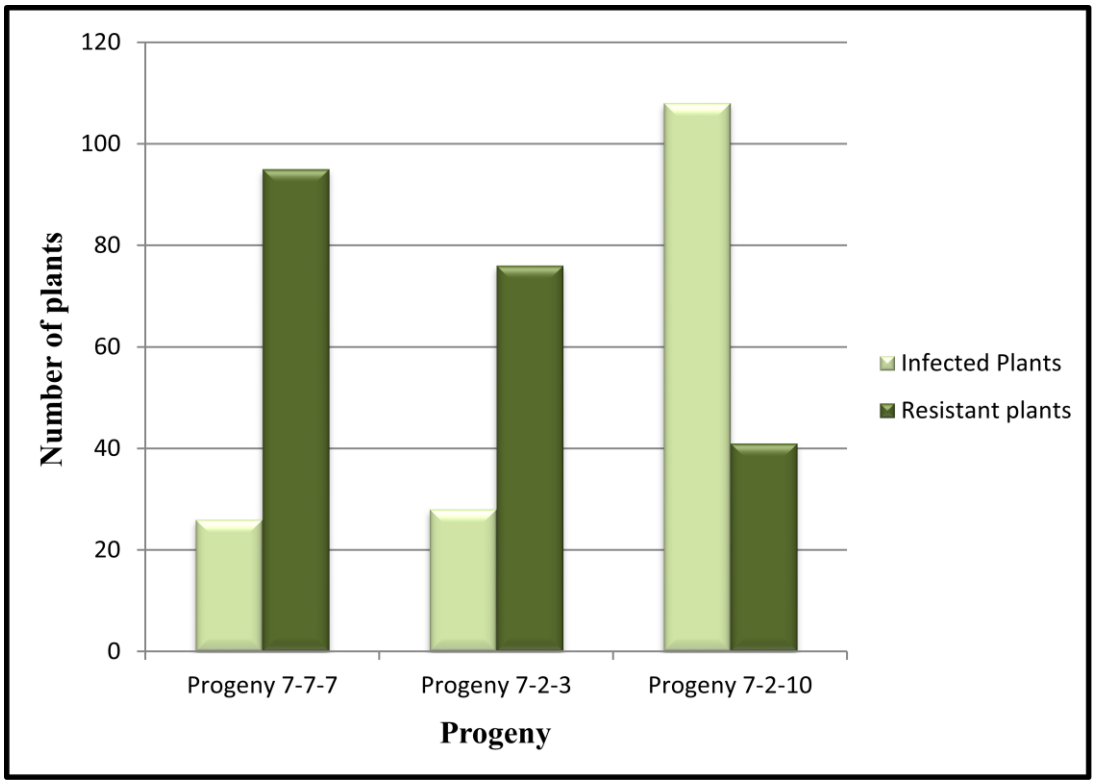

Fig. 7. Response to sorghum downy mildew disease among BC_3F_2 progenies

PCV (6.48), low GCV (6.00), high heritability (85.57%) and moderate genetic advance as per cent of mean (11.43%) (Table 3).

For progeny no. 7-2-3, the value varied from 50 to 68 days with a mean of 59.55 days which was similar to the recurrent parent. PCV (7.64) and GCV (7.25) values recorded were found to be low. High heritability (90.09%) and moderate genetic advance as per cent of mean (14.17%) was recorded for this trait (Table 4).

4.2.4.2. Days to silking

The mean days taken for silking in BC_3F_2 progeny no. 7-7-7 was recorded as 61.20 days similar to recurrent parent, UMI 79 (62.20 days) with minimum 55 and maximum 68 days. The variability parameters *viz.,* PCV (6.14) and GCV (5.57) were found to be low for the trait. High heritability (82.27%) with moderate genetic advance as per cent of mean (10.40%) was recorded (Table 3).

In case of progeny no. 7-2-3, mean value for the trait was 62.45 days with range from 54 to 70 days. The flowering was similar to the recurrent parent whereas PCV (7.26) and GCV (6.86) values recorded were observed to be low. High heritability (89.07 %) and moderate genetic advance as per cent of mean (13.33 %) was recorded for this trait (Table 4).

4.2.4.3. Days to maturity

Days to maturity for progeny no. 7-7-7 had a mean of 91 days with minimum 87 and maximum 102 days. It is almost similar to the recurrent parent, UMI 79 (93.60 days). Low PCV (3.92), low GCV (2.44), medium heritability (38.82%) and low genetic advance as per cent of mean (3.14 %) was observed for the trait in this progeny (Table 3).

For the same trait, value in progeny no. 7-2-3 was observed to be varying from 85 to 103 days with a mean of 91.08 days which is almost similar to UMI 79 (93.60 days), the recurrent parent. The trait showed low PCV (4.48), low GCV (3.27), moderate heritability (53.21 %) and low genetic advance as per cent of mean (4.91 %) (Table 4).

4.2.4.4. Plant height (cm)

The plant height of progeny no. 7-7-7, ranged from 58.60 cm to 133.90 cm. It showed a mean value of 99.92 almost similar to the recurrent parent, UMI 79 (98.47 cm).

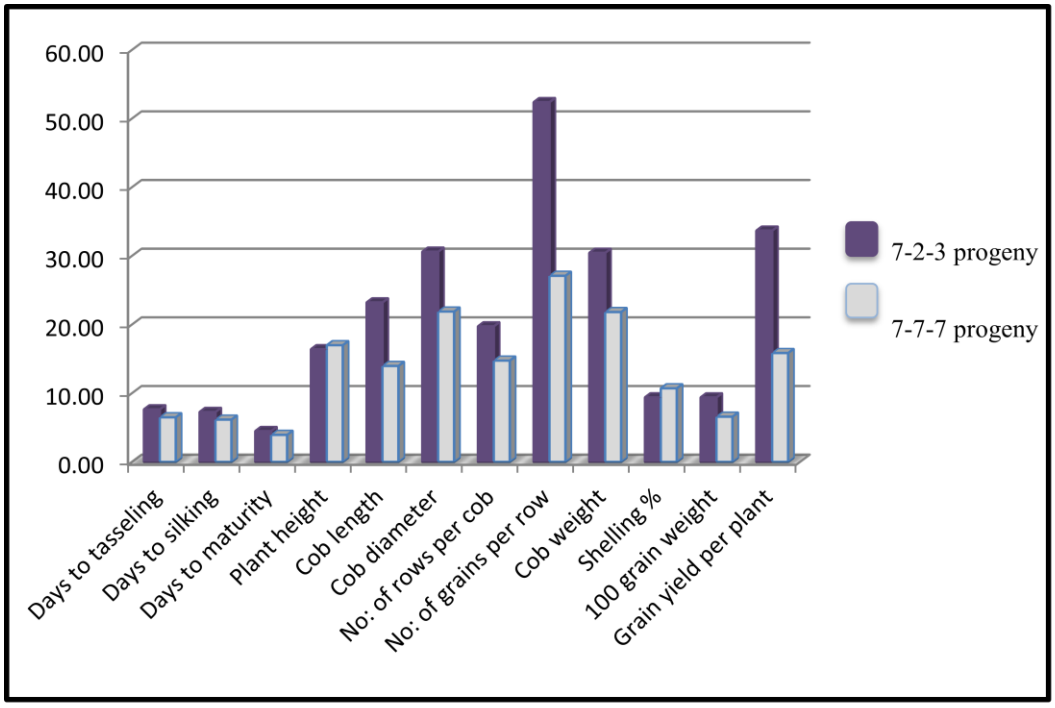

Fig. 8. PCV estimates (in%) in BC_3F_2 generation

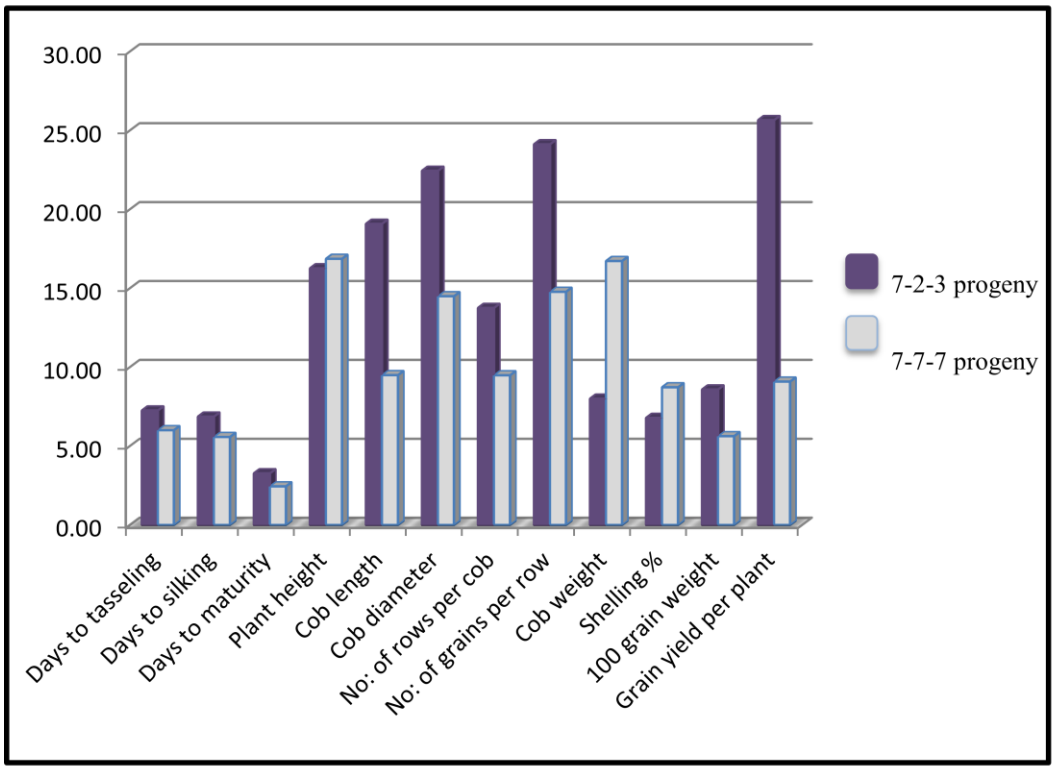

Fig. 9. GCV estimates (in %) in BC_3F_2 generation

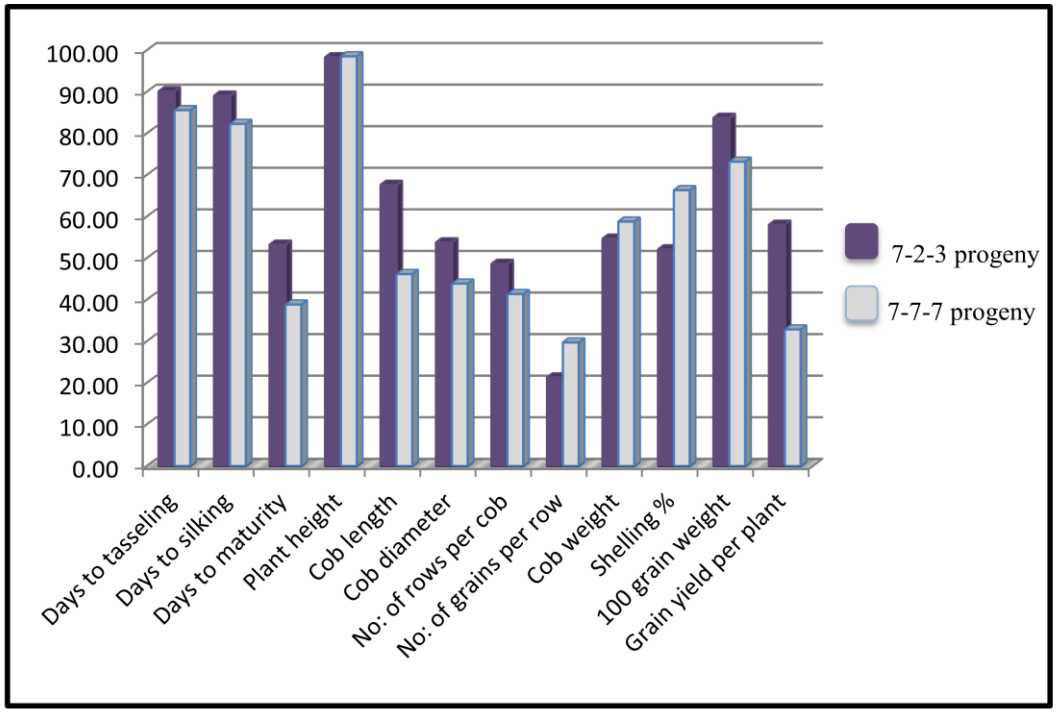

Fig. 10. Heritability estimates (in %) in BC_3F_2 generation

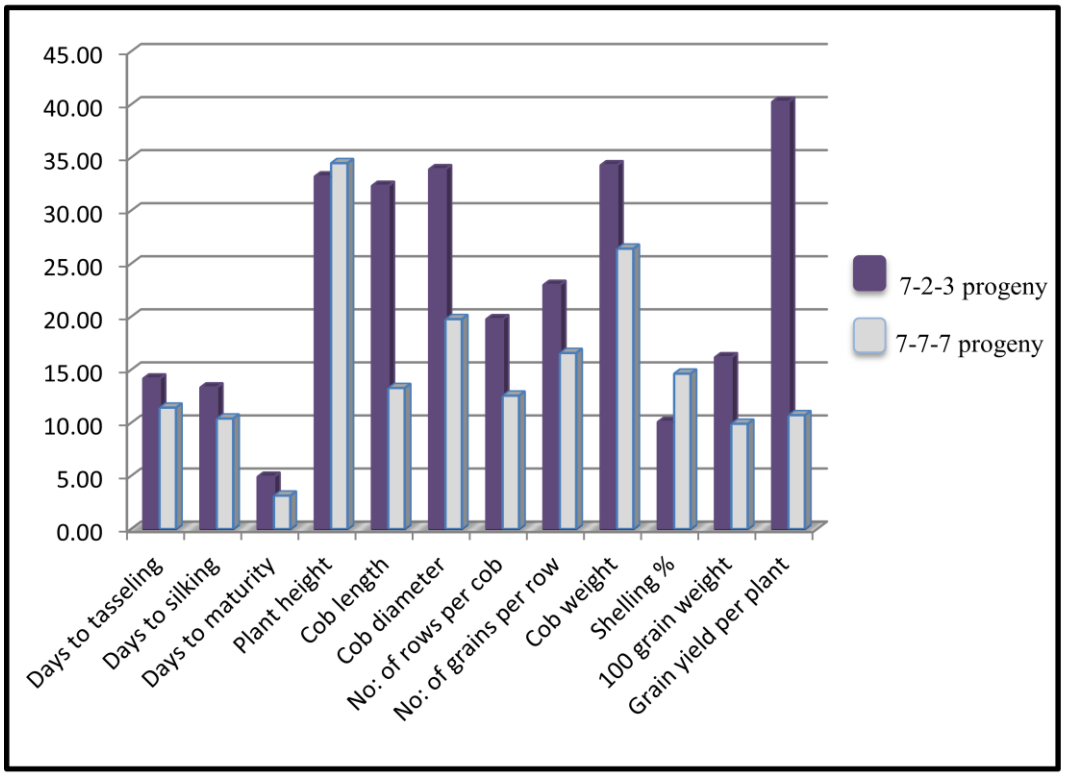

Fig. 11. GA as mean (in %) estimates in BC_3F_2 generation

The trait also exhibited moderate levels of PCV (16.98), GCV (16.86), high heritability (98.52%) and genetic advance as per cent of mean value (34.47 %) (Table 3).

For progeny no. 7-2-3, the observations of the trait varied between a minimum of 64.40 cm and maximum of 128.40 cm with a mean of 96.07 cm in which most of the plants were similar to recurrent parent. The trait exhibited moderate levels of PCV (16.40) and GCV (16.26). The trait also showed high heritability of (98.28 %) and high genetic advance as per cent of mean value (33.21 %) (Table 4).

4.2.4.5. Cob length (cm)

For cob length, progeny no. 7-7-7 exhibited a range from 8.00 to14.10 cm with a mean of 10.83 which is similar to the recurrent parent, UMI 79 (10.68 cm). The trait exhibited moderate PCV (13.96), low GCV (9.49), medium heritability (46.15 %) and moderate genetic advance as per cent of mean (13.28%) in this progeny (Table 3).

