FINAL REPORT OF NICHE AREA OF EXCELLENCE (2009 - 2014)

"Molecular Breeding for Improvement of Wheat and Pigeonpea in Eastern Indo-Gangetic Plains"

Centre: Institute of Agricultural Sciences

Banaras Hindu University, Varanasi- 221 005

SCIENTIST INVOLVED IN THE PROJECT

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Principal Investigator	**Prof. V.K. Mishra**, Department of Genetics and Plant Breeding Institute of Agricultural Sciences Banaras Hindu University, Varanasi

Name of Crop	**Scientist**
Wheat	1. Prof. V.K. Mishra, Genetics and Plant Breeding 2. Prof. Ramesh Chand, Mycology and Plant Pathology
Pigeonpea	1. Prof. M.N. Singh, Genetics and Plant Breeding 2. Prof. Ramesh Chand, Mycology and Plant Pathology

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FINAL REPORT FOR NICHE AREA OF EXCELLENCE

Project summary

With the inception of the Niche Area of Excellence project in October 2009 on "Molecular Breeding for Improvement of Major Crops of Eastern Indo-Gangetic Plains" at BHU Centre, we have made significant achievements with the objectives; to develop mapping populations in wheat and pigeonpea for identification of the genes/QTLs of interest,to integrate molecular approaches viz. marker assisted selection (MAS) with conventional breeding programmes,to facilitate practical and hands-on training to students in the area of molecular breeding,to undertake HRD/training in the niche area of excellence, and development of improved varieties and deposition of improved germplasm lines as the national public domain.

We are now focussing on integration of new technologies related to expression profiling, transcriptomics for plant-pathogen interaction studies, fine mapping along with ongoing marker assisted backcross breeding programmes under the project to come out with such results which would be rewarding not only to the science globally but also to the farming community of the nation to ensure their food and nutritional security. The major achievements during the five years tenure i.e., 2009-14 are summarised as:

- 90 wheat germplasm lines were maintained for breeding programme and basic studies related to spot blotch (*B. sorokiniana)*.
- Two major QTLs, *QSB.bhu2B* and *QSB.bhu7D* associated with spot blotch resistance were successfully validated and now being used for marker assisted selection.
- Crosses were made between most popular and promising wheat varieties (e.g., HUW234, HUW468, PBW154, HUW510, DBW14, NW2036 and DL803-3) of this region with potential spot blotch resistant wheat lines and marker assisted backcross breeding program is in progress to identify such lines which are agronomically as good as recurrent parent with resistant to spot blotch.
- Different *B. sorokiniana* isolates were characterised to identify the MAT locus and maintained in their pure form.
- Crosses were made among high yielding wilt susceptible varieties (Bahar, MAL13, MAL18, MAL31 and MA6) and potential resistant donors (BWR 23, BDN-2029, BSMR-846) to *Fusarium udum* and the materials are in advanced breeding stage to identify some high yielding lines with enhanced resistance to *Fusarium* wilt.
- Pigeonpea SSR markers namely, ASSR-1, ASSR-23, ASSR-148, ASSR-229, ASSR-363 and ASSR-366 were identified as tightly linked with *Fusarium* wilt resistance.
- Some potent resistant sources *viz*., BSMR-846, BWR-23, BDN-2029 having one to three major genes were identified from inheritance studied on Fusarium wilt resistance.
- More than 100 Undergraduate, Post-graduate and Ph.D. students availed the facilities under the Niche Area of Excellence project their for plant molecular breeding works.

1.	**Name of the University**	**:**	Banaras Hindu University
2.	**Name of the Niche Area**	**:**	"Molecular Breeding for Improvement of Wheat and Pigeonpea in Eastern Indo-Gangetic Plains"
3.	**Year of start**	**:**	Sanctioned- 12/16-10-2009 Start of the project- 25-01-2010
4.	**Name of the PI with designation**	**:**	**Dr. V.K. Mishra**, Professor, Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, BHU, Varanasi
5.	**Goal**	**:**	Rapid and precise incorporation of genes of interest in major crops through molecular breeding including marker assisted selection

6. Objectives:

1) Introgression of spot blotch resistance QTLs into wheat varieties, having high yield potential developed at B.H.U. and other centres, through marker assisted backcross breeding programme.
2) Characterization, genetics and molecular mapping of components of slow disease development (higher latent period, smaller lesion size) in wheat for durable spot blotch resistance.
3) Molecular mapping of *Fusarium* wiltresistance in pigeonpea using identified polymorphicSSR markers among resistant and susceptible lines and development of wilt resistant high yielding pigeonpea cultivars.
4) To assist wheat and pigeonpea breeders in molecular screening of advance breeding lines developed at BHU centre against respective pathogen in wheat and pigeonpea.
5) Pathogenic variability in the *B. sorokiniana* and *F. udum* and their fitness on the newly released resistance variety of wheat and pigeonpea, respectively.
6) To undertake HRD/hands on training to students in the area of molecular breeding.
7) Registration of improved wheat and pigeonpea lines to the national germplasm.

7. Funds released by the ICAR and utilized:

S.No.	**Year**	**Opening balance(Rs.)**	**Fund received(Rs.)**	**Expenditure(Rs.)**	**ClosingBalance (Rs.)**
1.	2009-10	NIL	20100000	3453240	16646760
2.	2010-11	16646760	2778000	11360090	8064670
3.	2011-12	8064670	2275000	9133735	1205935
4.	2012-13	1205935	3440965	4636984	9916
5.	2013-14	9916	2990084	2900645	99355

8. Approved technical programme and achievements for the period under report(2009-2014):

S. No.	Activities	Half yearly period								
		2009-10		2010-11		2011-12		2012-13		2013-14
		I	II	I	II	I	II	I	II	I
1.	Development of mapping population viz., RILs, NILs and DHLs in wheat, maize, rice, pigeonpea and pea.	✓	✓	✓	✓	✓	-	-	-	-
2.	A state of the art laboratory, glass house and storage facilities will be developed to support molecular breeding	✓	✓	✓	✓	-	-	-	-	-
3.	Robust molecular markers shall be used for rapid and precise incorporation of genes through marker assisted back cross breeding	-	-	✓	✓	✓	✓	✓	✓	✓
4.	Development of training manual (including its web display) with regard to molecular breeding in crops for faculty, students and other stakeholders	-	-	✓	✓	✓	✓	✓	✓	✓
5.	Generate better germplasm and human resource development to continue with use of emerging molecular tools in a sustained manner	✓	✓	✓	✓	✓	✓	✓	✓	✓

9. Approved activity milestones for the year (2009-14):

S. No.	Milestones
2009-2010	1. Development of the state of the art Molecular Breeding Laboratory 2. Development of new mapping populations viz., RILs and NILs in wheat/barley, maize, rice, pigeonpea and pea 3. Phenotyping and genotyping of already available RILs and NILs and DH populations 4. Making crosses and generation advancement of new RILs 5. Exposing already available F_2 and advanced breeding populations to known molecular markers of important traits.
2010-2011	1. Phenotyping/genotyping of available RILs, NILs and DH populations to continue only in wheat for spot blotch resistance and pigeonpea for *fusarium* wilt resistance (***as per advice of expert committee during 1st Annual Review Meeting***) 2. Identifying QTLs for traits of interest 3. Development of new RILs and NILs 4. Use of molecular markers in F_2, BC_1, BC_2 and advanced breeding populations to continue 5. Training to students and other stake holders
2011-2012	1. Identifying QTLs for traits of interest 2. Pyramiding one or more of the genes/QTL(s) for traits of interest in wheat and pigeonpea varieties cultivated in eastern Gangetic plains. 3. Use of molecular markers in F_2 and advanced breeding populations to continue 4. Training to studentsand stake holders
2012-2013	1. Validating QTLs associated with traits of interest in wheat and pigeonpea 2. Elite lines with enhanced and stable expression of traits of interest 3. Superior agronomic lines with improved traits (single or in combination) 4. Training to students and other stake holders
2013-2014	1. Backcrossing FG selected BC_1F_1 plants in wheat 2. Fresh F_1s generated using new wheat lines backcrossed to their respective recurrent parents 3. Customization & procurement of microarray 4. Fitness studies of *Bipolaris* isolates on wheat 5. Phenotyping of pigeonpea F_2 and BC populations

10. Approved monitorable targets for the year and achievements (2009-14):(Year 2009-2010)

Targets

1. Development of the state of the art Molecular Breeding Laboratory
2. Development of new mapping populations viz., RILs and NILs in wheat/barley, maize, rice, pigeonpea and pea
3. Phenotyping and genotyping of already available RILs and NILs and DH populations
4. Making crosses and generation advancement of new RILs
5. Exposing already available F_2 and advanced breeding populations to known molecular markers of traits of interest.

Achievements

(A) Wheat and barley

1. A well furnished Molecular Breeding Laboratory developed.
2. Phenotyping of already available RIL population (Ning × Sonalika) in wheat.
3. Classification and identification of RILs into resistant and susceptible 'Sonalika' types differing each other by presence or absence of major QTLs for spot blotch resistance.
4. Screening of 104 CSISA lines, received from CIMMYT, against spot blotch resistance through artificial inoculation and identification of three resistant lines CSISA-SB-72, CSISA-SB-91, CSISA-SB-97.
5. In barley, crosses were made during 2003-2004 Jyoti (susceptible to spot blotch) and Karan-15 (resistant) were advanced in F_6 generation during 2009-2010.
6. Twenty lines were found to have complete resistance reaction.

(B) Pigeonpea

1. Eight agronomically good parents but wilt susceptible genotypes (MA3, MAL13, MAL18, MAL23, MAL31, NDA-1, Bahar and Amar) were crossed with two wilt resistant (BWR-23, ICP-9174) genotypes to obtain 16 crosses.
2. All F_1s were selfed to produce seeds for F_2 generation and four crosses were backcrossed with their recurrent parents
   (i) (MAL18 × ICP9174) × MAL 18
   (ii) (MA3 × BWR23) × MA3
   (iii) (MA6 × BWR23) × MA6
   (iv) (MAL13 × BWR23) × MAL13
3. F_2 progenies raised:
   (i) MA3 × BWR23
   (ii) MA6 × BWR23
   (iii) MAL13 × BSMR-103
   (iv) MAL13 × BWR23

4. The pathogen *Fusarium udum* was isolated from fresh infected pigeonpea plants collected from the agricultural farm of Banaras Hindu University and farmer's field surrounding Banaras Hindu University

5. The cultures of *Fusarium udum* were purified and inoculation procedure was standardised.

(C) Fieldpea

1. To develop a mapping population for identification of markers linked to powdery mildew (*Erysiphe pisi*) resistance involving a cross between susceptible (KPMR-467) and resistant (FC1) parents were attempted.

2. To develop a mapping population for identification of markers linked to slow rusting (*Uromyces fabae*) resistance F_1 crosses involving susceptible (JPBB 1) and resistant (FC 1) parents were attempted.

(D) Maize

1. A total of 36 QPM inbred lines from various places were procured and planted for visual evaluation and seed multiplication during Rabi. Two-three plants were selfed in each line for seed purpose. Data on morphological traits, maturity traits, vigour and uniformity were recorded.

2. Three best inbred lines were identified to serve as donor for *opaque-2* genes along with modifiers.

3. Sixty inbred lines from Ludhiana, HAU, DMR, BHU and CIMMYT were also planted for evaluation and identification of recipient lines to be converted into QPM lines.

4. Observations on morphological traits, maturity traits, vigour and uniformity were recorded.

(E) Rice

1. Off season crop of rice variety HUR-105, Srjoo-52 and HUR4-3 were planted in three dates of sowing with 10 days interval at DRR research farm, Hyderabad.

2. Bacterial leaf blight (BLB) resistant rice varieties improved Pusa Basmati-1 and BPT-5204 Sambha Mahsury (possessing resistant gene *Xa5*, *Xa13* and *Xa21*) were also planted side by side at DRR farm.

3. Crosses have been attempted at DRR (off season nursery) for incorporation of BLB resistant genes from improved Pusa Basmati-1 and BPT-5204 in popular rice varities of Eastern Indo-Gangetic plain HUR-105, HUR 4-3 and Sarjoo-52.

4. Resistance sources for blast and BPH are being explored through national (DRR and CRRI) international (IRRI) organization.

(Year 2010-2011)

Targets

1. Phenotyping/genotyping of available RILs, NILs and DH populations to continue for spot blotch resistance in wheat and *Fusarium* wilt in pigeonpea (***as per advice of expert committee during 1st Annual Review Meeting***).
2. Identifying QTLs for some traits of interest
3. Development of new RILs and NILs
4. Use of molecular markers in F_2, BC_1, BC_2 and advanced breeding populations to continue
5. Training to students, farmers and other stake holders

Achievement

(A) Wheat

1. Three hundred ten germplasm lines received from CSISA-CIMMYT were sown in two replications alongwith Sonalika as a susceptible check.
2. Genomic DNA was isolated from leaf samples collected from 15 days old seedlings from 310 CSISA lines of CIMMYT and individual plants of a RIL population developed from cross Sonalika x Yangmai-6 using DNeasy Plant Mini Kit (Quiagen, Germany).
3. After artificial inoculation, data on disease severity was recorded on three different dates along with days to heading and biomass (g). Differential response of spot blotch severity is shown in Figure 1.
4. Ten elite wheat lines (1stCSISA-SB 6720, 1stCSISA-SB 6723, 1stCSISA-SB 6736, 1stCSISA-SB 6916, 1stCSISA-SB 6943, 2ndCSISA-SB 6705, 2ndCSISASB 6710, 2ndCSISA-SB 6713, 2ndCSISA-SB 6741, 2ndCSISA-SB 6745) had been selected which were resistant to spot blotch were crossed with two leading wheat varieties of Eastern Indo-Gangatic Plains i.e., HUW-234 and HUW-468.
5. Validation of SSR markers linked to major QTLs for spot blotch resistance in RIL (Sonalika×Yangmai-6) and CSISA lines
   (a)QSB.bhu2B- (Xgwm 148)
   (b)QSB.bhu7D- (Xgwm 111)
6. SSR marker *Xgwm148* linked with *QSb.bhu-2B* was validated in the RILs (Fig. 2) and further tested in 34 elite germplasm lines (Fig. 3). In RIL population, marker *Xgwm148* amplified a polymorphic product of approximately 180 and 170 bp in resistant (Yangmai-6) and susceptible parent (Sonalika), respectively. Selection efficiency of marker *Xgwm148* which is linked with spot blotch resistance QTL *QSb.bhu-2B* is 70%.
7. Fungal DNA of more than 100 isolates of *B. sorokiniana* was extracted and identity of *B. sorokiniana* was confirmed through the primer ITS1 (Fig. 4).

8. Variation in the population of *B.sorokinina* for aggressiveness was studied. The MAT locus usually contains genes for one or more transcription factors with structural motifs such as alpha-box (in MAT-1) and the high-mobility-group (HMG) DNA-binding domain (in MAT-2). The aim of this study was to identify the MAT locus in *B. sorokiniana* and correlate the phenotypic mating type designations with genotypic mating type designations.PCR primers were designed to amplify the MAT-1 and MAT-2 regions specifically. Amplification of MAT-1 regions revealed a ~650 to 850 bp product (fig. 5a), while the MAT-2 region amplified a 650 bp product (fig. 5b). The amplification results suggest that the population of B. *sorokiniana* consist of three distinct groups i.e. MAT-1, MAT-2 and mixed group containing MAT-1 and MAT-2 loci.

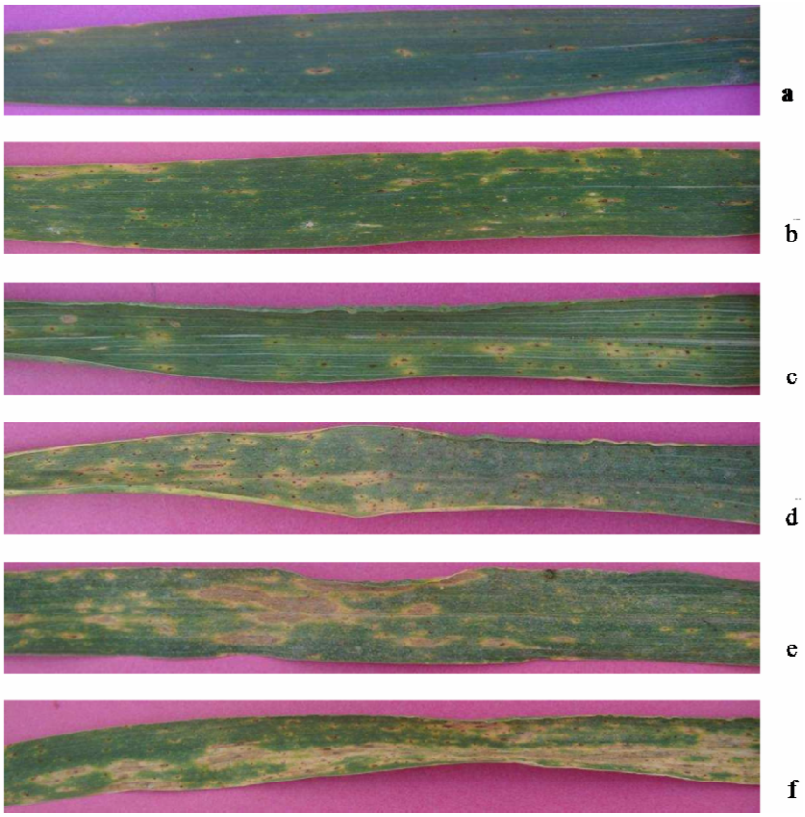


Fig.1.Differential response of spot blotch caused by *B. Sorokiniana* in wheat. **a** to **e**; elite wheat germplasm lines and **f**; susceptible check (Sonalika).

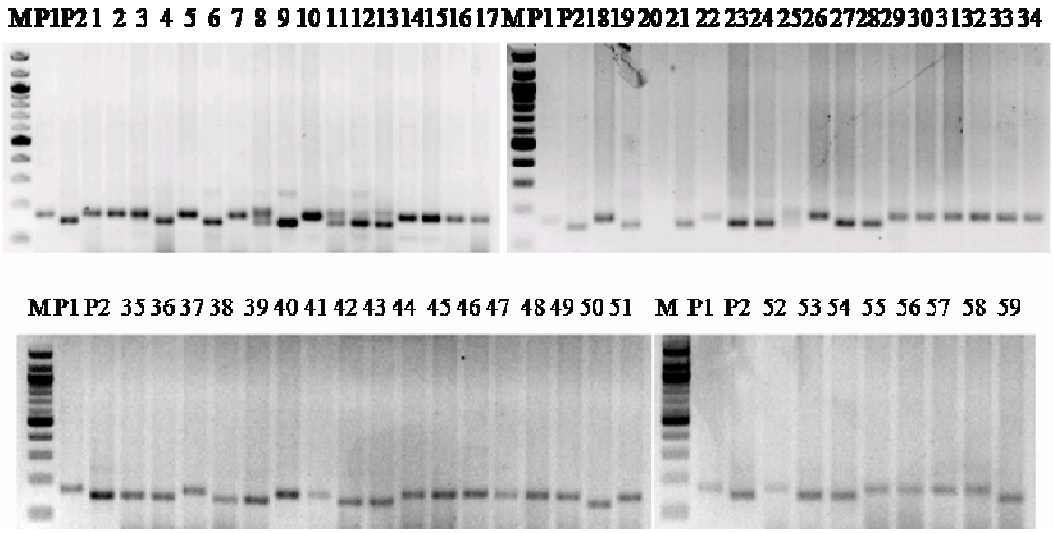


Fig. 2. Validation of SSR marker *Xgwm148* linked with spot blotch resistance QTL*QSB.bhu2B* in the RIL population (1-59 lines) derived from the cross Yangmai-6 (resistant; P1) × Sonalika (susceptible; P2). M is 100 bp DNA size marker.