The value of this trait in 7-2-3 progeny varied from 6.00 to 12.40 cm with a mean value of 8.40 cm. The cob length was found to be less compared to the parental lines. The trait recorded high PCV (23.21) and GCV (19.08) values. High heritability (67.61%) and high genetic advance as per cent of mean (32.33 %) was observed for the trait (Table 4).

4.2.4.6. Cob diameter (cm)

Cob diameter of 7-7-7 progeny, ranged between 6.20 and 14.20 cm with mean value of 10.08 cm similar to the recurrent parent (10.76 cm). Regarding variability parameters, high PCV (21.88), moderate GCV (14.49), medium heritability (43.86%) and moderate genetic advance as per cent of mean (19.77%) was observed (Table 3).

In case of progeny no. 7-2-3, cob diameter ranged from 5.00 to 12.9 cm with a mean value of 7.95 cm which was less than both the parents. The trait exhibited high PCV (30.60) and GCV (22.44), medium heritability (53.78%) and high genetic advance as per cent of mean (33.89%) (Table 4).

4.2.4.7. Number of rows per cob

In 7 7-7-progeny, number of kernel rows per cob was varying from 10.00 to 16.00 were observed with mean value of 12.52 similar to the recurrent parent UMI 79 (11.20). Moderate PCV (14.74) but low GCV (9.48) was noted. The trait showed medium heritability (41.34%) and moderate genetic advance as per cent of mean (12.56%) (Table 3).

Table 3. Variability parameters observed in the BC_3F_2 generation for progeny 7-7-7

Trait	Grand mean			Range		BC_3F_2						
	P1	P2	BC_3F_2	Min.	Max.	Vp	Vg	PCV (%)	GCV (%)	h^2 (%)	GA	GA (as percent of mean)
Days to tasseling	53.40±0.81	56.40±0.4	58.12±0.47	52.00	65.00	14.20	12.15	6.48	6.00	85.57	6.64	11.43
Days to silking	56.60±0.86	59.20±0.4	61.20±0.47	55.00	68.00	14.10	11.60	6.14	5.57	82.27	6.36	10.40
Days to maturity	97.60±1.57	93.60±0.81	91.00±0.44	87.00	102.00	12.75	4.95	3.92	2.44	38.82	2.86	3.14
Plant height (cm)	90.12±0.96	97.60±0.88	99.92±1.90	58.60	133.90	287.92	283.65	16.98	16.86	98.52	34.44	34.47
Cob length (cm)	9.06±0.32	10.68±0.79	10.83±0.19	8.00	14.10	2.29	1.06	13.96	9.49	46.15	1.44	13.28
Cob diameter (cm)	8.54±0.82	12.76±1.10	10.08±0.27	6.20	14.20	4.87	2.14	21.88	14.49	43.86	1.99	19.77
No: of rows per cob	9.60±0.49	10.80±0.75	12.52±0.23	10.00	16.00	3.41	1.41	14.74	9.48	41.34	1.57	12.56
No: of grains per row	8.60±1.08	9.40±1.69	13.25±0.44	7.00	21.00	12.88	3.83	27.09	14.77	29.71	2.19	16.58
Cob weight (g)	18.38±1.74	22.98±1.29	27.30±0.74	17.88	40.97	35.47	20.85	21.81	16.72	58.78	7.21	26.41
Shelling %	64.68±0.29	70.82±1.52	68.10±0.90	54.13	77.96	53.13	35.24	10.70	8.72	66.33	9.96	14.63
100 grain weight (g)	16.09±0.29	19.30±0.27	18.34±0.15	13.92	22.40	1.45	1.06	6.57	5.62	73.18	1.82	9.91
Grain yield per plant (g)	11.89±1.24	17.44±1.09	18.28±0.36	13.68	27.65	8.38	2.75	15.84	9.08	32.84	1.96	10.72

Table 4. Variability parameters observed in the BC_3F_2 generation for progeny 7-2-3

Trait	Grand mean			BC_3F_2								
				Range		Vp	Vg	PCV (%)	GCV (%)	h^2 (%)	GA	GA(as percent of mean)
	P1	P2	BC_3F_2	Min.	Max.							
Days to tasseling	53.40±0.81	56.40±0.4	59.55±0.74	50.00	68.00	20.69	18.64	7.64	7.25	90.09	8.44	14.17
Days to silking	56.60±0.86	59.20±0.4	62.45±0.74	54.00	70.00	20.58	18.33	7.26	6.86	89.07	8.32	13.33
Days to maturity	97.60±1.57	93.60±0.81	91.08±0.66	85.00	103.00	16.67	8.87	4.48	3.27	53.21	4.48	4.91
Plant height (cm)	90.12±0.96	97.60±0.88	96.07±1.56	64.40	128.40	248.33	244.06	16.40	16.26	98.28	31.9	33.21
Cob length (cm)	9.06±0.32	10.68±0.79	8.40±0.32	6.00	12.40	3.80	2.57	23.21	19.08	67.61	2.72	32.33
Cob diameter (cm)	8.54±0.82	12.76±1.10	7.95±0.39	5.00	12.90	5.91	3.18	30.60	22.44	53.78	2.69	33.89
No. of rows per cob	9.60±0.49	10.80±0.75	10.00±0.32	8.00	14.00	3.89	1.89	19.73	13.75	48.61	1.98	19.76
No. of grains per row	8.60±1.08	9.40±1.69	6.47±0.55	3.00	17.00	11.50	2.45	52.38	24.12	21.29	1.49	22.99
Cob weight (g)	18.38±1.74	22.98±1.29	16.68±0.82	11.24	28.39	25.78	14.09	30.43	8.00	54.67	5.72	34.27
Shelling %	64.68±0.29	70.82±1.52	65.06±0.99	55.93	76.88	37.35	19.46	9.39	6.78	52.09	6.56	10.08
100 grain weight (g)	16.09±0.29	19.30±0.27	16.50±0.25	12.44	18.67	2.39	2.00	9.38	8.58	83.72	2.67	16.17
Grain yield per plant (g)	11.89±1.24	17.44±1.09	10.87±0.59	7.47	18.75	13.41	7.78	33.67	25.65	58.01	4.38	40.24

Among the plants of 7-2-3 progeny, row number per cob was ranging from 8.00 to 14.00 was observed for the trait. A mean value of 10.00 was recorded. The trait showed moderate PCV (19.73), moderate GCV (13.75), medium heritability (48.61%) and moderate genetic advance as per cent of mean (19.76%) (Table 4).

4.2.4.8. Number of grains per row

Number of grains per row ranged from 7.00 to 21.00 in progeny no. 7-7-7 with a mean value of 13.25. High PCV (27.09), moderate GCV (14.77), low heritability (29.71%) and moderate genetic advance as per cent of mean (16.58%) was recorded (Table 3).

For this trait values ranging from 3.00 to 17.00 was recorded for progeny no. 7-2-3. A mean value of 6.47 which is less than both the parental lines was recorded. The trait was observed to show high PCV (52.38), high GCV (24.12), low heritability (21.29%) and high genetic advance as per cent of mean (22.99%) (Table 4).

4.2.4.9. Cob weight (g)

For progeny no. 7-7-7, the cob weight registered a minimum of 17.88g and a maximum of 40.97g with a mean value of 27.30g. as far as variability parameters is concerned, high PCV (21.81), moderate GCV (16.72), medium heritability (58.78%) and high genetic advance as per cent of mean (26.41%) were noted (Table 3).

In progeny no. 7-2-3, cob weight was observed to vary from 11.24 to 28.39 g with mean value of 16.68 g which is less than the parental lines. The trait exhibited high PCV (30.43) but low GCV (8.00). The trait had medium heritability (54.67%) and high genetic advance as per cent of mean (34.27%) (Table 4).

4.2.4.10. Shelling %

Progeny no. 7-7-7 showed a range from 54.13 to 77.96% for shelling outturn. A mean value of 68.10 % almost similar to the recurrent parent UMI 79 (66.47%) was noted. Moderate PCV (10.70), low GCV (8.72), high heritability (66.33%) and moderate genetic advance as per cent of mean (14.63%) was observed for this trait (Table 3).

In case of progeny no. 7-2-3, values ranging from 55.93 to 76.88% were observed for this trait. A mean value of 65.06% which is similar to the recurrent parent UMI 79 (66.47%) was recorded. The trait showed low PCV (9.39), low GCV (6.78), medium

heritability (52.09%) and moderate genetic advance as per cent of mean (10.08%) (Table 4).

4.2.4.11. 100 grain weight (g)

In progeny no. 7-7-7, the hundred grain weight varied from 13.92 to 22.40 g with mean value of 18.34 g similar to the recurrent parent UMI 79 (19.00g). Low PCV (6.57), low GCV (5.62), high heritability (73.18%) and low genetic advance as per cent of mean (9.91%) was recorded (Table 3).

For 7-2-3 progeny, the trait ranged from 12.44 to 18.67g with a mean value of 16.50g which was less than parental lines. Variability parameters expressed low PCV (9.38) and GCV (8.58) for this trait. High heritability (83.72%) and moderate genetic advance as per cent of mean (16.17%) were observed for 100 grain weight (Table 4).

4.2.4.12. Grain yield per plant (g)

Grain yield in the progeny no. 7-7-7 varied from 13.68 to 27.65g with a mean value of 18.28g similar to the recurrent parent UMI 79 (17.44g). Moderate PCV (15.84), low GCV (9.08), medium heritability (32.84%) and medium genetic advance as per cent of mean (10.72%) was recorded (Table 3).

For 7-2-3 progeny, grain yield ranged from 7.47 to 18.75g with a mean value of 10.87g. This was lesser when compared to the parental lines. While variability parameters *viz.*, PCV (33.67) and GCV (25.65) values observed for this trait were high. The heritability (58.01%) was medium and genetic advance as per cent of mean (40.24%) was high (Table 4).

4.2.5. Frequency distribution

In progeny no. 7-7-7, frequency distribution indicated that days to maturity (1.51), cob diameter (0.64), number of grains per row (0.64), cob weight (0.71) and grain yield per plant (1.04) were positively significant for skewness. All other traits showed non-significant skewness. Days to maturity (1.69) and number of grains per row (1.44) showed significant and positive kurtosis and 100 grain weight (-1.86) and grain yield per plant (-1.99) showed negative kurtosis. All other traits expressed non-significant kurtosis (Table 5).

The frequency distribution for progeny 7-2-3 indicated that days to maturity (1.34), number of grains per row (1.44), cob diameter (0.89), cob weight (1.07) and grain

Table 5. Skewness and kurtosis in BC_3F_2 generation

Traits	7-7-7 progeny			7-2-3 progeny		
	No. of progenies	Skewness	Kurtosis	No. of progenies	Skewness	Kurtosis
Days to tasseling	65	0.53	-0.84	38	-0.10	-0.78
Days to silking	65	0.54	-0.81	38	-0.03	-0.99
Days to maturity	65	1.51**	1.69**	38	1.34**	1.51*
Plant height (cm)	65	-0.33	-0.12	38	-0.06	-0.42
Cob length (cm)	65	0.25	-0.69	38	0.34	-1.27
Cob diameter (cm)	65	0.64*	-0.88	38	0.89*	-0.63
No. of rows per cob	65	0.19	-0.81	38	0.71	-0.44
No. of grains per row	65	0.64*	1.44*	38	1.44**	1.60*
Cob weight (g)	65	0.71*	-0.84	38	1.07**	0.06
Shelling %	65	-0.52	-0.79	38	0.62	-0.91
100 grain weight (g)	65	-0.33	-1.86**	38	-0.26	-1.63**
Grain yield per plant (g)	65	1.04**	-1.99**	38	1.31**	-1.65**

***and ** indicate significance at 0.05 and 0.01 probability levels respectively**

yield per plant (1.31) were positively significant for skewness. All other traits exhibited non-significant skewness. Positively significant kurtosis was observed for days to maturity (1.51) and number of grains per row (1.60). 100 grain weight (-1.63) and grain yield per plant (-1.65) showed negatively significant kurtosis. All the remaining traits showed non-significant kurtosis (Table 5).

The mean of BC_3F_2 progenies were classified into different classes at regular class intervals and the population distribution was obtained pictographically for all the characters under study in which the number of progenies resembling the parental range can be identified.

In progeny 7-7-7, the number of progenies resembling the recurrent parent was recorded to be high for plant height (Fig. 18), cob length (Fig. 20), cob diameter (Fig. 22), number of grains per row (Fig. 26), cob weight (Fig. 28), shelling % (Fig. 30) and grain yield per plant (Fig. 34). In 7-2-3 progeny, the number of progenies resembling the recurrent parent was found to be high for days to tasseling (Fig. 13), days to silking (Fig. 15), plant height (Fig. 19), cob length (Fig. 21) and number of rows per cob (Fig. 25).

4.3. Study of BC_3F_3 generation

4.3.1. Development of BC_3F_3 population

Thirty eight phenotypically resistant individuals from 7-2-3 progeny and sixty five phenotypically resistant individuals from progeny no. 7-7-7 were selfed to generate the BC_3F_3 generation. Among these, eight superior progenies were selected. The BC_3F_3 population was raised in the Eastern Block of the Central Farm Unit, Department of Agronomy, at Tamil Nadu Agricultural University, Coimbatore during Rabi, 2014.

4.3.2. Genotyping of BC_3F_3 population

From the BC_3F_3 population eight phenotypically superior progenies comprising a total of 68 individuals were selected for genotyping with the selected polymorphic markers of chromosome 3 and 6. The SSR markers *viz.*, phi053 located on the chromosome 3 and nc013 located on the chromosome 6 were found to show good polymorphism between the parental lines and used for genotyping among the selected BC_3F_3 progenies.

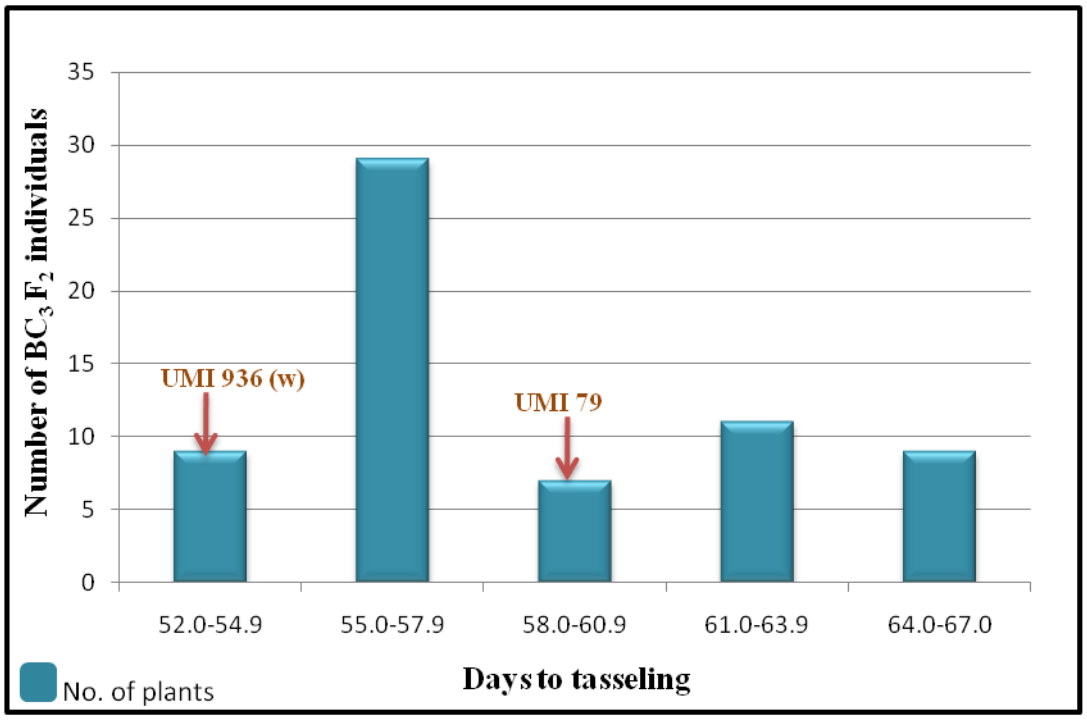

Fig. 12. Frequency distribution for days to tasseling in BC_3F_2 generation for progeny 7-7-7