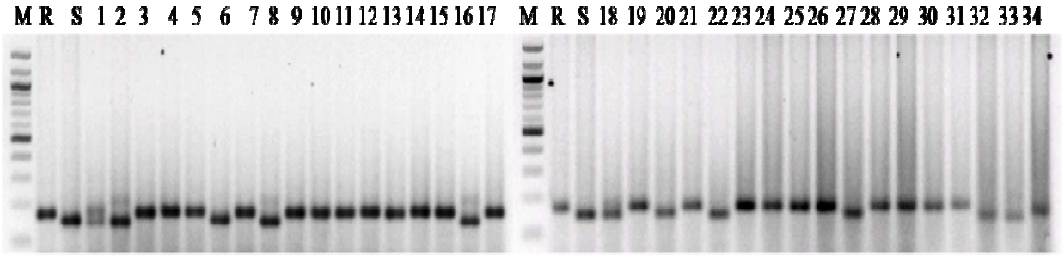


Fig. 3. Validation of SSR marker *Xgwm148* linked with QTL*QSB.bhu2B* in some elite wheat germplasm lines (1-34). Lane M; 100 bp DNA size marker, lane R; Yangmai-6 (resistant genotype) and lane S; Sonalika (susceptible genotype).

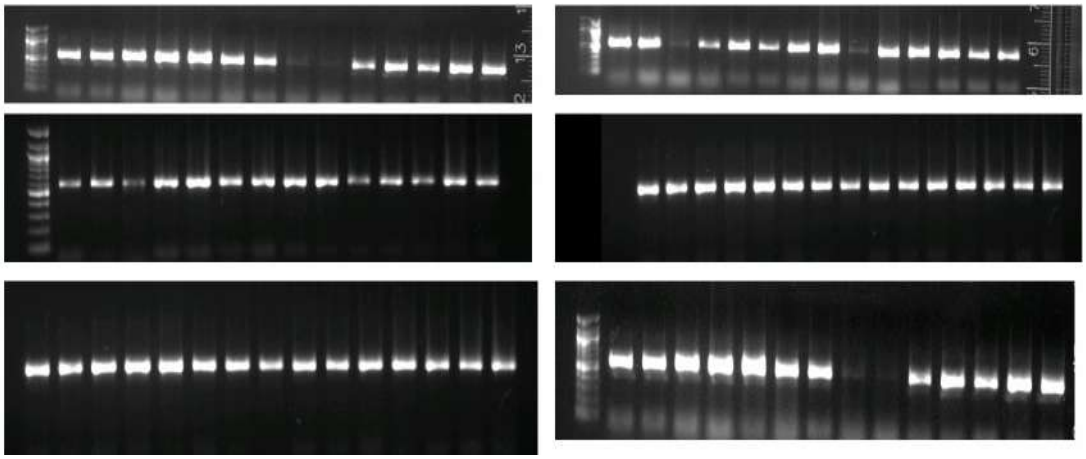


Fig. 4.PCR amplified product of ITS1 region of 57 *B. sorokiniana* isolates M= 100 bp DNA size marker.

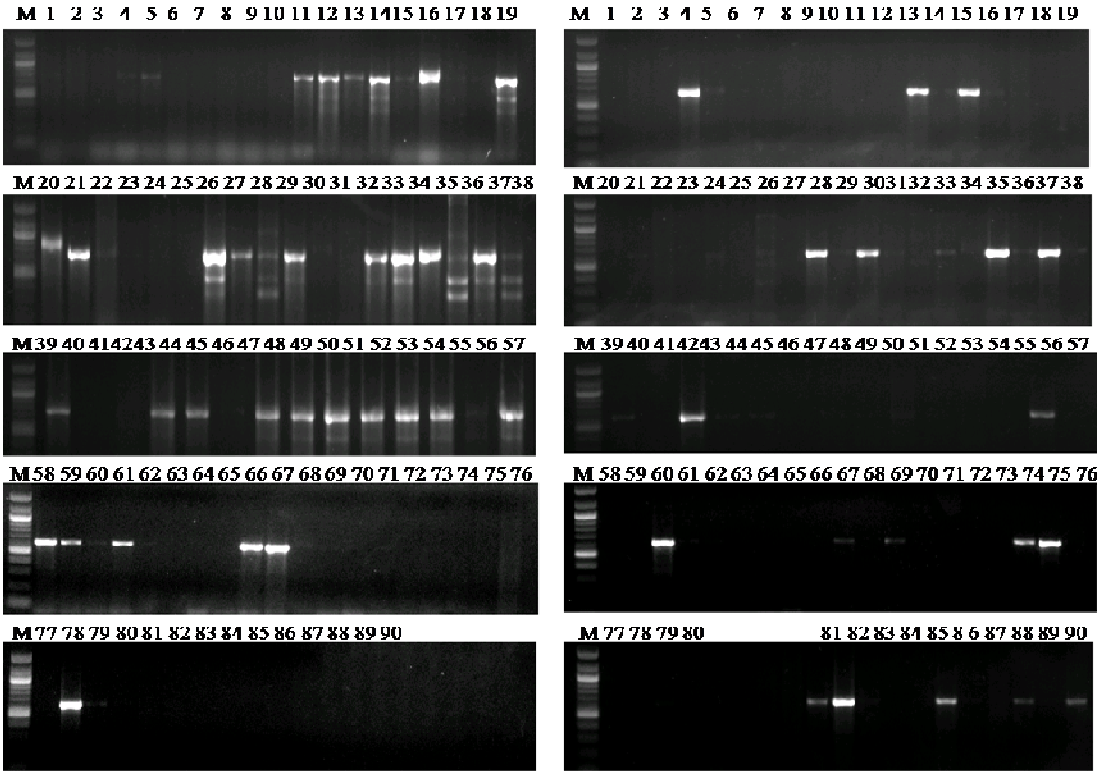


Fig. 5a. Amplification of Alpha box (MAT- 1) in 90 *B. sorokiniana* isolates.

Fig. 5b. Amplification of HMG box (MAT-2) in 90 *B. sorokiniana* isolates.

(B) Pigeonpea

I. Hybridization programme

Few highly wilt susceptible genotypes (Bahar, MAL 18) were crossed with wilt resistant donors such as ICP 9174, BWR 23, BSMR 846, IPA 8F, IPA 9F, BDN 2001-9, BDN 2004-1, BDN 2029 and BDN 2010 to obtain fresh F1seeds.

II. F_1 and advanced generations raised

1. F_1s raised

A total 18 F_1s involving agronomically good but susceptible/ highly susceptible genotypes (MAL13, MAL18, MAL 31, MA 3, MA 6, MAL 23, NDA 1, Bahar, Amar) and resistant donors (BWR 23, ICP 9174) were raised.

2 BC_1F_1 raised

The following F_1s were backcrossed with their recurrent parents and BC_1F_1were raised and selfed to procure seed for F_2 generation**:**

(a) (MAL 18 × ICP 9174) × MAL 18
(b) (MA 6 × BWR 23) × MA 6
(c) (MA 3 × BWR 23) × MA 3

3. F_2 raised

The following F_2s comprising of >1000 progenies each were raised:MAL 18 × BWR 23; MAL 18 × ICP 9174; MAL 13 × ICP 9174; MAL 13 × BWR 23; MA 6 × BWR 23; MA 6 × ICP 9174; MA 6 × KPL 43; MA 3 × BWR 23

4. F_3 raised

(a) MAL 13 × BWR 23(b) MA 3 × BWR 23(c) MAL 13 × ICP 9174
(d) MAL 13 × BWR 23 (e) MAL 13 × IPA 16 F

III. 78 microsatellite primers were selected from Odeny et al. (2007 and 2009). Initially, parental polymorphism survey was performed to select the polymorphic microsatellite primers which have to be used in mapping studies for *Fusarium* wilt resistance. For this, two highly wilt resistant parental genotypes, ICP 9174, BWR 23 and one highly susceptible line Bahar were used. Out of 78 microsatellite primers, 36 primers were screened and parental polymorphism survey with other primers is in progress (Fig. 6).