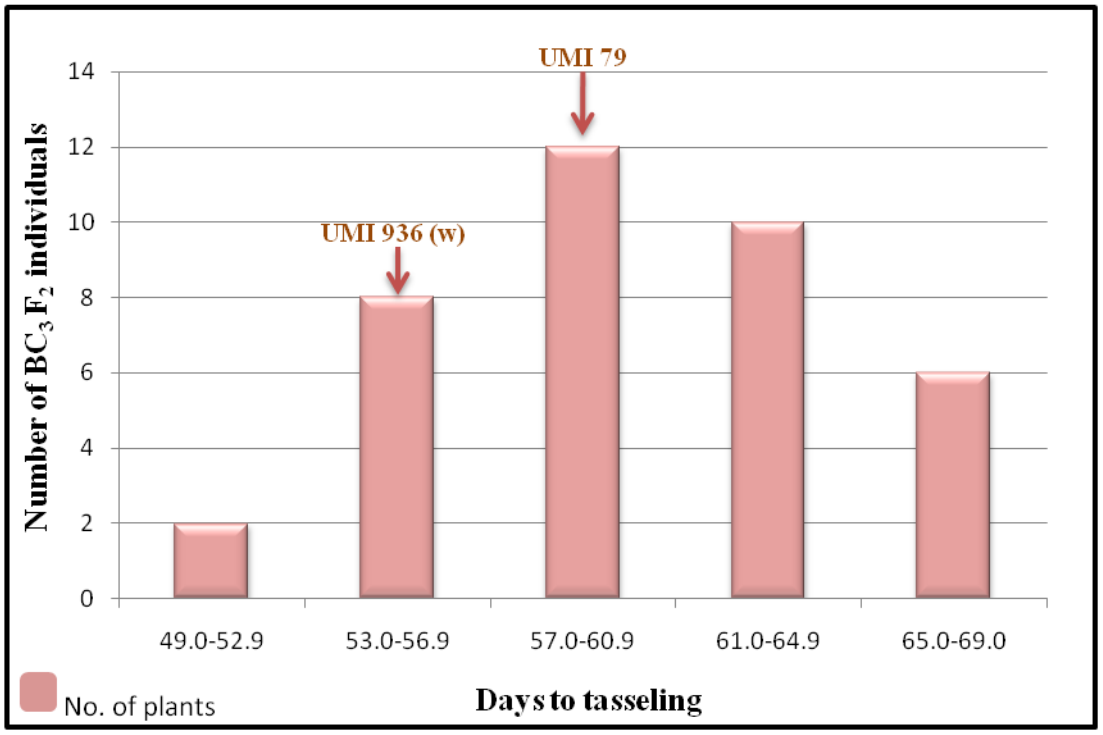

Fig. 13. Frequency distribution for days to tasseling in BC_3F_2 generation for progeny 7-2-3

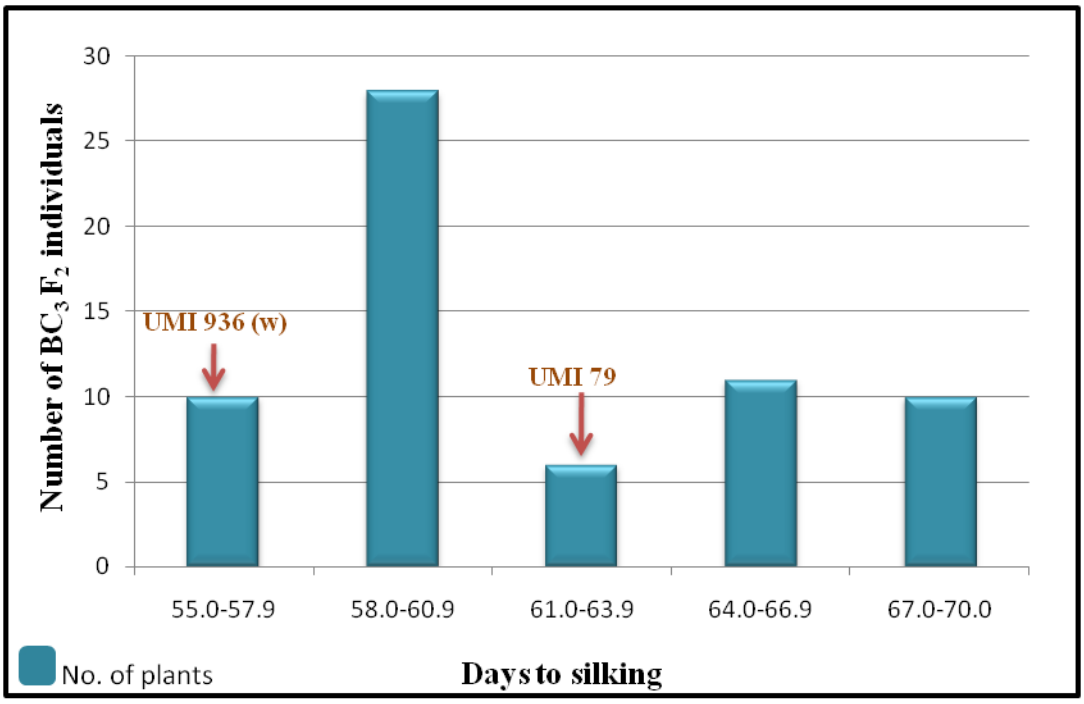

Fig. 14. Frequency distribution for days to silking in BC_3F_2 generation for progeny 7-7-7

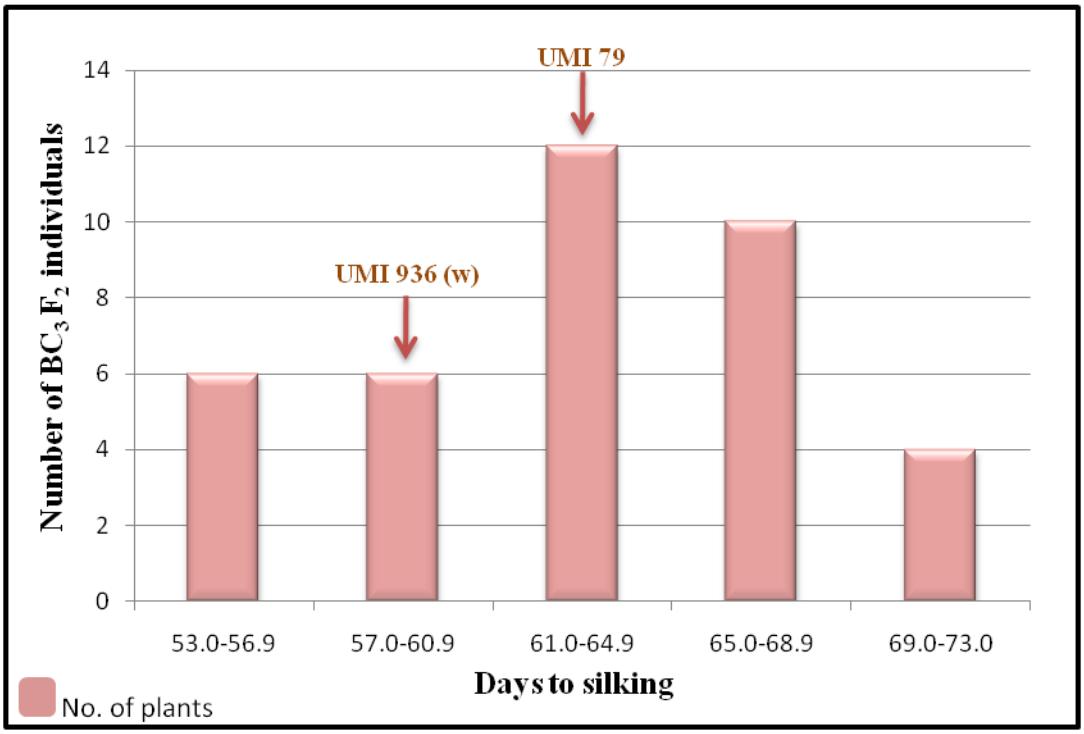

Fig. 15. Frequency distribution for days to silking in BC_3F_2 generation for progeny 7-2-3

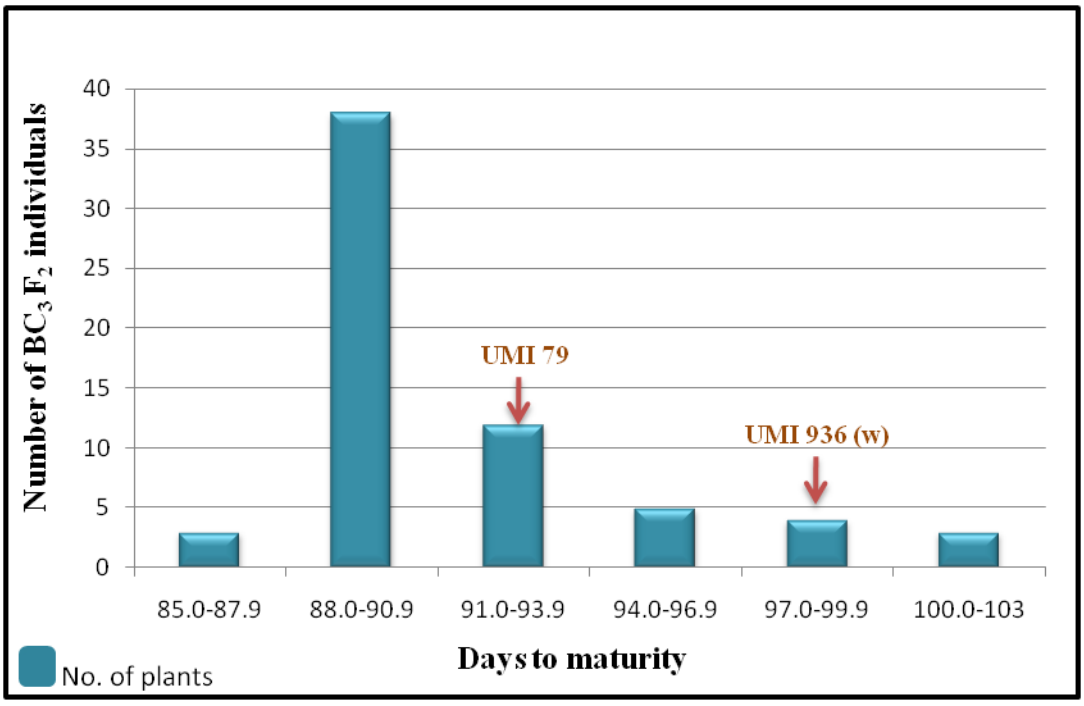

Fig. 16. Frequency distribution for days to maturity in BC_3F_2 generation for progeny 7-7-7

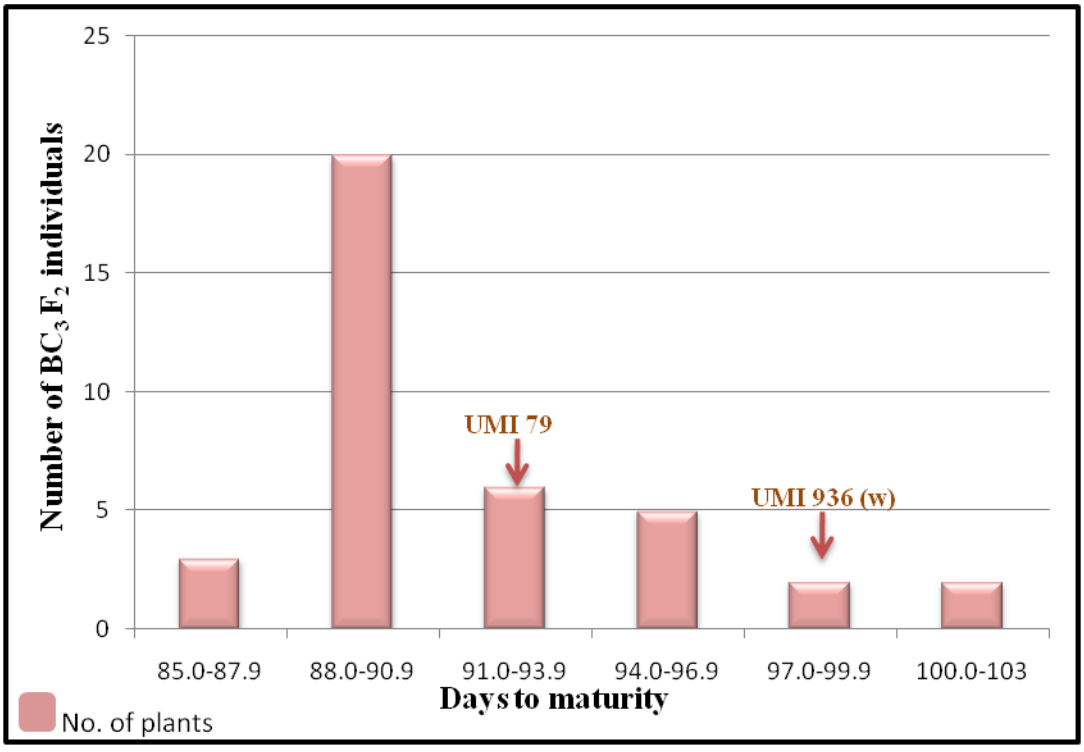

Fig. 17. Frequency distribution for days to maturity in BC_3F_2 generation for progeny 7-2-3

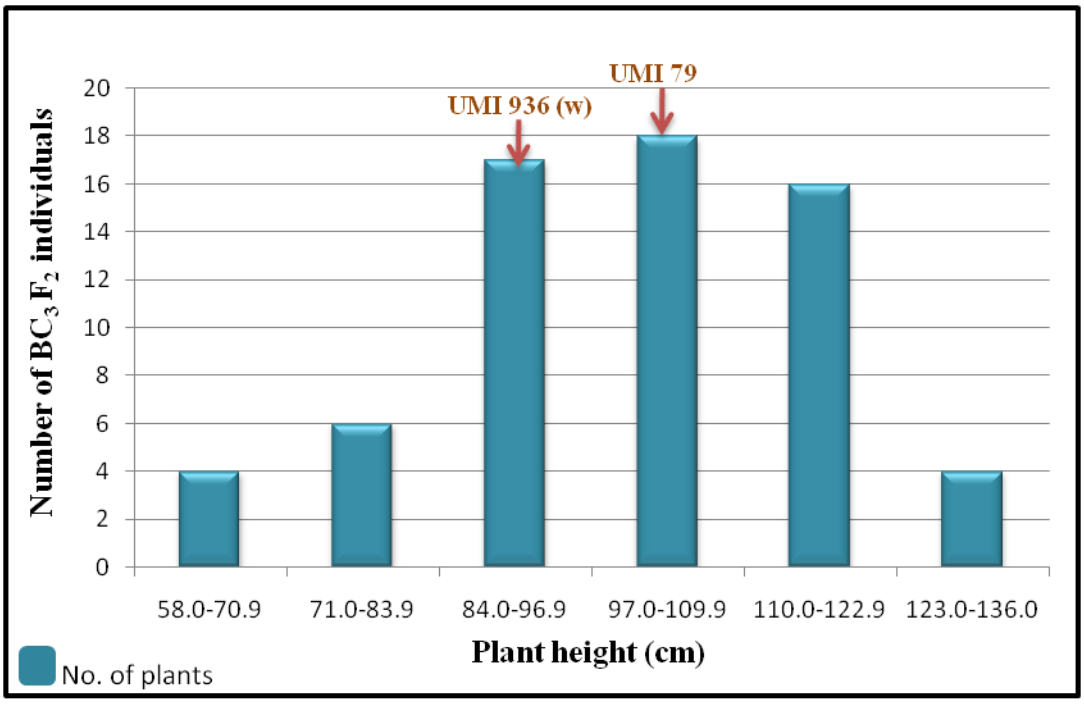

Fig. 18. Frequency distribution for plant height in BC_3F_2 generation for progeny 7-7-7