IV. The pathogen, *Fusarium udum* from fresh infected pigeonpea plant were collected from adjoining area and cultures were purified. The Pigeonpea genotypes were screened to re-confirm their reaction to *F. udum.*

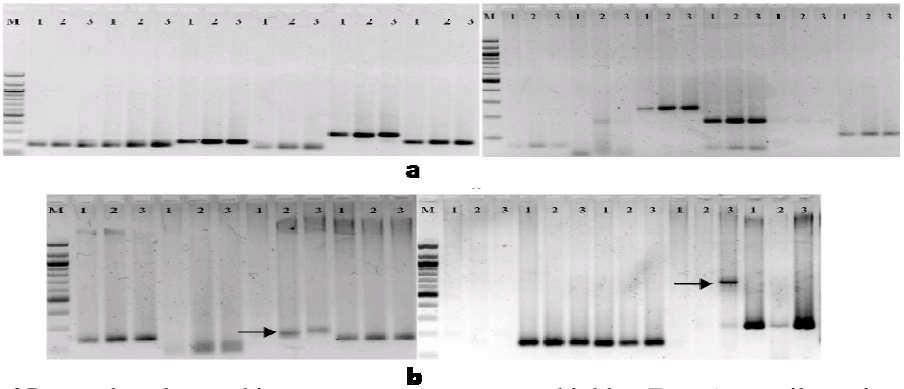


Fig. 6. Parental polymorphism survey among two highly *Fusarium* wilt resistant genotypes ICP 9174 (lane number 1), BWR 23 (lane number 2) and one highly susceptible genotype Bahar (lane number 3) with 34 primers. M is 100bp DNA size

(Year 2011-2012)

Targets

1. Identifying additional QTLs for traits of interest.
2. Pyramiding one or more of the genes/QTL for important traits in major crops having relevance to eastern Gangetic plains.
3. Use of molecular markers in F_2 and advanced breeding populations to continue.
4. Training to students, farmers and stake holders.

Achievements

(A) Wheat

1. A core collection of 310 wheat spot blotch resistant lines obtained from CIMMYT, Mexico have been maintained, and out of that 90 wheat lines have been reconstituted which are early in maturity as 'Sonalika' but having different levels of resistant to spot blotch. These 90 lines were grown in replicated trials and screened for spot blotch during Rabi season (2011-12) along with other yield and component traits.
2. RILs developed from Ning × Sonalika and Chiriya 3 × Sonalika are being maintained. QTLs associated with spot blotch resistance present on 2B (*QSB.bhu2B*) and 7D (*QSB.bhu7D*) chromosomes were validated. Molecular work on saturation of genetic region in between the flanking markers associated with spot blotch resistance is in progress in order to get some more closely linked markers.
3. Ten elite wheat lines (1st CSISA-SB 6720, 1st CSISA-SB 6723, 1st CSISA-S 6736, 1st CSISA-SB 6916, 1st CSISA-SB 6943, 2nd CSISA 6705, 2nd CSISA 6710, 2nd CSISA 6713, 2nd CSISA 6741 and 2nd CSISA 6745) which were identified as resistant to spot blotch were crossed with two high yielding but susceptible wheat varieties, HUW-234 and HUW-468 during Rabi 2010-11. These 20 F_1s were sown at IARI Regional Station, Wellington, Tamil Nadu and backcrossed with their respective recurrent parents i.e., HUW-234 and HUW-468.
4. Twenty BC_1F_1 progenies obtained from off-season (Wallington centre) were space-planted in Rabi season 2011-12 along with the parental lines (HUW-234 and HUW-468) for foreground and background selection at BHU centre. Leaf samples were collected from individual plants of each backcross for foreground selection using SSR markers associated with spot blotch resistance.

5. 6 BC_1F_1s *viz*., [(HUW-234 × 1st CSISA 6736) × HUW-234], [(HUW-234 × 2nd CSISA 6705) × HUW-234], [(HUW-234 × 2nd CSISA 6713) × HUW-234], [(HUW-468 × 1st CSISA 6916) × HUW-468], [(HUW-468 × 2nd CSISA 6705) × HUW-468] and [(HUW-468 × 2nd CSISA 6713) × HUW-468] which had good population size (> 120 individual plants) were finally selected for foreground selection for spot blotch resistance. Individual BC_1F_1 plants which were heterozygous for the SSR marker *Xgwm148* are confirmed to possess spot blotch resistance QTL *QSb.bhu-2B (*Fig. 7 and 8). The individual plants selected from 6 BC_1F_1s will be further backcrossed to increase the recovery of recurrent parent genome (validated through background selection) having resistance to spot blotch.

6. Some fresh crosses were also made during Rabi season 2011-12 between three highly resistant genotypes (CSISA-6705, CSISA-6713 and Frankolin) with new promising wheat lines (PBW-154, HUW-510, DBW 14, NW 2036 and DL 803-3). These lines were selected on the recommendations of AICRP on wheat. The F_1 seeds thus obtained will be sown as off-season crop at Wellington centre and backcrosses will be made with their respective recurrent parents. This new set of backcrossed material will be added to the ongoing marker assisted backcross breeding programme to develop high yielding cultivars with spot blotch resistance.

7. In year 2010-11, it was described that whole isolated population of *B. sorokiniana* was divided in to three distinct groups i.e. MAT-1, MAT-2 and mixed group containing MAT-1 and MAT-2 loci. Now, the amplified product of these loci were sequenced by ABI 3010 (Applied Biosystems automated sequence analyzer). All nucleotide sequences were blast using the NCBI server at http://www.ncbi.nlm.nih.gov/blast/Blast.cgi. BlastN analysis revealed that all the selected isolates showed 95-98% similarity with *B. Sorokiniana* which further strengthens the identity of the fungal isolates. The MAT sequences of *B. Sorokiniana* were deposited in GenBank under accession number mentioned in Table 1. The phylogenetic tree derived from MAT sequences of *B. Sorokiniana* isolates were constructed with 18 standard *B. Sorokiniana* MAT sequences obtained from Genebank. Result revealed that all selected isolates were positioned between the *Cochliobolus* genera and related species.

8. One of the component of slow disease development i.e. lesion size has been identified in the 90 wheat lines which were constituted on the basis of their maturity similar to 'Sonalika' variety, but having different levels of resistant to spot blotch. These 90 lines were grown in replicated trial and screened for spot blotch reaction during Rabi season (2011-12) along with other yield and its component traits. The lesion size has been found ranging from less than 0.50mm - more than 3.5mm. The 90 elite wheat lines were grouped into 5 categories depending upon spot blotch lesion size (Table 2).

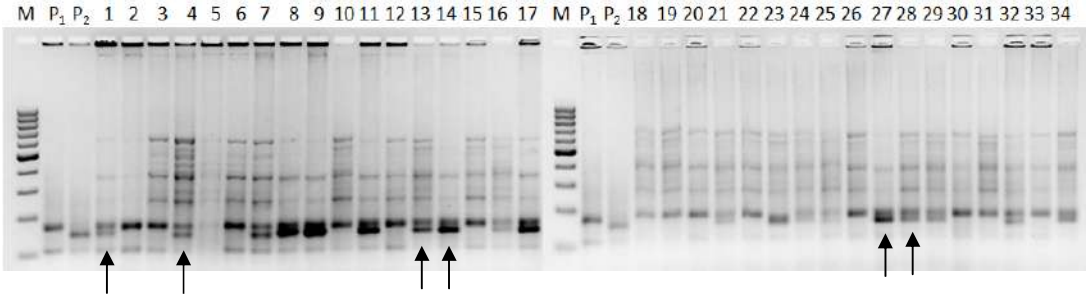


Fig.7. Foreground analysis of BC_1F_1 population derived from the cross [(HUW-234 × 2nd CSISA 6705) × HUW-234] with SSR marker *Xgwm148* associated with spot blotch resistance QTL *QSb.bhu-2B*. Lane M =100 bp DNA size marker; P_1= 2nd CSISA 6705 (resistant parent); P_2 = HUW-234 (susceptible parent) and 1-34 are individual backcross plants. *Arrow* indicates some of the heterozygous BC_1F_1 individual plants selected through

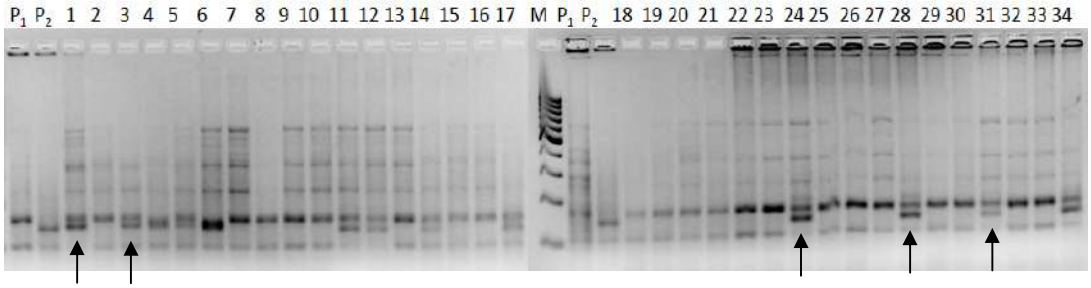
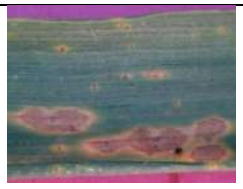
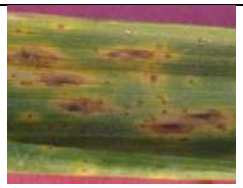


Fig.8. Foreground analysis of BC_1F_1 population derived from the cross [(HUW-468 × 2nd CSISA 6713) ×HUW-468] with SSR marker *Xgwm148* associated with spot blotch resistance QTL *QSb.bhu-2B*. Lane M =100 bp DNA size marker; P_1= 2nd CSISA 6713 (resistant parent); P_2 = HUW-468 (susceptible parent) and 1-34 are individual backcross plants. *Arrow* indicates some of the heterozygous BC_1F_1 individual plants selected through foreground selection.

Table 1. Sequenced strains of *B. sorokiniana* belonging to MAT-1 and MAT-2 group.

MAT-1			MAT-2		
S. No.	**Strains**	**Accession No.**	**S. No.**	**Strains**	**Accession No**
1.	B-11 (23)	JN128872	7.	T-10 (4)	JN128878
2.	HUW-510 (9)	JN128873	8.	B-8-3 (2)	JN128879
3.	S-10-4 (19)	JN128874	9.	B-1 (3)	JN128880
4.	NV (4)	JN128875	10.	B-7-2 (14)	JN128881
5.	S-11-3 (6)	JN128876	11.	T-2 (3)	JN128882
6.	HD3069	JN128877	12.	D-5- (5)	JN128883

Table 2. Categorization of lesion size in wheat infected by *B. sorokiniana*.

S. No.	Lesion photograph	Lesion size (mm)	Group
1.		Less than 0.5	1
2.		0.6 – 1.5	2
3.		1.6 – 2.5	3
4.		2.6 – 3.5	4
5.		More than 3.5	5

(B) Pigeonpea

The development of high yielding and wilt resistant genotypes is an important objective to enhance the productivity of pigeonpea. Some of the donor sources for resistance to wilt such as ICP 9174, BWR 23, IPA 8F, IPA 9F, IPA 16 F, KPL 43, BSMR 846, BSMR 301, BDN 2004-1, BDN 2001-9, BWR 133 have been used in crossing programme and for development of resistant genotypes through marker assisted selection (MAS).

i. A total 18 F_1s, generated by crossing agronomically good but susceptible/ highly susceptible genotypes to Fusarium Wilt (MAL13, MAL18, MAL 31, MA 3, MA 6, MAL 23, NDA 1, Bahar, Amar) and resistant genotypes (BWR 23, ICP 9174), were raised.
ii. The BC1F1s population obtained from three crosses viz., (MAL 18 × ICP 9174) x MAL 18, (MA 6 × BWR 23) × MA 6 and (MA 3 × BWR 23) × MA 3 were also raised.
iii. Fresh F1s were made during 2011-12 between wilt susceptible genotype MAL-34 and three wilt resistant genotypes BSMR-846, BDN-2029 and BWR-133.
iv. 12 backcrosses were attempted using both the parents to obtain BC1F1 seeds:
    [(MAL-13 × BSMR-846) × MAL-13]
    [(MAL-13 x BSMR-846) × BSMR-846]
    [(MA-6 × BSMR-846) × MA-6]
    [(MA-6 × BSMR-846) × BSMR-846]
    [(Bahar × BSMR-846) × Bahar]
    [(Bahar × BSMR-846) × BSMR-846]
    [(ICP-2376 × BSMR-846) × ICP-2376]
    [(ICP-2376 × BSMR-846) × BSMR-846]
    [(MAL-18 × BSMR-846) × MAL-18]
    [(Bahar × BSMR-846) × BSMR-846]
    [(MAL-33 × BSMR-846) × MAL-33]
    [(MAL-33 × BSMR-846) × BSMR-846]
v. 120 microsatellite primers were selected from different published reports of pigeonpea to perform parental polymorphism survey (Table 3). These primers were selected on the basis of high polymorphism information content (PIC) values. Two highly resistant genotypes ICP 9174, BWR 23 and one highly susceptible line 'Bahar' along with one moderately susceptible line MA-6 were used for initial screening of the polymorphic primers (Fig. 9). Out of 120 microsatellite primers, 90 primers have been screened so far. The SSR primers found polymorphic between these four genotypes were further used to screen a diverse set of 35 pigeonpea genotypes including some wild pigeonpea species (Table 4).

Dutta et al. (2011) developed 550 genic-SSR markers and then validated using deep transcriptome sequencing in pigeonpea. These genic-SSRs will provide an important genomic resource for diversity analysis and genetic mapping in pigeonpea, and were predicted to perform some specific function, e.g. marker ASSR-3 is predicted to perform Cytochrome P450 possessing cinnamate 4- hydroxylase activity and amplified a product size of approximately 145 bp.

Pigeonpea genotypes with an amplification product of approx. 145 bp were predicted to have Cytochrome P450 gene for cinnamate 4- hydroxylase activity (Fig. 10). Similarly, genic-SSR markers, ASSR-363 (unknown Protein) (Fig. 10), ASSR-148 (Ethylene-responsive transcription factor) and ASSR-352 (Major intrinsic protein) was amplified a band of 190, 110 and 130 bp, respectively (Fig. 11).

Table 3. Summary of SSR primers used for screening of 38 pigeonpea genotypes.

S. No.	SSR Primer Series	No. of primers selected	References
1.	ASSR	29	Dutta et al. (2011), BMC Plant Bio. 11:17
2.	ICPeM	15	Raju et al. (2010), BMC Plant Bio. 10: 45
3.	ICPM	11	Saxena et al. (2010), Plant Breed. 129: 142-148
4.	CCB, BMd	22	Datta et al. (2010), Physiol. Mol. Bio. Plants 16(2)
5.	CCtt	11	Odeny et al. (2009), BMC Res. Notes 2: 35
6.	SP	15	-do-
7.	-	17	Odeny et al. (2007), Plant Breed. 126: 130-136

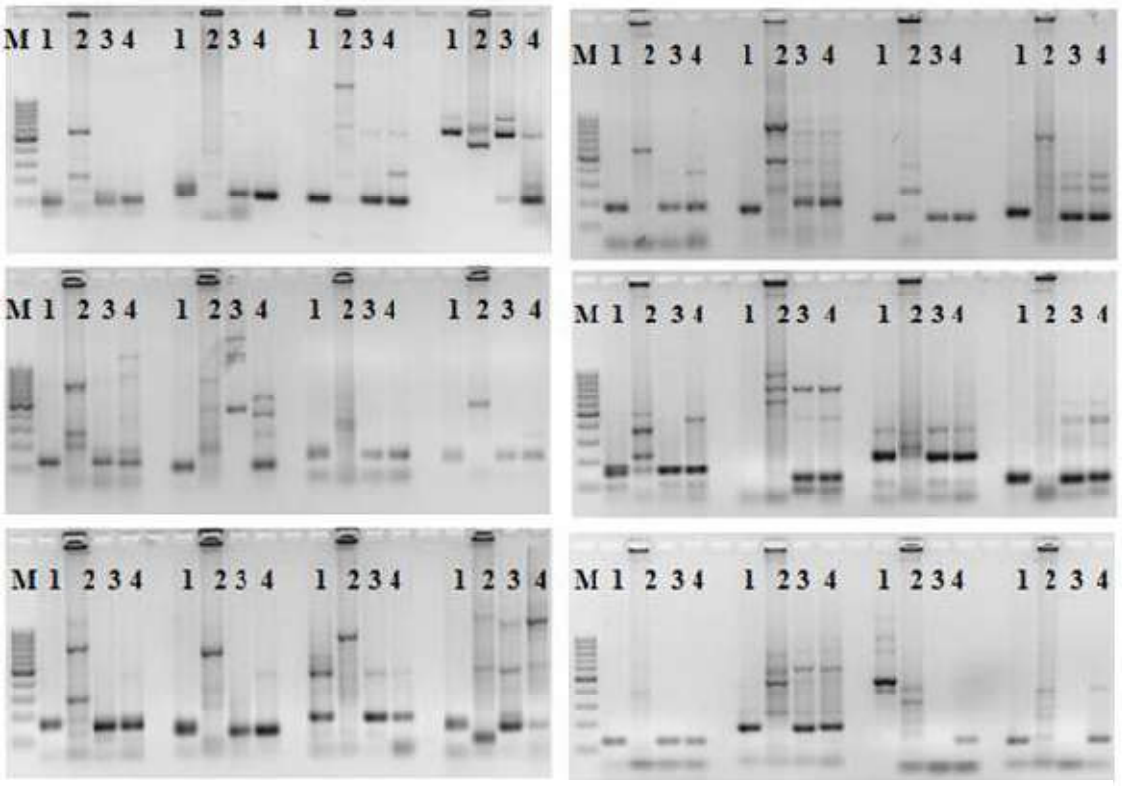


Fig. 9. Lane M, 100 bp DNA size marker; lane 1, FW resistant genotype ICP-9174; lane 2, FW resistant genotype BWR-23; lane 3, FW susceptible genotype Bahar and lane 4, FW moderately susceptible genotype MA-6.

Table 4. List of pigeonpea genotypes used for screening of SSR markers.

S. No.	Genotypes	Fusarium Wilt reaction*	Characteristic features
1.	Bahar	S	Compact, yellow flower, purple pod
2.	IPA-204	R	Semi compact, tall yellow flower, pod green with streaks
3.	KPL-43	R	Compact, yellow flower, purple pod
4.	BDN-2010	R	Semi-compact, reddish yellow flower with red base, purple pod
5.	BDN-2029	R	Semi compact, dark red flower, pod green with purplish streaks
6.	IPA-8F	R	Semi spreading, dark red flower, purple pod
7.	BDN-2001-9	R	Spreading, yellow flower, pod green with streaks
8.	IPA-234	HR	Compact, yellow flower, pod green with streaks
9.	ICP-9174	MR	Semi-Spreading, yellow flower, pod green with streaks
10.	BWR-23	HR	Flower red, pod green with streaks
11.	BSMR-846	HR	Semi compact, yellow flower with red streaks, pod green with streaks
12.	IPA-9F	R	Compact, light yellow flower with light red base, pod green with streaks
13.	IPA-16F	R	Spreading yellow flower, tall, pod green with streaks
14.	BDN-2004-1	R	Semi spreading, medium dwarf, purple flower, pod green with streaks, purple stem
15.	BWR-133	R	Semi spreading, yellow flower, pod green with streaks
16.	MAL-31	S	Semi spreading, light yellow flower, pod green with streaks, brown bold seed (14g/100 seeds)
17.	NDA-1	MS	Compact, yellow flower with purple veins, green pod
18.	ICP-9150	MR	Compact, purple stem, yellow flower, pod green with streaks
19.	Amar	MR	Compact, yellow flower, purple pod with constricted locule
20.	MAL-13	MR	Spreading, light yellow flower, pod green with streaks and constricted locule, resistant