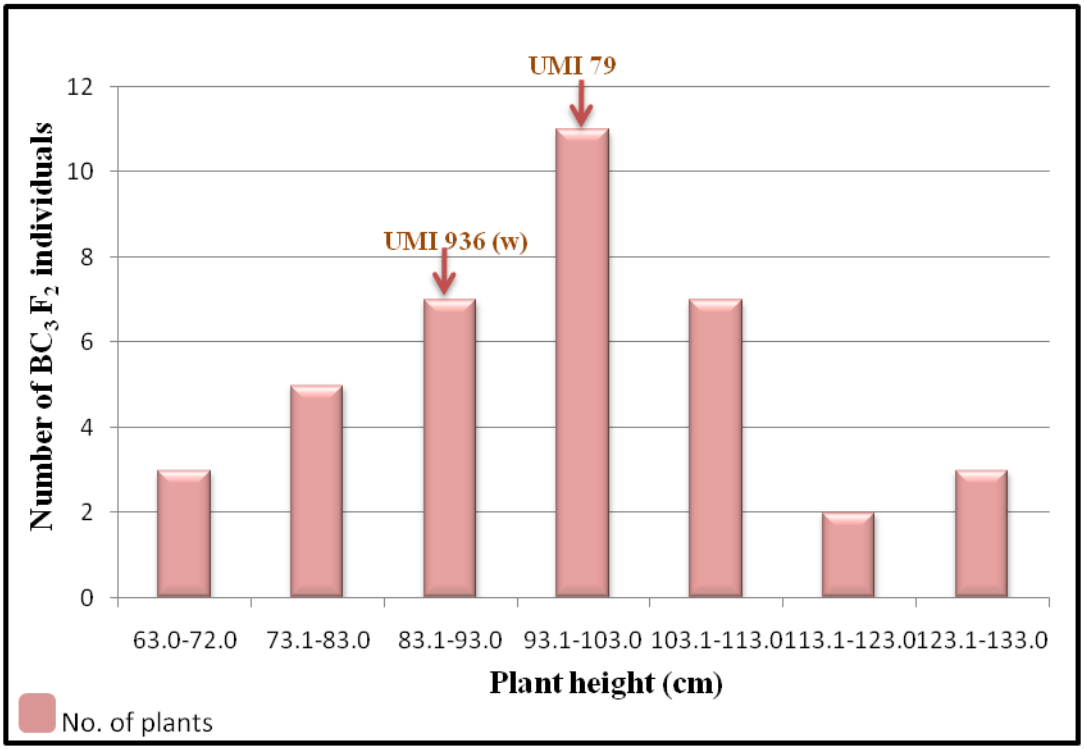

Fig. 19. Frequency distribution for plant height in BC_3F_2 generation for progeny 7-2-3

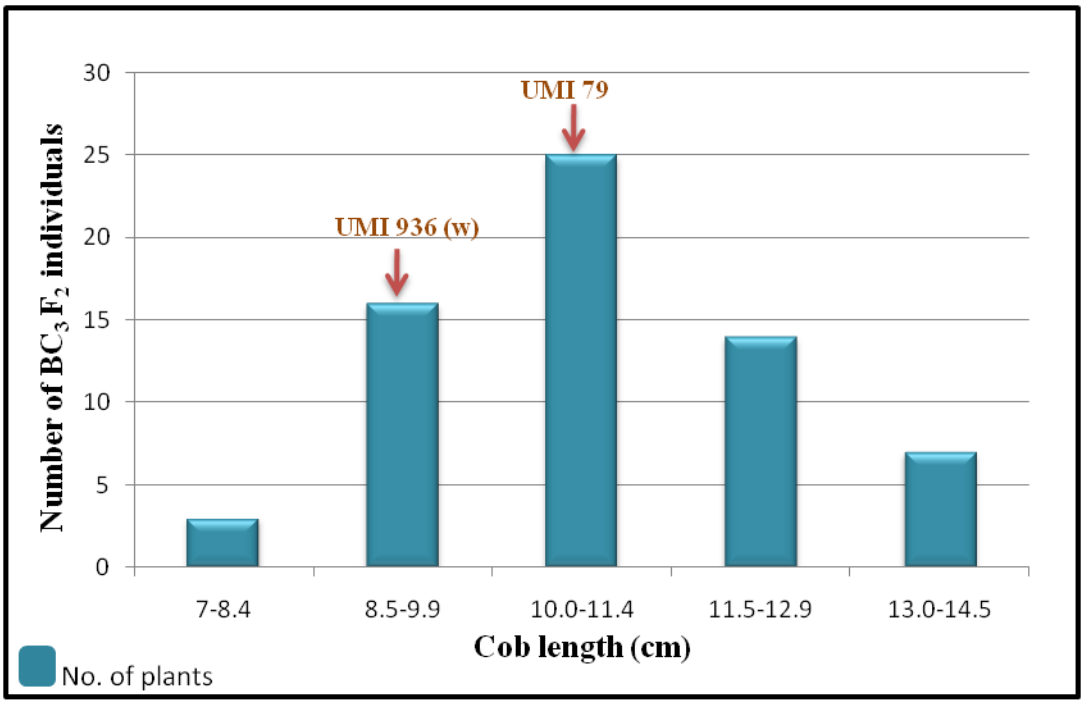

Fig. 20. Frequency distribution for cob length in BC_3F_2 generation for progeny 7-7-7

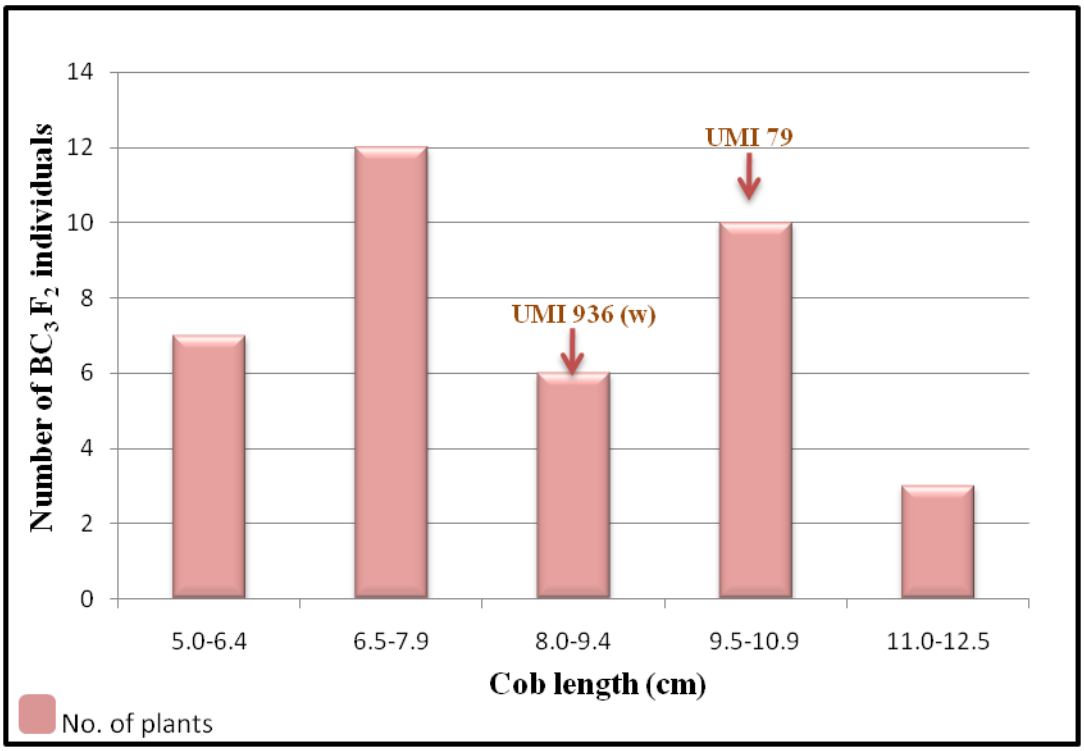

Fig. 21. Frequency distribution for cob length in BC_3F_2 generation for progeny 7-2-3

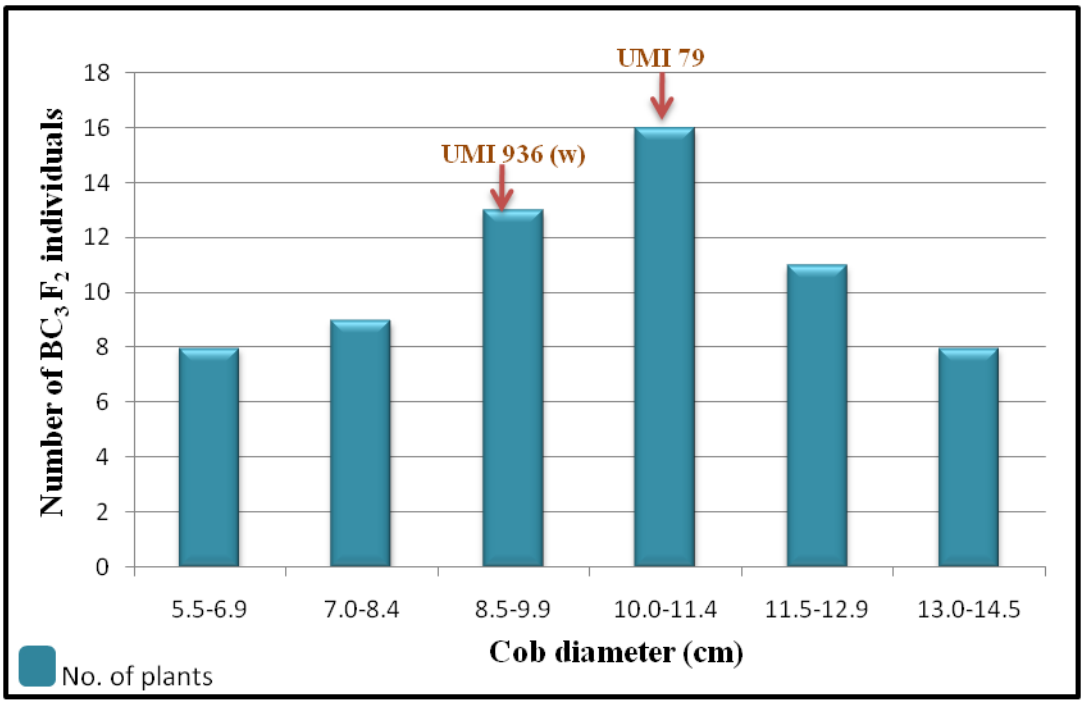

Fig. 22. Frequency distribution for cob diameter in BC_3F_2 generation for progeny 7-7-7

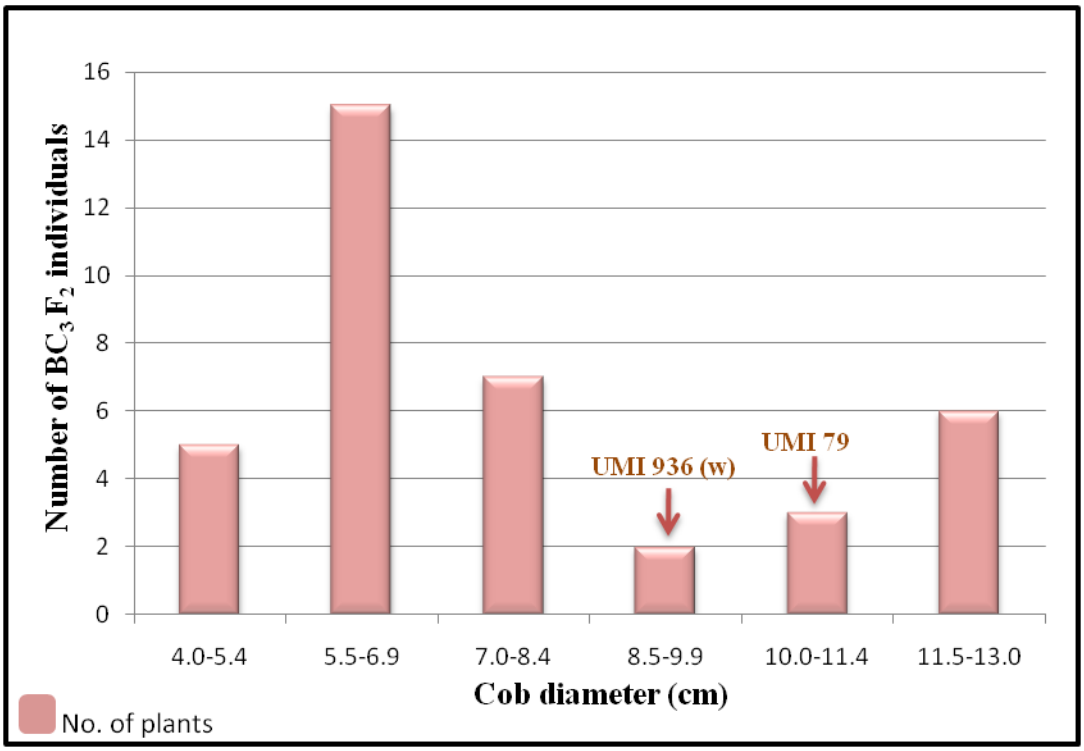

Fig. 23. Frequency distribution for cob diameter in BC_3F_2 generation for progeny 7-2-3

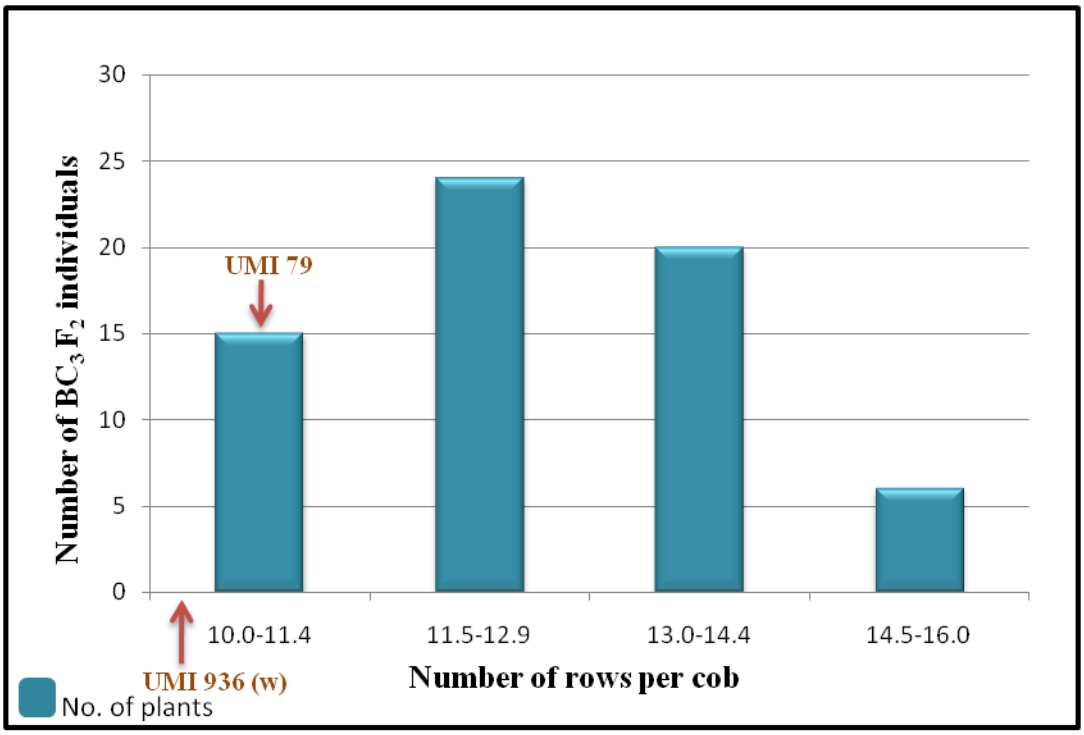

Fig. 24. Frequency distribution for number of rows per cob in BC_3F_2 generation for progeny 7-7-7

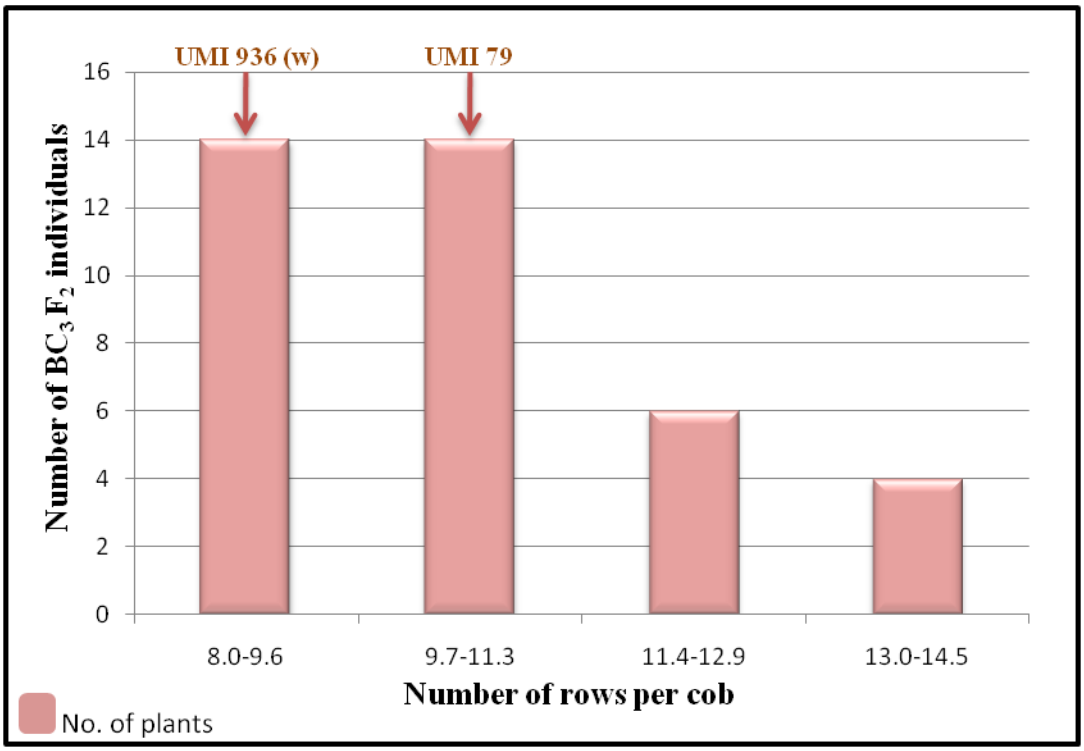

Fig. 25. Frequency distribution for number of rows per cob in BC_3F_2 generation for progeny 7-2-3

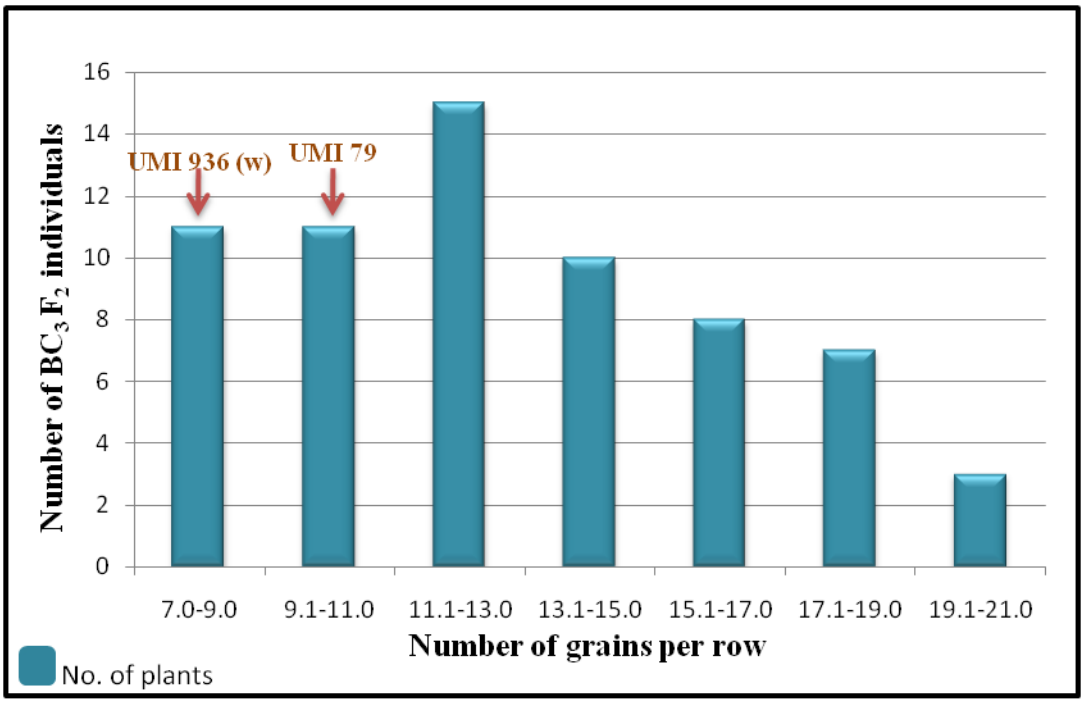

Fig. 26. Frequency distribution for number of grains per row in BC_3F_2 generation for progeny 7-7-7

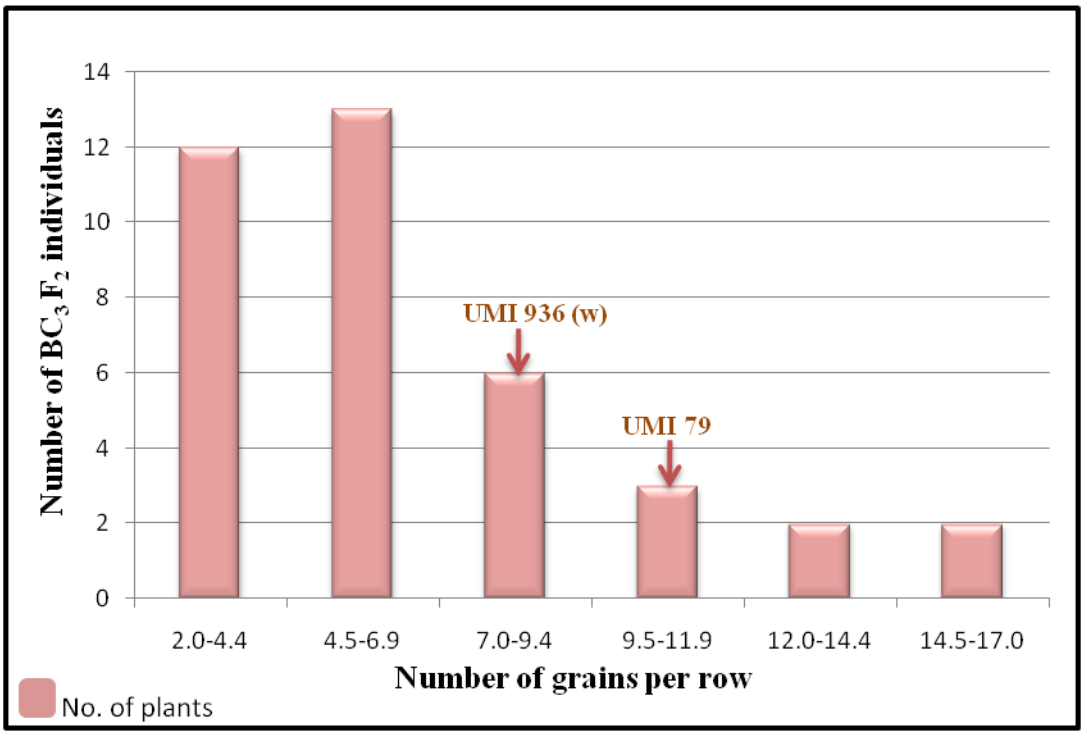

Fig. 27. Frequency distribution for number of grains per row in BC_3F_2 generation for progeny 7-2-3

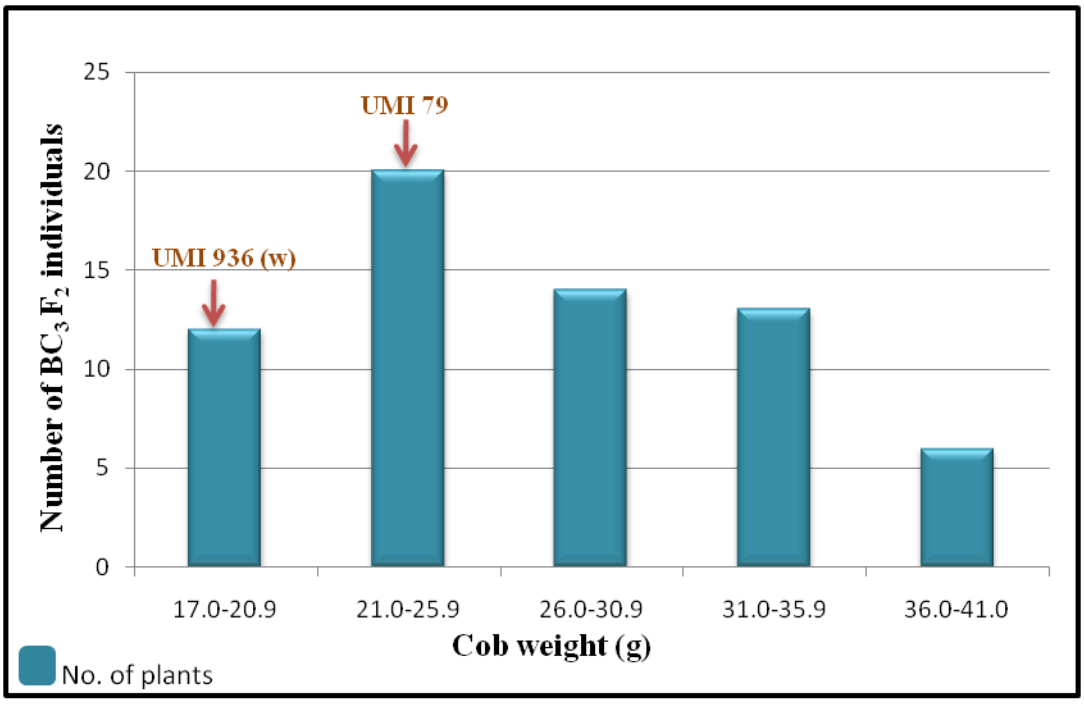

Fig. 28. Frequency distribution for cob weight in BC_3F_2 generation for progeny 7-7-7

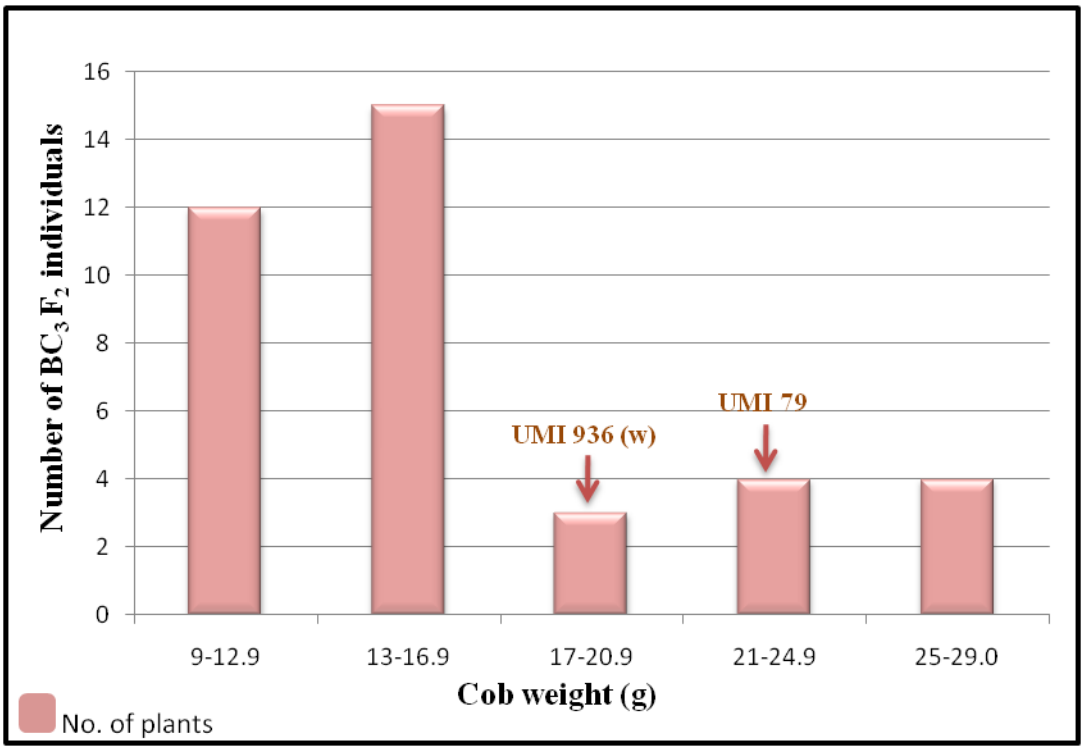

Fig. 29. Frequency distribution for cob weight in BC_3F_2 generation for progeny 7-2-3

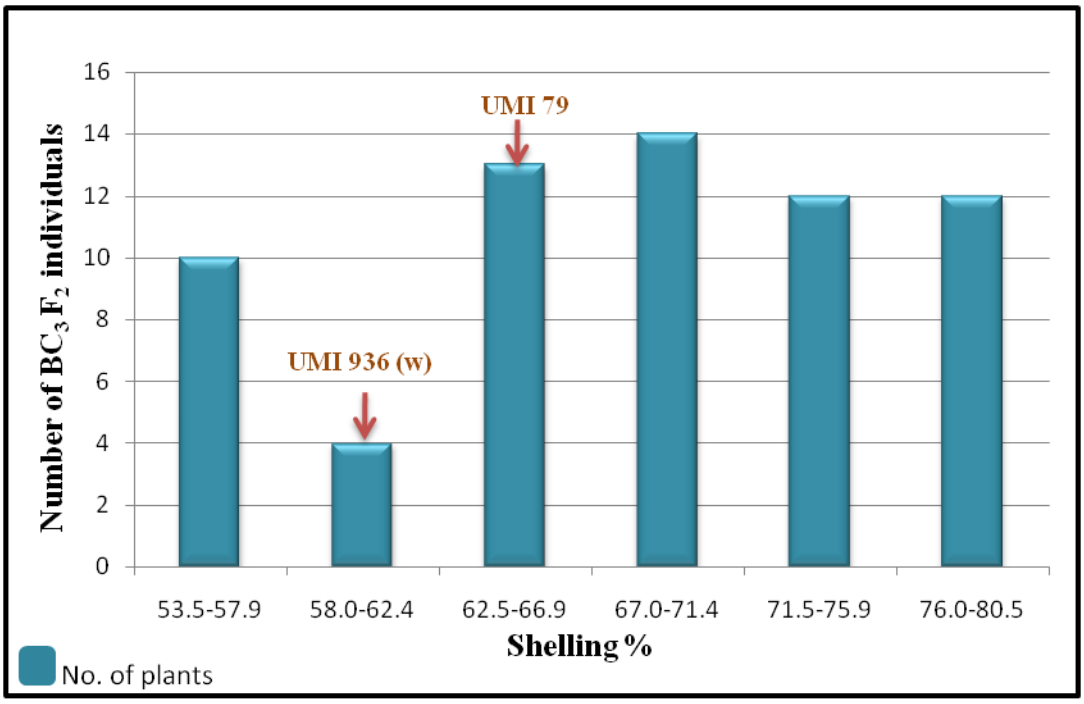

Fig. 30. Frequency distribution for shelling % in BC_3F_2 generation for progeny 7-7-7

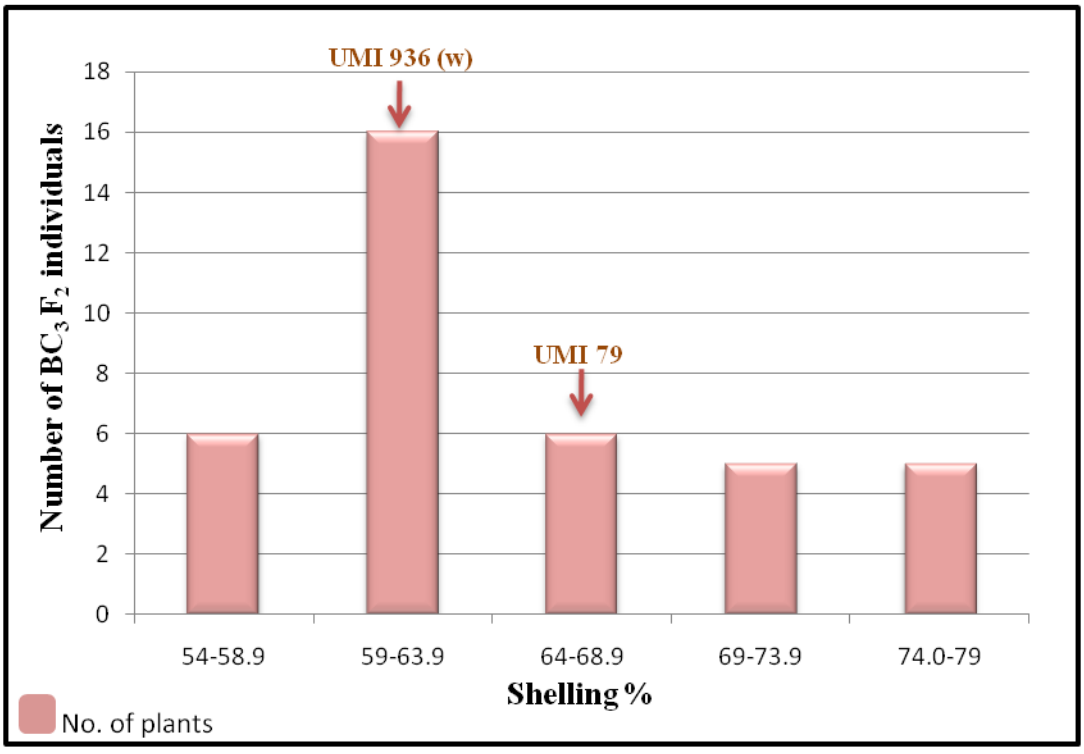

Fig. 31. Frequency distribution for shelling % in BC_3F_2 generation for progeny 7-2-3