S. No.	Genotypes	Fusarium Wilt reaction*	Characteristic features
			to sterility mosaic virus
21.	MA-6	MR	Semi-spreading, yellow flower, purple pod, highly resistant to sterility mosaic virus
22.	MAL-18	S	Spreading, yellow flower, purple pod, highly resistant to sterility mosaic virus
23.	ICP-2376	HS	Semi spreading , yellow flower, pod green with streaks
24.	LRG-41	MR	Semi spreading, purple pod
25.	BSMR-301	MR	-
26.	MAL-23	MS	Spreading, yellow flower, purple pod, susceptible to sterility mosaic virus
27.	MA-3	MS	Semi spreading, yellow flower, small green pod with streaks and constricted locules , small seeds (9g/ 100 seeds)
28.	MAL-34	MS	Spreading, red flower, pod green with streaks
29.	Deo-89	S	Compact, flower yellow with purple streaks
30.	PTH-2	S	Compact, red flower and pod, red large seeds, highly resistant to sterility mosaic virus
31.	JKM 7	-	-
32.	11887	-	-
33.	ICP 8860	-	-
34.	ICP 8863	S	highly resistant to sterility mosaic virus
35.	*C. scarabaeoides*	R	Trailing habit, small pods with small black seeds (less than 3g), resistant to pod fly and pod borer,
36.	*C. cajanifolius*	MS	Yellow flower, semi spreading, pod green, small black seeds
37.	*C. volubilis*	R	-
38.	*Rhynchosia species*	R	-

*Fusarium Wilt reaction, R = resistant; S = susceptible; MR = moderately resistant and MS = moderately susceptible.

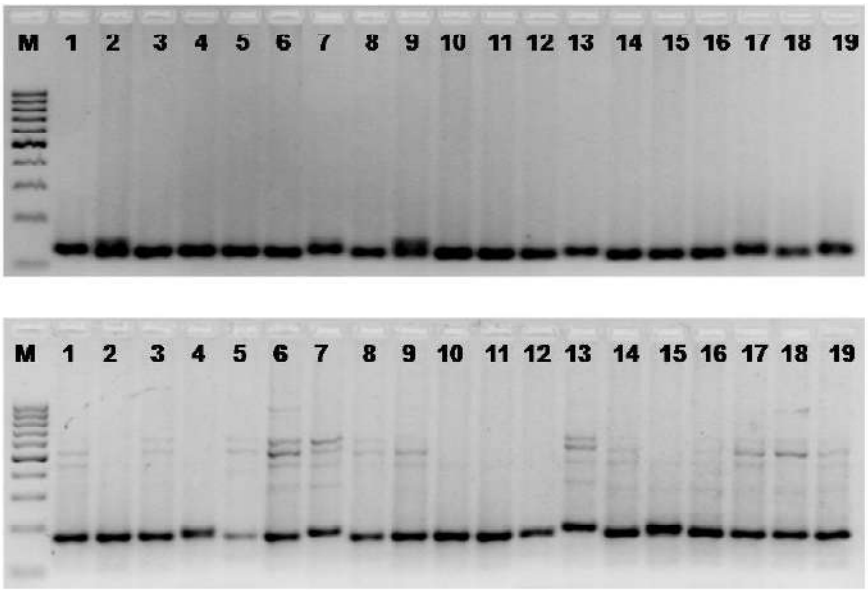


Fig.10. Lane M, 100 bp DNA size marker; lane 1-19 are pigeonpea genotypes differing as listed in table 4. Banding profile generated by pigeonpea SSR primers ASSR-3 (top) and ASSR-363 (bottom).

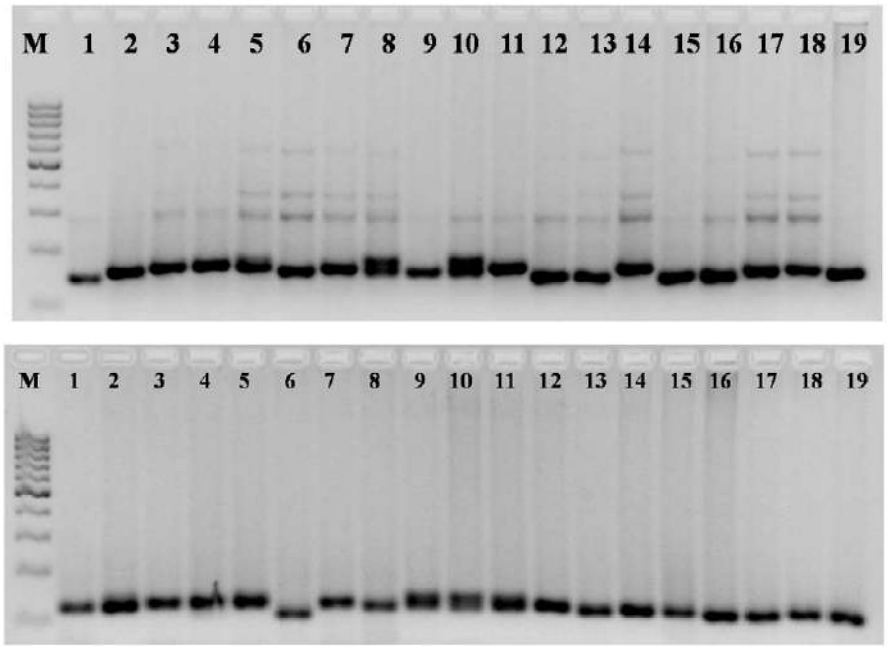


Fig. 11. Lane M, 100 bp DNA size marker; lane 1-19 are pigeonpea genotypes as listed in table 4. Banding profile generated by pigeonpea SSR primers ASSR-148 (top) and ASSR-352 (bottom).

(v) A total of 80 isolates of the pathogen, *Fusarium udum* collected from adjoining area to Varanasi were cultured and purified. These isolates were used for molecular diversity studies using 25 RAPD primers and 25 gene based markers. Genetic diversity analysis using gene-based primers are going on for categorization of identified Fusarium isolates on the basis of its functional role in pathogenesis e.g., NPS6 gene which encodes an enzyme nonribosomal peptide synthetase. The present population of *F. udum* was tested for the presence of this locus (*NPS6*) and result revealed that 22 isolates out of 80 were devoid of NPS6 gene (Fig. 9). The result obtained by different gene based primers will aid in determining the pathogenic ability of different isolates of *F. udum*.

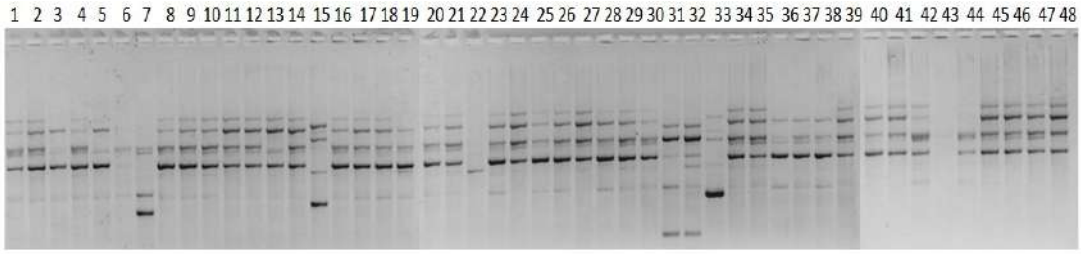


Fig. 12. PCR amplification in Fusarium isolates using a RAPD primer OPL-15. 1-48 are different *Fusarium* isolates.

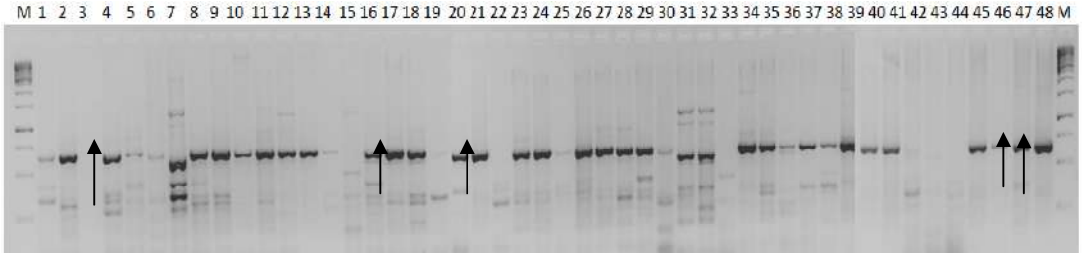


Fig.13. Genetic diversity studies in *Fusarium* isolates using a gene-based primer NPS6. M = 100 bp DNA size marker; 1-48 are different *Fusarium* isolates. *Arrow* indicated some of the isolates devoid of *NPS6* gene activity.