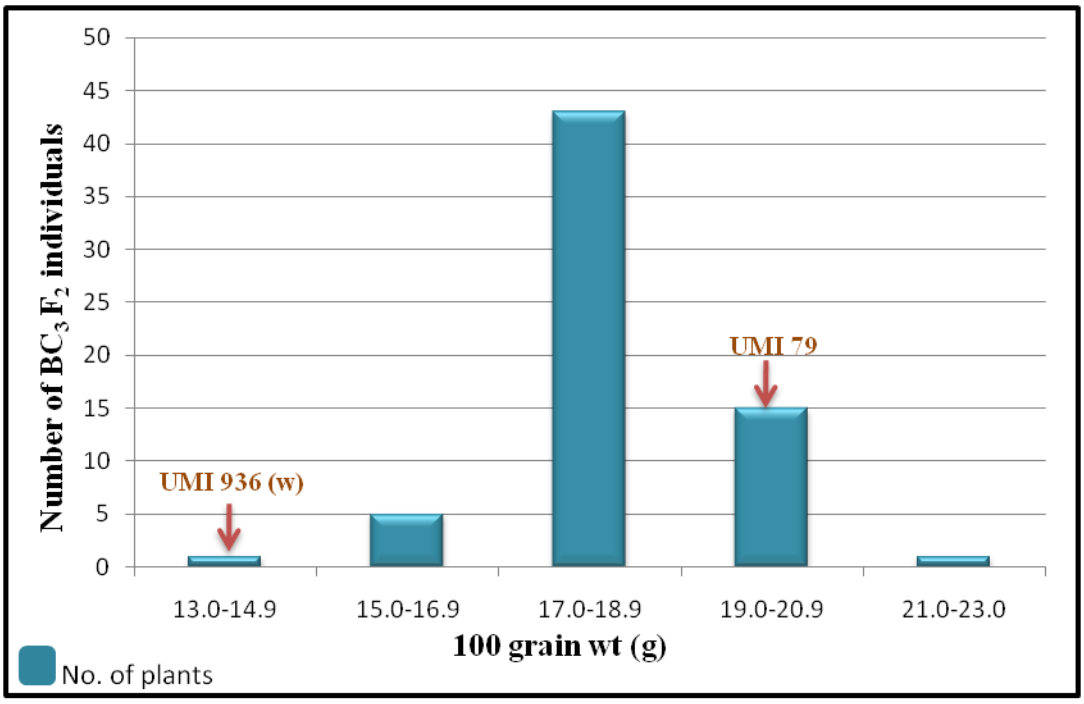

Fig. 32. Frequency distribution for 100 grain weight in BC_3F_2 generation for progeny 7-7-7

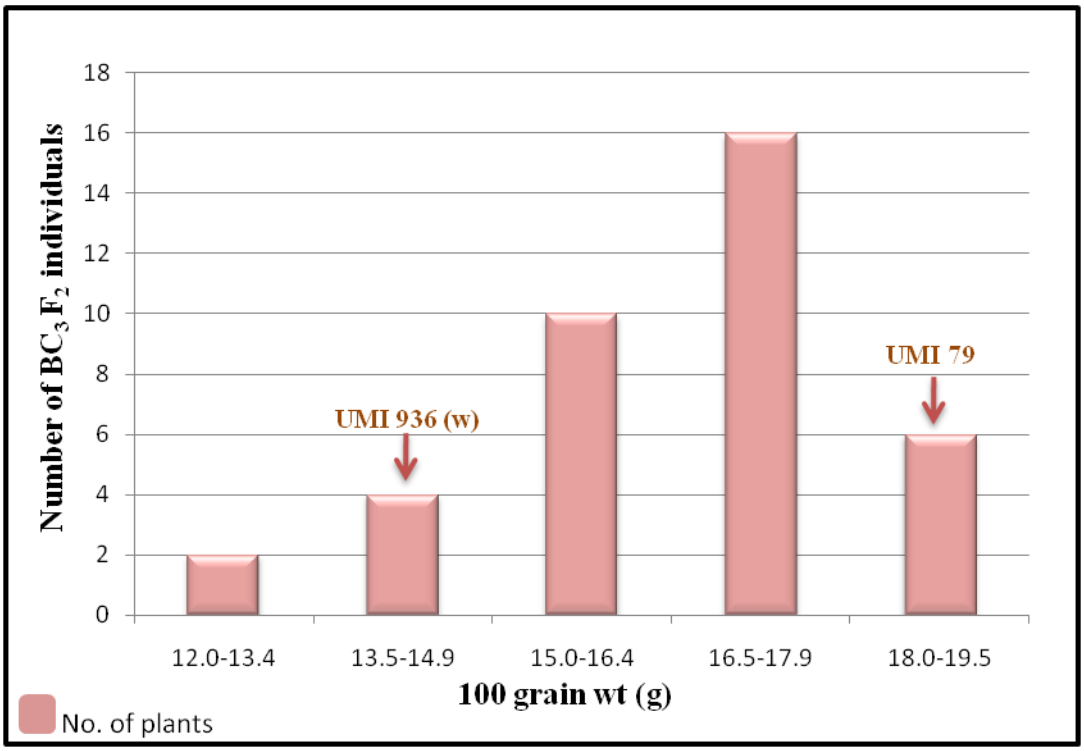

Fig. 33. Frequency distribution for 100 grain weght in BC_3F_2 generation for progeny 7-2-3

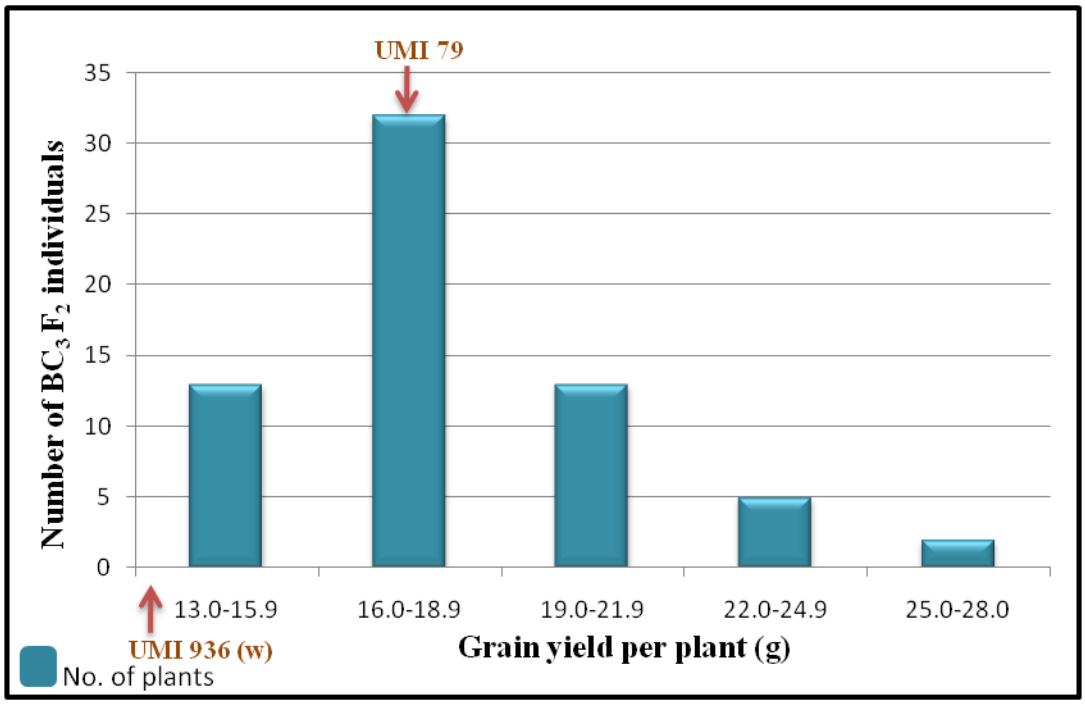

Fig. 34. Frequency distribution for grain yield per plant in BC_3F_2 generation for progeny 7-7-7

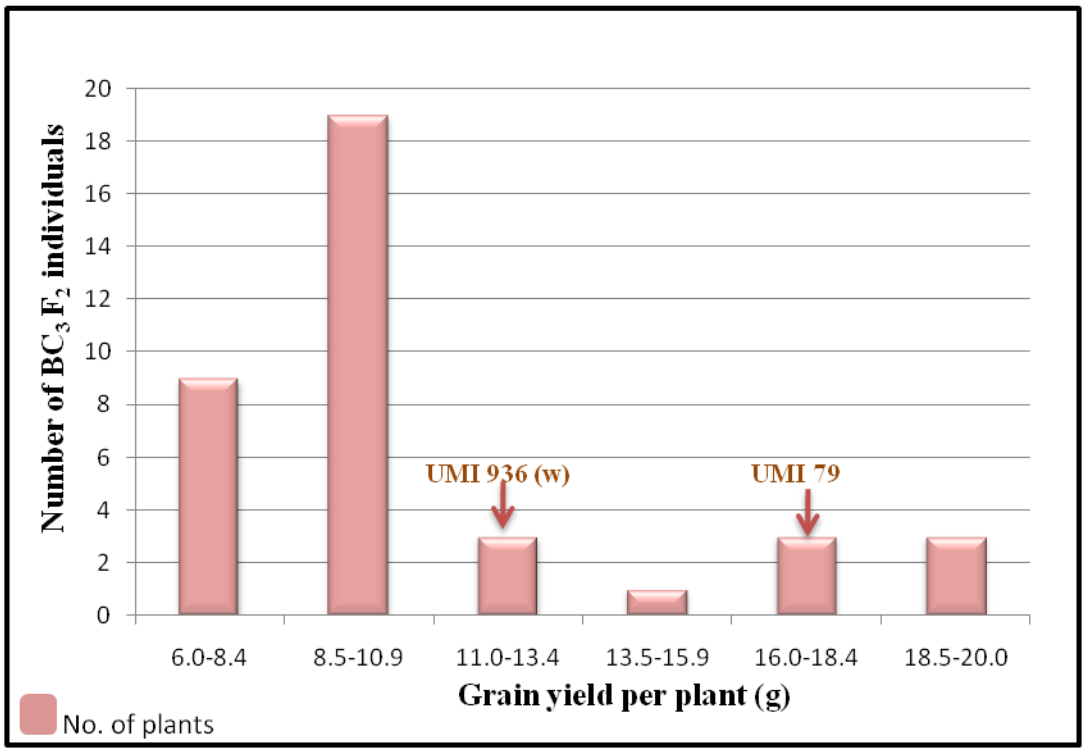

Fig. 35. Frequency distribution for grain yield per plant in BC_3F_2 generation for progeny 7-2-3

The SSR marker phi053 on chromosome 3 amplified the polymorphic segment of 180bp for UMI 79 and 196bp for UMI 936(w). The banding pattern obtained is given in the Plate 10. The marker nc013 on chromosome 6 amplified the polymorphic region between 114 bp for UMI 79 and 134bp for UMI 936(w). The identified heterozygotes were labelled as “H”, individuals similar to female parent UMI 79 were labelled as “A” and individuals similar to the resistant donor parent UMI 936 were labelled as “B” (Plate 11).

Among the eight progenies studied, the progeny 7-7-7-53 alone had homozygous status for UMI 936(w) allele for both phi053 and nc013. Other progenies had heterogenous nature for either one or both alleles (Table 6).

Table 6. Genetic constitution of BC_3F_3 progenies

S. No.	BC_3F_3 Progeny	Number of plants screened	Number of Heterozygotes (H)		Number of Homozygotes similar to UMI 79 (A)		Number of Homozygotes similar to UMI 936(w) (B)	
			phi053	nco13	phi053	nc013	phi053	nc013
1	7-7-7-53	6	0	0	0	0	6	6
2	7-7-7-59	8	5	2	1	6	2	0
3	7-7-7-84	6	3	0	2	2	1	4
4	7-7-7-85	11	1	0	9	0	0	11
5	7-7-7-86	6	5	1	1	4	0	1
6	7-7-7-89	18	0	8	18	0	0	10
7	7-2-3-2	6	4	6	1	0	1	0
8	7-2-3-8	7	5	0	0	0	2	7

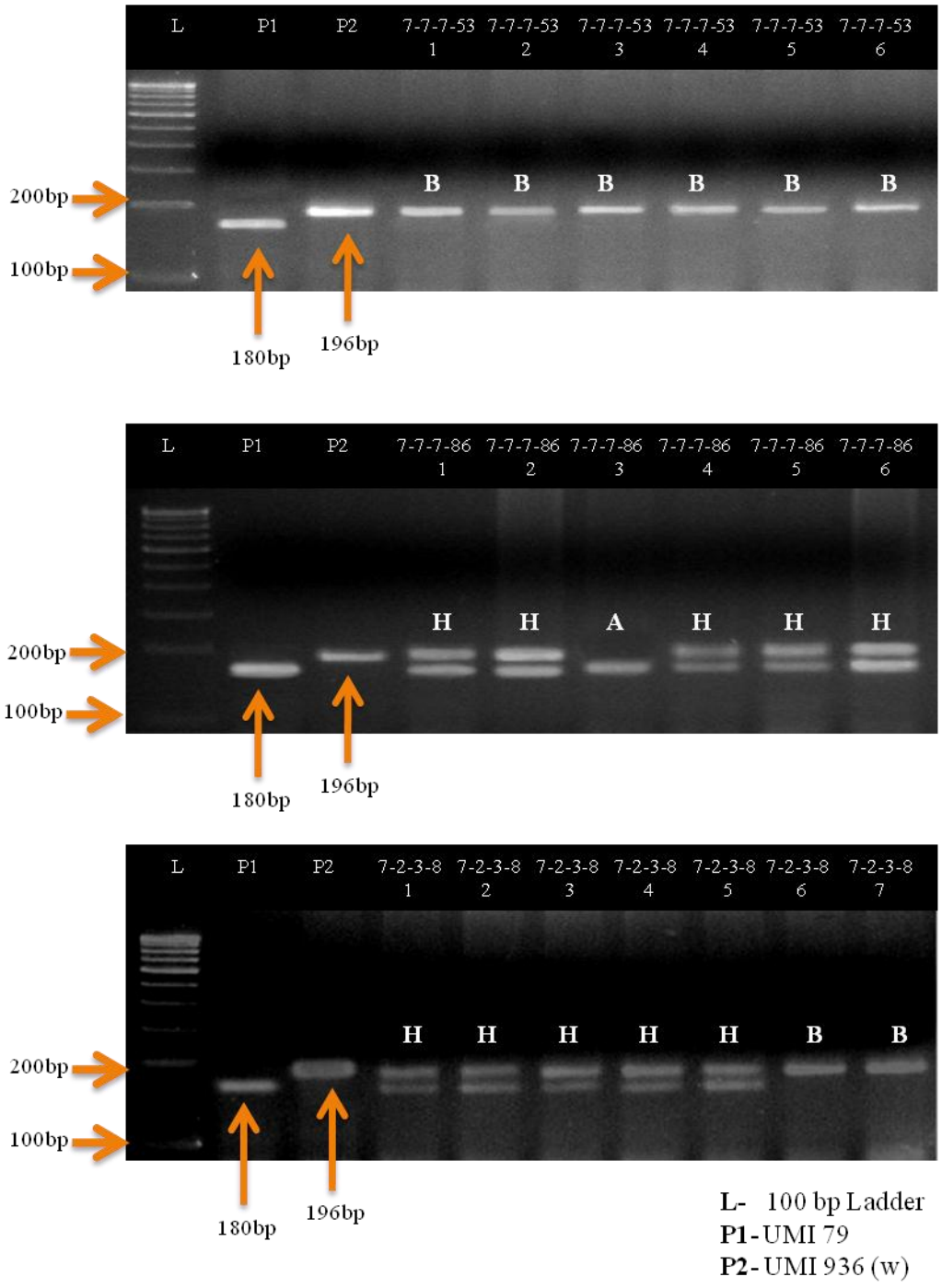

Plate 10. Segregation pattern of SSR marker phi053 in BC_3F_3 progenies of (UMI 79 x UMI 936 (w)) x UMI 79