(Year 2012-2013)

Targets

1. Marker assisted selection in backcross populations of wheat
2. Characterization of components of slow spot blotch disease development
3. Standardization of RNA isolation from wheat leaves and pigeonpea roots
4. Interaction studies between *Bipolaris* isolates and wheat genotypes
5. Phenotyping of the F_2 and backcross population of pigeonpea against *Fusarium* wilt
6. Customization of wheat and pigeonpea microarray

Achievements

(A) WHEAT

The main goal of NAE program at BHU centre is introgression of major QTLs for spot blotch resistance into the popular varieties HUW-234 and HUW-468 through integrated classical breeding and marker aided selection (MAS) approach. In addition to this, newly identified superior genotypes (DBW 14, NW 2036, DL 803-3 and HUW-510) were also included during 2011-2012 for the improvement of spot blotch resistance through MAS. Whole genome expression analysis using wheat microarray have been designed and wheat microarray were procured which will enable us for better understanding of the mechanism of spot blotch resistance. The progress made in wheat during year 2012-2013 is briefly described as under:

1. For the introgression of spot blotch QTLs into recipient parents, HUW-234 and HUW-468 from donors 2nd CSISA 6705, 2nd CSISA 6713, 1st CSISA 6736 and 1st CSISA 6916; six backcross breeding populations *viz*., [(HUW-234 × 1st CSISA 6705) × HUW-234], [(HUW-234××x 2nd CSISA 6713) × HUW-234], [(HUW-234 × 2nd CSISA 6736) × HUW-234], [(HUW-468 × 1st CSISA 6916) × HUW-468], [(HUW-468 × 2nd CSISA 6705) × HUW-468] and [(HUW-468 × 2nd CSISA 6713) × HUW-468] were developed. Out of six BC_2F_1 populations, two populations [(HUW-234× 2nd CSISA 6713) × HUW-234] and [(HUW-468 × 2nd CSISA 6705) × HUW-468] were finally selected for foreground selection for spot blotch QTLs depending on the parental polymorphism survey for background analysis and good population size (>200 individual plants).
2. In foreground analysis, individual BC_2F_1 plants of both the crosses were first tested with marker *Xgwm111* associated with QTL *QSb.bhu-7D* and +ve plants were identified. After that +ve plants were tested with marker *Xgwm148* associated with QTL *QSb.bhu-2B*.Plants heterozygous (+ve) for both SSR markers, *Xgwm111* and *Xgwm148*,were confirmed to possess both QTLs *QSb.bhu-7D* andQTL *QSb.bhu-2B* (Fig. 14 and Fig. 15), respectively. After morphological comparison of selected plants with their respective recurrent parents; and background analysis, a total of 8 and 6 plants were selected from BC_2F_1populations of HUW- 234 × 2nd CSISA 6713 and HUW-468 × 2nd CSISA 6705 crosses respectively (Table

5). Selected BC_2F_1 plants were further backcrossed to increase the recovery of recurrent parent genome having resistance to spot blotch.

Table 5. Summary of MAS program for the introgression of spot blotch resistance QTLs into HUW-234 and HUW-468 during 2012-2013.

S. No.	*BC_2F_1 population of cross	No. of plants +ve for marker *Xgwm111* associated with QTL *QSb.bhu-7D*	No. of plants +ve for marker *Xgwm148* associated with QTL *QSb.bhu-2B*	No. of plants selected for back crossing after morphological comparison and background analysis
1.	HUW- 234 × CSISA 6713	114 /240	52/114	8
2.	HUW-468 × CSISA 6705	136/270	58/136	6

***Recurrent:** HUW-234, HUW-468; **Donor:** CSISA 6713, CSISA 6705

3. During previous year (2011-2012), new set of material was added to the ongoing marker assisted backcross breeding programme to develop newly identified high yielding cultivars with spot blotch resistance. Some fresh crosses were made during Rabi season 2011-12 between three highly resistant genotypes (2nd CSISA 6713, PMBWR 4, 3rd CSISA DR 5207) with new promising wheat lines (PBW-154, DBW 14, NW 2036 and DL 803-3). These lines were selected from the list of recommended lines of AICRP on wheat. One line, HUW-510 of BHU was also included in new crossing program. The F_1 seeds thus obtained were sown as off-season crop at Wellington centre and backcrosses were made with their respective recurrent parents. Due to less number of seeds, the crosses of PBW-154 were not further carried. BC_1F_1 populations thus obtained were grown in Rabi season 2012-13 at Agriculture Research Farm, B.H.U., Varanasi.
4. The detail of the foreground selection with markers *Xgwm111* and *Xgwm148* and final selection of plants based on morphological comparison and background analysis in all the crosses are listed in table 6. The representative gel images of foreground selection with marker *Xgwm111* in three crosses are shown in Fig 14. The selected plants in each backcross population were further backcrossed with their respective recurrent parents.

Table 6. Summary of MAS program for the introgression of spot blotch resistance QTLs into HUW-510, PBW-154, DBW 14, NW 2036 and DL 803-3 during 2012-2013.

S. No.	*BC_1F_1 population of cross	No. of plants +ve for marker *Xgwm111* associated with QTL *QSb.bhu-7D*	No. of plants +ve for marker *Xgwm148* associated with QTL *QSb.bhu-2B*	No. of plants selected for BC after morphological comparison and background analysis
1.	HUW – 510 × 3rd CSISA DR 5207	72/158	34/72	11
2.	HUW – 510 × 2nd CSISA 6713	67/140	38/67	18
3.	DBW – 14 × 3rd CSISA DR 5207	82/170	46/82	7
4.	DBW – 14 × PMBWR 4	72/146	40/72	18
5.	DBW – 14 × 2nd CSISA 6713	68/156	28/68	8
6.	NW – 2036 × 2nd CSISA 6713	61/126	34/61	13
7.	NW – 2036 × PMBWR 4	36/80	21/36	7
8.	NW – 2036 × 3rd CSISA DR 5207	46/96	22/46	2
9.	DL 803-3 × 2nd CSISA 6713	56/118	26/56	10

***Recurrent:** HUW –510, DBW –14, NW – 2036, DL 803-3; **Donor:** 2nd CSISA 6713, PMBWR 4, 3rd CSISA DR 5207.

5. For whole genome expression analysis of resistant (Yangmai-6 and Chirya-3) and susceptible (Sonalika) genotypes against spot blotch pathogen, the genotypes were grown in glass house with two replications each for control and treatment. At flowering stage high humidity was maintained and artificial inoculation with spot blotch pathogen was done. Leaf samples from control and treated plants of each genotype were collected after 1h, 24h, 72h and one week of inoculation and stored at -80 °C. Wheat microarrays were procured to conduct the experiments on whole genome expression studies.

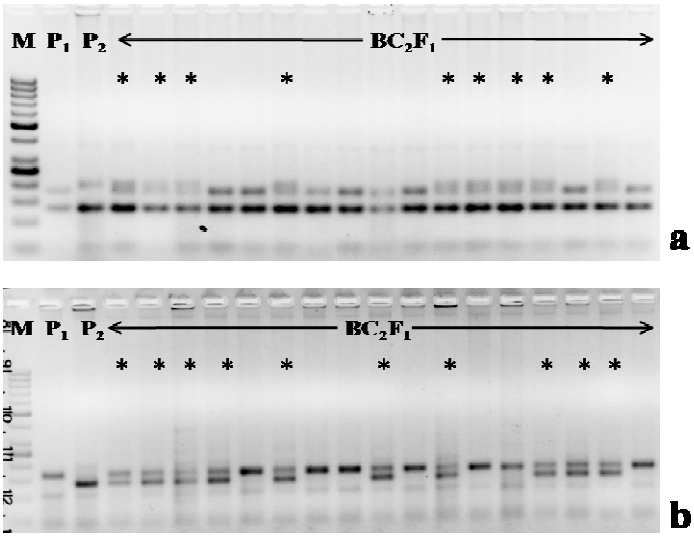


Fig.14. Foreground analysis of BC_2F_1 population derived from the cross [(HUW-234 × 2nd CSISA 6713) × HUW-234] with SSR markers (a) *Xgwm111* associated with QTL *QSb.bhu-7D* and (b) *Xgwm148* associated with QTL *QSb.bhu-2B*. Lane M =50 bp DNA size marker; P_1 = HUW-234 (recurrent parent); P_2= 2nd CSISA 6713 (donor parent) and BC_2F_1 are individual backcross plants. * indicates heterozygous BC_2F_1 individual plants selected through foreground selection.

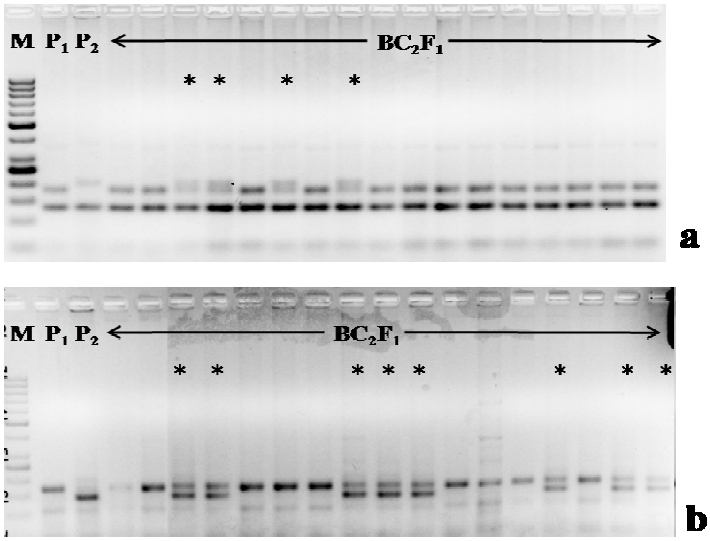


Fig.15. Foreground analysis of BC_2F_1 population derived from the cross [(HUW-468 x 2nd CSISA 6705) x HUW-468] with SSR markers (a) *Xgwm111* associated with QTL *QSb.bhu-7D* and (b) *Xgwm148* associated with QTL *QSb.bhu-2B*. Lane M =50 bp DNA size marker; P_1 = HUW-468 (recurrent parent); P_2= 2nd CSISA 6705 (donor parent) and BC_2F_1 are individual backcross plants. * indicates heterozygous BC_2F_1 individual plants selected through foreground selection.