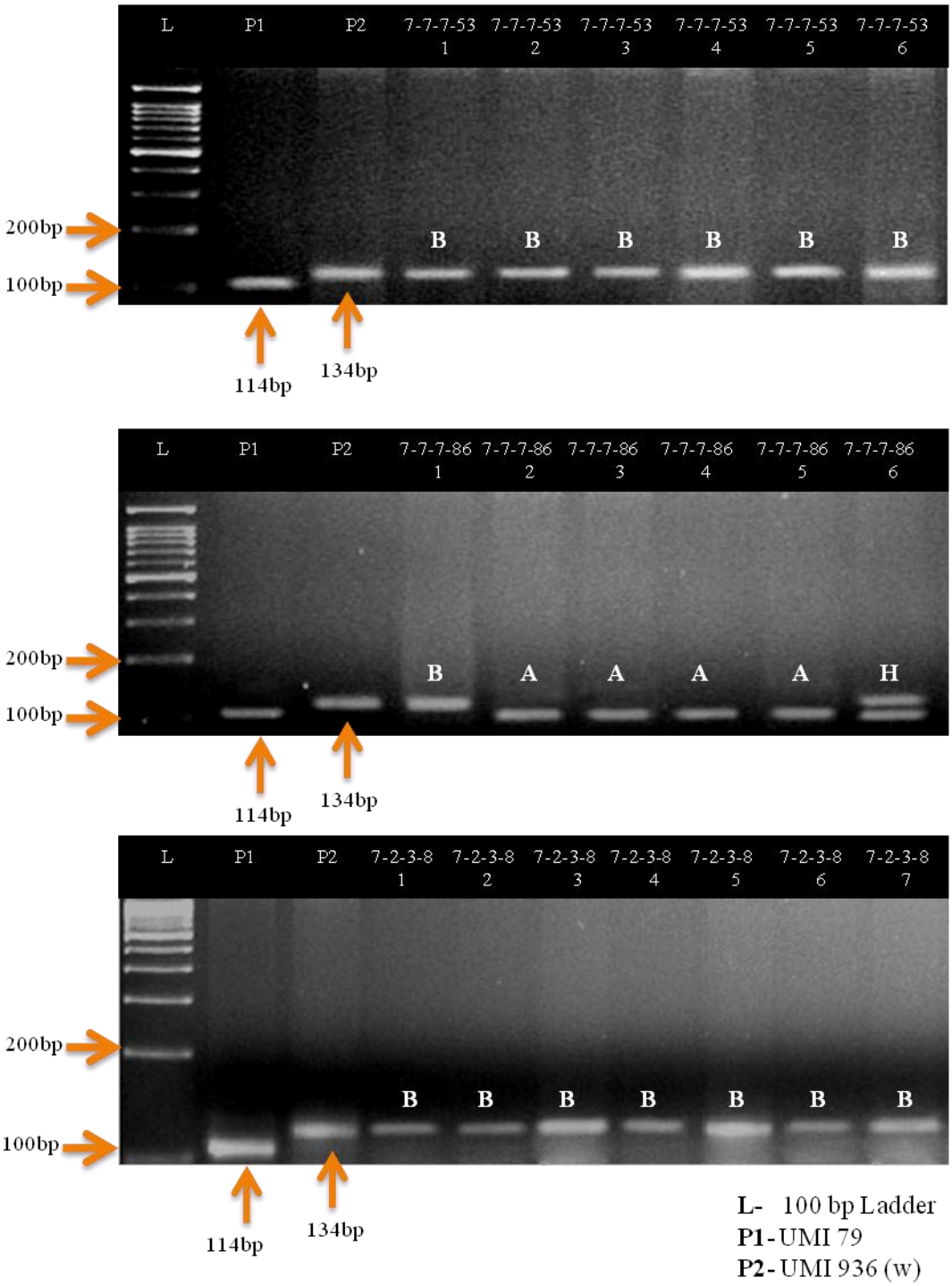

Plate 11. Segregation pattern of SSR marker nc013 in BC_3F_3 progenies of (UMI 79 x UMI 936 (w)) x UMI 79

Discussion

CHAPTER V

DISCUSSION

Maize **(***Zea mays.* L) is the world's most widely cultivated cereal crop and an essential food source for millions of world's poor. It ranks among one of the four principal crops of the world. It is grown widely throughout the world in a range of agro ecological environments.Today maize is one of the handfuls of crop plants that support the world's food supply. Versatile utility of corn as food and feed enhances the area under production and the productivity day by day (Netravati *et al*., 2013). Together with rice and wheat, maize provides at least 30% of the food calories to more than 4.5 billion people in 94 developing countries (Von Braun *et al.*, 2010). Demand for maize crop in the developing countries is expected to surpass the demand for both wheat and rice by the year 2020 (Pingali and Pandey, 2001and Prasanna and Hoisington, 2003).

Maize is a widely grown C_4 crop with a high rate of photosynthetic activity leading to high grain and biomass yield potential. It is predominantly a cross-pollinating species, a feature that has contributed to its broad morphological variability and geographical adaptability. In the tropics, corn is primarily grown for human consumption. FAO predicts that an additional 60 million tonnes of maize grain will be needed from the annual global harvest by 2030. The demand for maize as an animal feed will continue to grow faster than the demand for its use as a human food, particularly in Asia, where a doubling of production is expected from the present level of 165 million tonnes to almost 400 million tonnes in 2030. (Paliwal *et al*., 2000).

In India, a significant progress has been made in relation to maize improvement using traditional breeding strategies. However, disease, insects and abiotic stresses are the major impediments to maize production at present. Minimizing losses from biotic stress offer tremendous opportunities for increasing and stabilizing maize productivity while maintaining the quality of produce. Numerous options have been recommended for the control and management of diseases and insect pests. Among the diseases sorghum downy mildew (SDM) caused by *Peronosclerospora sorghi* (Butler, 1907), a disease of sorghum which also affects maize (Williams, 1984) can cause severe epidemics, resulting in heavy yield losses.

Worldwide, the percentage of area with reported economic losses to downy mildew is 30%, both in tropical lowland maize and in subtropical, mid-altitude, transition zone and highland maize (Jeffers *et al.*, 2000). Despite the use of metalaxyl fungicide, severe incidence of the disease still occurs in localized areas (Dalmacio, 2000). Seed treatment with fungicide by commercial companies makes the seeds expensive and generally beyond the financial reach of resource-poor farmers. These cost concerns and the emerging problem of build up of chemical resistance in the pathogen point to the use of resistant varieties as a more cost-effective and environmentally safe alternative for managing the downy mildews.

One main objective of resistance breeding is the introgression of one or more resistant genes from a donor into the background of an elite variety (recurrent or recipient parent) and to recover the recurrent parent genome as rapidly as possible. Introgression of sorghum downy mildew resistance through Marker Assisted Backcrossing (MAB) involving the resistance source would be the practical option for the breeders to develop disease resistance cultivars. In order to precisely identify the introgressed line, in addition to phenotypic screening of genotypes under sick plot, the genotypes identified as resistant are again screened for identified sorghum downy mildew resistant QTLs through molecular marker analysis. Backcross progenies are also screened with background markers to identify the recovery of recurrent parent genome.

Markers are effective aids for selection in backcrossing in three ways. First, markers can aid selection on target alleles whose effects are difficult to observe phenotypically. Second, markers can be used to select for rare progeny which contain the target gene with less agronomic introgression from the donor parent. Third, markers can be used to select rare progeny that are the result of recombination near the target gene, thus minimizing the effects of linkage drag (Joshi and Nayak, 2010).Thus marker assisted backcrossing (MAB) is a major inventory step in the development of the sorghum downy mildew resistant lines rapidly within a very few generations with the aid of linked markers for resistant QTLs.

It is important to identify cultivars not only with sorghum downy mildew resistance but also having good yield and quality traits. The yield attributing characters

are essential for utilizing these lines in developing superior single cross hybrids especially in maize. Since the agronomic performance of the introgressed line is also utmost important for practical utility, biometric observations were recorded in the BC_3F_2 generation to determine the biometric value of introgressed lines.

5.1. Marker assisted selection

Molecular marker technology facilitates the identification of genomic locations linked to the traits of interest and helps in indirect selection of such complex traits without depending on difficult phenotypic measurements and will speed up the selection process in breeding programme. Marker Assisted Backcrossing (MAB) combines 'Foreground' selection of donor alleles linked to markers and 'Background' selection of recurrent parent alleles in the later generation and become most efficient and feasible breeding approach for speedy introgression of resistant genes.

Marker assisted foreground analysis is the selection of individuals that are possessing target genes in a segregating population using functional, linked or flanking markers. The markers linked to genes of interest or QTLs should be developed as a prerequisite and these can be used to screen the transferred genes in different backgrounds. One of the main advantages of this strategy is that the selection is more towards specific genes of interest and not the phenotype. The availability of large number and types of molecular markers and no environmental influence makes the selection of desirable traits simple in plant breeding.

George *et al.* (2003) using RILs population derived from cross between Ki3 (downy mildew resistant) and CML139 (susceptible) found out that five genomic region on chromosome 1, 2, 6, 7 and 10 are involved in sorghum downy mildew resistance. A strong QTL on chromosome 6 was stable across environments, significantly affecting disease resistance at the five locations in four Asian countries (Nair *et al*. 2005). They identified three QTLs (one each on chromosomes 2, 3 and 6) for sorghum downy mildew resistance and verified that locus on chromosome 6 contributed resistance to diverse downy mildews in tropical Asia, including sorghum downy mildew in India. The three QTLs appeared to have additive effects on resistance, identifying one major gene on

chromosome 2 and two minor genes on 3 and 6 that contribute to downy mildew resistance (Sabry *et al*., 2006).

Based on the linkage map obtained from Nair *et al*. (2005), the polymorphic markers were selected for the backcross population [(UMI 79 x UMI 936(w)) x UMI 79]. Interestingly the two SSR loci on chromosome 6 (bnlg1154 and nc013) and two on chromosome 3 (phi053 and bnlg420) included in the genotyping were found to be polymorphic between the susceptible, UMI 79 and the resistant parent, UMI 936(w).

The highly susceptible parent UMI 79 along with the sorghum downy mildew resistant parent UMI 936(w) identified under sick plot screening were surveyed using SSR markers linked to sorghum downy mildew resistance QTLs. Among 46 markers screened, only 12 expressed polymorphism between the parents covering the 2, 3 and 6 linkage groups. Higher percentage of polymorphism was observed in the linkage group 6 around 42.8 per cent (Kashmiri, 2010).

In the current study, donor parent UMI 936(w) was crossed with the recurrent parent UMI 79 in order to transfer the sorghum downy mildew resistance QTL. Using the sequence information obtained from the maizegdb and from Nair *et al*. (2005) and Kashmiri (2010), those markers, *viz.*, phi053 on chromosome 3 and nc013 on chromosome 6 found polymorphic for the recurrent parent and the donor parent was used to screen the progenies for identifying the heterozygosity. The selected progenies were repeatedly backcrossed with the recurrent parent as pollen parent upto BC_3F_1 generation with phenotyping in sick plot coupled with genotyping for resistant QTLs. From BC_3F_1, three progenies heterozygous for all the four foreground SSR markers were selfed to raise the BC_3F_2 generation. The three selected progenies *viz.*, 7-2-3, 7-2-10 and 7-7-7 were phenotypically screened under sick plot for sorghum downy mildew disease and phenotypically resistant individuals were identified and forwarded to BC_3F_3 generation.

5.2. Phenotypic screening of BC_3F_2 population

The development of a reliable method for evaluating maize plants for the expression of sorghum downy mildew was crucial to the success of the study. Consistent heavy disease pressure is required to assess accurately the potential of plant genotypes to

resist the onset and progress of sorghum downy mildew and to determine the magnitude of the effect of genetic factors that contribute to resistance.

In this study we have screened three BC_3F_2 progenies, 7-2-3, 7-7-7 and 7-2-10 in the sorghum downy mildew sick plot. The disease pressure developed was found to be high as the susceptible check CM 500 showed 100% infection. Similarly CM500 showed 94% and 100% disease infection in kharif 2008 and 2009 respectively during work of Kashmiri (2010). The two parents (UMI 79 and UMI 936(w)) used in the backcrossing programme exhibited the most extreme phenotypes to sorghum downy mildew infection (plate. 1 and 2). The inbred parent UMI 936(w) and UMI 79 were found to be highly resistant and susceptible respectively to *P. sorghi* pathogen (Arulselvi, 2007). The progeny 7-2-10 showed high percentage of infection (72.38%) and was therefore eliminated from the study. Among the progenies, progeny no. 7-7-7 had more number of resistant plants.

5.3. Studying the biometrical performance of BC_3F_2 progenies

5.3.1. Performance of BC_3F_2 progenies for variability parameters

Plant breeders are commonly faced with problems of handling segregating populations and selection procedures. Mean and variability are the important factors for selection. Mean serves as a basis for eliminating undesirable crosses or progenies. Variability helps to choose a potential cross or progeny since variability indicates the extent of recombination for initiating effective selection procedures. The genotypic co-efficient of variation provide a mean to study the genetic variability generated in quantitative characters (Johnson *et al.*, 1955). Selection for the improvement of quantitative traits can be effective only when segregating generation's posses the potential variability. Breeding potentials of a cross between varieties or inbreds are judged by the number of desirable transgressive segregants. The probability of obtaining superior lines can be worked out in early generations through the estimates of first and second order degrees of statistics if genetic variation is available (Pooni *et al.*,, 1992). In order to know the breeding utility of this variability and selection value of various quantitative traits, it is essential to determine various components and heritable proportion of variability.

Selection of parents is one of the most important steps in any breeding programme. Selection method can extract good cultivars if the parents used in the breeding programme are ideal. Therefore, an elite inbred UMI 79 was selected to introgress sorghum downy mildew resistance from UMI 936(W). Inclusion of elite inbred as parent (UMI 79) will largely help to ensure the recovery of a high proportion of progenies with adaptation and quality that would in turn helpful in developing superior hybrids with sorghum downy mildew resistance. The parents selected in the study UMI 79 and UMI 936(w) were having extreme variations in case of sorghum downy mildew resistance parameters while UMI 79 is having superior phenotypical expression for morphological and yield traits.

The important biometrical traits *viz.*, days to tasseling, days to silking, days to maturity, plant height, cob length, cob diameter, number of rows per cob, number of grains per row, cob weight, shelling %, 100 grain weight and grain yield per plant were studied in the BC_3F_2 generation to identify resistant progeny with better biometric performance.

In the BC_3F_2 generation the progeny 7-7-7 which exhibited high sorghum downy mildew resistance recorded moderate variability (GCV-10% to 20%) for plant height, cob diameter, number of grains per row and cob weight. However, it recorded low variability (GCV<10) for remaining traits.

In progeny 7-2-3, the variability for days to tasseling, days to silking, days to maturity, cob weight, shelling % and 100 grain weight was low as it is evident from low GCV (<10 per cent). However, moderate variability (GCV-10% to 20%) was observed for plant height, cob length, and number of rows per cob and high variability was observed in cob diameter, number of grains per row and grain yield per plant. The moderate to high GCV gives an indication of justifiable variability among the genotypes with respect to these characters and therefore gives scope for further improvement of these traits through selection. Similar findings in maize were also reported by Rafique *et al.* (2004), Abhirami *et al.* (2005), Alake *et al.* (2008) and Reddy *et al.* (2013). Panwar *et al.* (2013) also reported moderate variability for number of grains per row and cob diameter. Vashishta *et al*., 2013 observed high to moderate variability for number of grains per row and grain yield per plant.

The progeny 7-7-7, therefore appears to have attained more uniformity with moderate variability for important biometrical traits like plant height, cob diameter, number of grains per row and cob weight. Hence this progeny would be useful in deriving Near Isogenic Lines (NILs) with high per se which could be utilized in developing hybrids in future.

For traits cob diameter, number of grains per row, cob weight and grain yield per plant, the PCV was higher than the corresponding GCV showing the role of environment in these characters. Minor variations between PCV and GCV values for rest of the traits under study shows the limited role of environment in these characters and they were mostly governed by the genetic factors. Selection for improvement of such characters will be rewarding in this situation.

In progeny 7-7-7, the number of progenies resembling the recurrent parent was recorded to be high for plant height, cob length, cob diameter, cob weight, shelling % and grain yield per plant which is a desirable feature as the objective was to introgress resistant QTLs by retaining superior biometrical traits of recurrent parent.

In 7-2-3 progeny, the number of progenies resembling the the recurrent parent, UMI 79 was found to be high for days to tasseling, days to silking, plant height, cob length and number of rows per cob.

5.3.2. Performance of BC_3F_2 progenies for Heritability and Genetic Advance

Heritability provides the magnitude of inheritance whereas the genetic advance is a measure of genetic gain under selection. In both 7-2-3 progeny and 7-7-7 progeny, plant height, days to tasseling, days to silking, cob length, cob diameter, number of rows per cob, cob weight, shelling %, 100 grain weight and grain yield per plant exhibited high to moderate heritability followed by high to moderate genetic advance. This is the indication of predominance of additive gene action. This is desirable for selection since these traits are least influenced by the environment. The selection for these traits is likely to accumulate more additive genes leading to further improvement in their agronomic performance. Similar results were obtained by Vashishta *et al.* (2013) for grain yield per plant (58.52), plant height (37.09) and ear height and also by Bekele and Rao (2014) and Panwar *et al.* (2013).

Low heritability followed by moderate to high genetic advance was reported in number of grains per row in progeny 7-2-3 and 7-7-7. Similar findings were reported by Vashishta et al. (2013). This indicates that the traits are governed by additive gene effects. The low heritability is being exhibited due to high environmental effects. Intensive selection may be effective in such cases.

Moderate heritability but low genetic advance was observed in days to maturity in both the progenies *viz.*, 7-2-3 and 7-7-7. Similar result has been reported by Bekele and Rao (2014) in maize. This indicates non additive gene action and selection for this trait may not be rewarding. This is the indication of epistatis and dominant gene action for these trait. The traits could be exploited through heterosis breeding after the introgression is complete.

5.3.3. Performance of BC_3F_2 progenies for skewness and kurtosis

Skewness describes the degree of departure of a distribution from symmetry and kurtosis characterizes the peakedness of a distribution. In a frequency distribution of a segregating generation, skewness could result when certain combinations of genes are lethal or when there is incomplete linkage of certain genes controlling the trait or when there is presence of epistasis or due to non additive effects (dominance or over dominance) or due to the presence of genotype x environment interaction or when one gene has much larger effect than others.

Skewness helps us to draw the conclusion about the gene action for a particular trait. The positive skewness indicates the presence of complementary epistatic gene action for the trait and the gain is slower with mild selection and gain is faster with intensive selection. The negative skewness indicates the presence of duplicate epistasis gene action and the gain is faster with mild selection and rapid with intense selection (Snape and Riggs, 1975).

Kurtosis will occur if either a few genes are controlling the phenotypic distribution or there are inequalities in the additive genetic effects at different loci. Traits for which data is showing leptokurtic distribution are usually those under control of relatively few segregating genes, whereas data showing a platykurtic distribution usually represent characters that are controlled by many genes. The positive values of kurtosis

indicate leptokurtic curve while negative kurtosis indicate platykurtic curve and if values are not significant or zero, it indicates mesokurtic *i.e.* normal distribution.

The present study reveals that positively significant skewness was observed for days to maturity, cob diameter, cob weight, number of grains per row and grain yield per plant. Therefore for these characters complementary gene action was confirmed. The gain is slower with mild selection but is faster with intensive selection. Similar results have been reported by Tamilkumar (2012). Positively significant kurtosis was observed for days to maturity and number of grains per row. The positive kurtosis indicates leptokurtic curve and the traits are controlled by few genes. Negative kurtosis was observed in 100 grain weight and grain yield per plant. Tamilkumar (2012) and Aarthi (2012) reported similar results for grain yield. This indicates platykurtic curve which means that flat values are present in the distribution and characters are controlled by many genes. If selection for these characters were made intensively, the gain will be faster.

5.3. Genotyping of BC_3F_3 progenies

From BC_3F_3 progenies, eight phenotypically superior progenies comprising 68 individuals were genotyped using two polymorphic SSR markers linked to chromosome 3 (phi053) and chromosome 6 (nc013). The progenies showing both parental alleles were scored as “H”, those similar to UMI 79 were scored as “A” and those similar to UMI 936(w) were scored as “B”.

Among the markers, phi053 (chromosome 3) amplified 180bp for UMI 79 and 196bp for UMI 936(w). The marker nc013 showed amplification of 114bp and134bp for UMI 79 and UMI 936(w) respectively.

Among the progenies, one progeny (7-7-7-53) was identified homozygous for UMI 936(w) allele for both markers, two progenies (7-7-7-85 and 7-2-3-8) were identified homozygous for UMI 936(w) allele for marker nc013 and four progenies were identified as heterozygous for both the markers. Similar to the present results, Aarthi (2012) could identify heterozygotes and homozygotes for phi053 and nc013 markers in BC_1F_1 population of (UMI 79 X UMI 936(w)) x UMI 79).

From the advanced backcross populations, near-isogenic lines (NILs) are created that contain specific QTLs from the resistant donors that are able to significantly improve the performance of the elite variety QTL-NILs represent the starting point to (i) fine map QTLs, (ii) eliminate undesirable effects caused by linkage drag and, eventually, (iii) perform map-based cloning of target QTLs (Frary *et al.*, 2000; Fridman *et al.*, 2000 and Salvi and Tuberosa, 2005).

The single plants of progeny 7-7-7-53 was identified to be homozygous for UMI 936(w) allele for both the markers and is expected to have the resistant QTLs on both chromosome 3 and 6 introgressed into it from the donor parent UMI 936(w). Single plants of two progenies (7-7-7-85 and 7-2-3-8) were identified to be homozygous for UMI 936(w) allele for marker nc013 (chromosome 6) alone. These three lines can be designated as NILs (Near Isogenic Lines) resistant to the sorghum downy mildew disease and can be used in further breeding programmes as one of the parents to develop sorghum downy mildew resistant maize hybrids. Single plants of four progenies (7-7-7-59, 7-7-7-84, 7-7-7-86 and 7-2-3-2) are identified heterozygous for both the markers and hence they are to be advanced into next generation by selfing so as to identify more NILs differing from each other for the resistance QTL alone. Similarly NILs have been produced by Belcher *et al.* (2012) for southern leaf blight resistance in maize and also by Guimaraes *et al.* (2014) for Aluminium tolerance in maize.

The objective of the present study was to develop sorghum downy mildew resistant lines with background of UMI 79, the recurrent parent. In the present study, through phenotypic screening followed by genotypic screening, three sorghum downy mildew resistant QTLs introgressed NILs (7-7-7-53, 7-7-7-85 and 7-2-3-8) have been identified which can be used for further hybrid maize breeding programmes. In addition, four progenies (7-7-7-59, 7-7-7-84, 7-7-7-86 and 7-2-3-2) have been identified which are to be forwarded to next generation in order to identify more NILs with introgressed sorghum downy mildew resistant QTLs.

From the analysis of morphological traits, progeny no. 7-7-7 is found to be phenotypically more superior than 7-2-3 progeny.

Table 7. Comparison of the two promising sorghum downy mildew resistant BC_3F_2 progenies for important biometrical parameters

Progeny	MEAN		GCV (%)		% of plants similar to recurrent parent	
	7_7_7	7_2_3	7_7_7	7_2_3	7_7_7	7_2_3
Plant height (cm)	99.92	96.07	16.86	16.26	27.69	28.95
Cob length (cm)	10.83	8.40	9.49	19.08	38.46	26.32
Cob diameter (cm)	10.08	7.95	14.49	22.44	24.62	7.89
Number of rows per cob	12.52	10.00	9.48	13.75	23.08	36.84
Number of grains per row	13.25	6.47	14.77	24.12	16.92	7.89
Cob weight (g)	27.30	16.68	16.72	8.00	30.77	10.53
Shelling %	68.10	65.06	8.72	6.78	20.00	15.79
100 grain wt (g)	18.34	16.50	5.62	8.58	23.08	15.79
Grain yield per plant (g)	18.28	10.87	9.08	25.65	49.23	7.89

The promising 7-7-7 progeny is found to have higher mean performance for important biometric traits than 7-2-3 progeny (Table 7). Also 7-7-7 progeny is found to have moderate variability for important biometrical traits like plant height, cob diameter, number of grains per row and cob weight. Besides this, the number of progenies resembling the recurrent parent was observed to be high in 7-7-7 progeny for desirable characters such as plant height, cob length, cob diameter, number of grains per row, cob weight, shelling %, 100 grain weight and grain yield per plant which is a desirable feature. Thus, considering the biometric value of progenies, 7-7-7-53, 7-7-7-59, 7-7-7-84, 7-7-7-85 and 7-7-7-86, (Table 8), may be more useful in deriving Near Isogenic Lines (NILs) with high per se which are ultimately exploited in hybrid maize breeding as one of the parents to develop sorghum downy mildew resistant maize hybrids.

Table 8. Genetic constitution and agronomic performance of selected elite BC_3F_3 progenies

Progeny	Genetic constitution		Plant height (cm)	Cob length (cm)	Cob diameter (cm)	No: of rows per cob	No: of grains per row	Cob weight (g)	Shelling %	100 grain wt (g)	Grain yield Per plant (g)
	phi 053	nc013									
7-7-7-53	B	B	98.6	13.50	11.4	12	11	22.98	62.10	18.21	14.27
7-7-7-59	H	H	123.5	10.90	7.6	12	10	25.08	69.10	18.44	17.33
7-7-7-84	H	H	110.8	11.00	10.1	10	16	24.36	77.11	19.74	18.78
7-7-7-85	H	B	94.7	10.10	9.8	12	10	17.88	76.53	18.40	13.68
7-7-7-86	H	H	99.9	14.10	12.3	14	16	20.62	74.05	16.82	18.27

Summary

CHAPTER VI

SUMMARY

The present study was undertaken with a view of developing sorghum downy mildew resistant elite maize inbreds by introgressing the sorghum downy mildew resistant QTLs. The development of sorghum downy mildew resistant inbred lines through marker assisted backcrossing was found to be a promising option to develop sorghum downy mildew resistant single cross hybrids. In the present study, UMI 936(w) was used as a donor parent to transfer downy mildew resistant QTLs into an lite inbred line, UMI 79 (recurrent parent) and further backcross progenies were generated using UMI 79 as male parent. Screening of the BC_3F_2 progenies was done under sick plot conditions and phenotypically resistant individuals were identified which were selfed and forwarded to BC_3F_3 generation. Using the sequence information obtained from the maizegdb and from the reports of Nair *et al.* (2005), two SSR markers which were polymorphic between the parents were used to screen the selected BC_3F_3 progenies.

1. Three BC_3F_2 progenies *viz.*, 7-2-3, 7-2-10 and 7-7-7 which were found to be resistant in BC_3F_1 generation were screened phenotypically under sick plot. Progeny no. 7-2-10 was found to be highly susceptible (72.38% infection) to sorghum downy mildew and was therefore eliminated from further studies. Among 7-7-7 (21.49% infection) and 7-2-3 (26.92% infection) progenies, progeny no. 7-7-7 was found to be phenotypically more resistant to the disease.

2. While considering the morphological traits of the advanced BC_3F_2 progenies, the progeny no. 7-7-7 seems to have attained more uniformity with moderate variability for important biometrical traits like plant height, cob diameter, number of grains per row and cob weight. Therefore, this progeny would be useful in deriving Near Isogenic Lines (NILs) with good field performance.

3. For all biometrically important characters, 7-7-7 progeny is found to have higher mean value than 7-2-3 progeny. Also in progeny 7-7-7, the number of progenies resembling the recurrent parent was recorded to be high for plant height, cob length, cob diameter, cob weight, shelling % and grain yield per plant. This is desirable

feature as the objective was to introgress resistant QTLs by retaining superior biometrical traits of recurrent parent.

4. The variability parameters of BC_3F_2 progenies revealed that the traits *viz.,* plant height, days to tasseling, days to silking, cob length, cob diameter, number of rows per cob, cob weight, shelling %, 100 grain weight and grain yield per plant have exhibited high to moderate heritability followed by high to moderate genetic advance. This clearly denoted that these traits are governed largely by additive gene effect which may be favourably exploited for further improvement by selection.
5. Low heritability followed by moderate to high genetic advance was observed in BC_3F_2 progenies for number of grains per row indicating that the traits are governed by additive gene effects. The low heritability being exhibited may be attributed to high environmental effects. Intensive selection may be effective in such cases.
6. Moderate heritability but low genetic advance observed in BC_3F_2 progenies for days to maturity indicates epistatis and dominant gene action and selection for this trait may not be rewarding. The traits could be exploited through heterosis breeding once the introgression is complete.
7. Positively significant skewness was observed BC_3F_2 progenies for days to maturity, cob diameter, cob weight, number of grains per row and grain yield per plant confirming complementary gene action for the traits. The gain is slower with mild selection but is faster with intensive selection.
8. Positively significant kurtosis was observed for days to maturity and number of grains per row. The positive kurtosis indicates leptokurtic curve and the traits are controlled by few genes. Negative kurtosis was observed in 100 grain weight and grain yield per plant. This indicates platykurtic curve which means characters are controlled by many genes. If selection for these characters were made intensively, the gain will be faster.
9. Thirty eight phenotypically resistant individuals from 7-2-3 progeny and sixty five phenotypically resistant individuals from progeny no. 7-7-7 were selfed to generate the BC_3F_3 generation. From this population eight phenotypically superior progenies

comprising sixty eight individuals were selected and genotyped with two SSR markers located on the chromosome 3 and chromosome 6.

10. Among the 12 SSR markers reported to be located on different chromosomes (George *et al*., 2003; Nair *et al*., 2005; Sabry *et al*., 2006 and Kashmiri, 2010), two markers *viz.,* phi053 on chromosome 3 and nc013 on chromosome 6 which showed good polymorphism between the parental lines of the present study were used for the analysis of BC_3F_3 progenies.

11. The genotyping of BC_3F_3 progenies for the marker phi053 (chromosome 3) revealed 6 progenies to be heterozygous (H), one progeny similar to UMI 79 and one progeny similar to UMI 936(w).

12. For marker nc013 (chromosome 6), 5 BC_3F_3 progenies were found to be heterozygous (H) and 3 were similar to UMI 936(w).

13. One BC_3F_3 progeny (7-7-7-53) was identified homozygous for UMI 936(w) allele for both the polymorphic SSR markers phi053 and nc013 and two progenies (7-7-7-85 and 7-2-3-8) were identified homozygous for UMI 936(w) allele for marker nc013 (chromosome 6) alone. These three lines can be designated as NILs (Near Isogenic Lines) resistant to the sorghum downy mildew disease and can be exploited in further maize hybrid breeding programmes.

14. Four BC_3F_3 progenies (7-7-7-59, 7-7-7-84, 7-7-7-86 and 7-2-3-2) were identified heterozygous for the polymorphic markers phi053 and nc013. They are to be selfed and advanced into next generation to identify more NILs differing from each other for the resistance QTL alone.

15. From the analysis of morphological traits of BC_3F_2 generation, progeny 7-7-7 is found to be phenotypically more superior to 7-2-3 progeny. Considering the biometric value of BC_3F_3 progenies, 7-7-7-53, 7-7-7-59, 7-7-7-84, 7-7-7-85 and 7-7-7-86 may be more useful in deriving Near Isogenic Lines (NILs) with high performance. Thus these five BC_3F_3 progenies can be used as NILs resistant to sorghum downy mildew which could further be used for developing sorghum downy mildew resistant single cross maize hybrids which is the need of the hour.

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