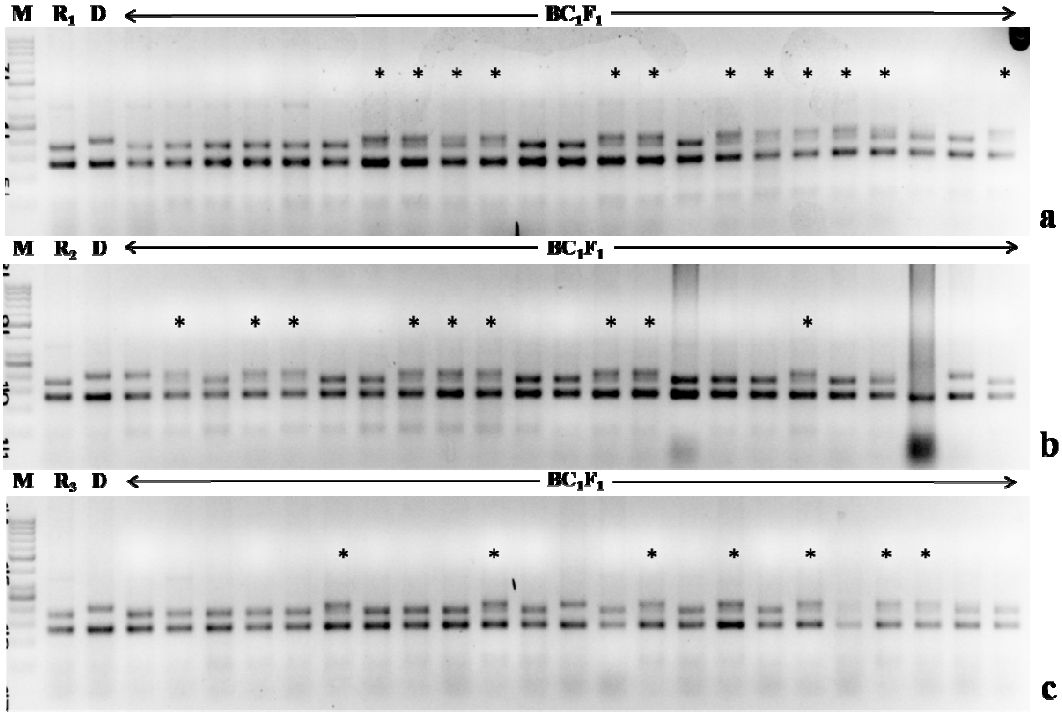


Fig.16. Foreground analysis of BC_1F_1 population derived from the crosses (a) (DBW-14 x 3rd CSISA DR 5207) x DBW-14 (b) (HUW-510 x 3rd CSISA DR 5207) x HUW-510 (c) (NW-2036 x 3rd CSISA DR 5207) x NW-2036 with SSR markers *Xgwm111* associated with QTL *QSb.bhu-7D*. Lane M =50 bp DNA size marker; R_1 = DBW-14, R_2= HUW-510, R_3 = NW-2036 (recurrent parent); D = HU 84 (donor parent) and BC_1F_1 are individual backcross plants. * indicates heterozygous BC_1F_1 individual plants selected through foreground selection.

6. The procedure of RNA isolation from infected wheat leaf samples was standardised. Total RNA was isolated by using the RIBOZOL™ RNA extraction reagent following manufacturer's instructions (Amresco, USA). Two replicate samples for each treatment were extracted and the quality of RNA samples was checked using 1.0% denaturing agarose gel (Fig. 17).

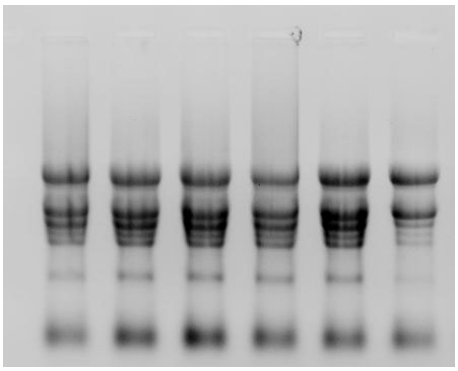


Fig.17. Quality analysis of RNA samples isolated from spot blotch infected wheat lines and their electrophoretic patterns in 1.0% denaturing agarose gel.

7. Cuticular waxes cover all aerial plant surfaces and form a protective barrier between a plant and its environment, which ultimately play an important role in plant resistance to a variety of biotic and abiotic stresses such as those caused by fungal pathogens. In an attempt to evaluate correlation of epicuticular wax content with resistance to spot blotch, previously reconstituted 90 wheat lines early in maturity as 'Sonalika' but having different levels of resistance to spot blotch, their leaves were sampled. The epicuticular wax of each genotype was measured and data of other yield related traits, *viz.*, days to flowering, plant height, AUDPC for spot blotch, biomass, plot yield and thousand grain weight were taken. Univariate statistical analysis showed that a large variation occurred in all traits including epicuticular wax content (Table 7). Highly significant negative correlation between epicuticular wax and AUDPC (Table 8) suggests that it may be a supporting trait for development of spot blotch resistance genotypes.

Table 7. Range, Mean ±SE and critical variation (%) in 90 wheat lines for seven quantitative traits.

Parameter	DF[@]	PH	AUDPC	BC	PY	TGW	Wax
Min.	61.00	76.0	254.94	875.0	156.0	27.24	0.33
Max.	83.00	117.0	1037.04	1600.0	721.0	43.72	0.74
Mean	76.01	91.98	570.08	1266.89	462.68	34.28	0.45
±SE	00.49	0.86	18.76	20.29	12.06	0.40	0.01
CV (%)	06.16	8.88	31.22	15.19	24.73	11.05	17.36

[@]DF = Days to 50% flowering; PH = Plant height; AUDPC = Area under disease progress curve; BC = Biomass content; PY = Plot yield (g); TGW = Thousand grain weight (g) and WC = Wax content (mg/cm^2).

Table 8. Pearson's correlation coefficient among seven quantitative traits in 90 wheat lines.

Trait	DF	PH	AUDPC	BC	PY	TGW
PH	0.245***					
AUDPC	-0.450***	0.031				
BC	0.260***	0.293***	-0.443***			
PY	0.106	-0.012	-0.552***	0.450***		
TGW	-0.094	0.162**	-0.302***	0.417***	0.416***	
WC	0.098	0.129*	-0.174**	0.094	0.169**	0.103

[@]DF = Days to 50% flowering; PH = Plant height; AUDPC = Area under disease progress curve; BC = Biomass content; PY = Plot yield (g); TGW = Thousand grain weight (g) and WC = Wax content (mg/cm^2).

*, **, *** Significant at P = 0.05, P = 0.01 and P = 0.001 respectively.

8. RILs developed from Ning × Sonalika and Chirya 3 × Sonalika are being maintained. Molecular work on saturation of genetic region in between the flanking markers associated with spot blotch resistance is in progress in order to get some more tightly linked markers.

(A) PIGEONPEA

The genetics of FW resistance is still not clear, and number of gene vary from a single dominant gene to two complementary genes and even involvement of multiple factors for resistance. In pigeonpea a fairly enough genomic resources are now being available including, a consensus genetic map comprising 339 loci spanning a distance of 1,059 cM with an average marker density of 3.1 cM (Bohra et al., 2012). A total of 156 SSR markers information was used until development of intra-specific consensus genetic map of Bohra et al. (2012). Advent of genomic resources will accelerate breeding in pigeonpea leading to development of several improved

cultivars/varieties with enhanced resistance/tolerance to biotic or abiotic stresses.Progress made in pigeonpea crop during 2012-13 is describes as below:

Genetic diversity studies

A set of 36 elite pigeonpea genotypes, that are adapted to different climatic zones with good agronomic performance, were evaluated for *Fusarium* Wilt (FW) including 22 FW resistant and moderately resistant genotypes and, 14 FW susceptible and moderately susceptible pigeonpea genotypes. These genotypes were screened in wilt sick plot at Agriculture Research Farm, Banaras Hindu University, Varanasi, India. To access the uniformity of disease incidence a row of susceptible check 'Bahar' was planted after every 10 rows. Four pigeonpea genotypes differing to their FW reactions *viz*., BWR-23 (resistant), ICP-9174 (moderately resistant), MA-6 (moderately susceptible) and Bahar (susceptible) were initially screened for polymorphism using pigeonpea SSR markers; finally 24 SSR markers found polymorphic among these four lines were used for diversity analysis across 36 genotypes (Table 9).

Table 9. Details of the polymorphic SSR markers used in the study.

S. No.	SSR marker	SSR motif	Expected size of PCR product (bp)	Observed size range (bp)	No. of alleles	PIC
1.	ASSR-1	$(GA)_{10}$	100	100-120	2	0.49
2.	ASSR-3	$(AGAAAG)_5$	145	140-175	2	0.45
3.	ASSR-8	$(AGA)_9$	140	120-140	3	0.40
4.	ASSR-23	$(CCTTCT)_5$	150	120-160	2	0.42
5.	ASSR-66	(CT)12	180	180-210	3	0.65
6.	ASSR-70	$(GGTAGA)_6$	170	170-200	2	0.50
7.	ASSR-77	$(CT)_{10}$	140	120-140	2	0.32
8.	ASSR-93	$(CATTTG)_5$	170	110-160	2	0.30
9.	ASSR-97	$(ATGGAC)_8$	150	110-200	4	0.55
10.	ASSR-148	(CAA)7	110	140-160	2	0.49
11.	ASSR-206	(GTAATA)6	150	150-170	3	0.50
12.	ASSR-228	(CTAAGG)5	140	100-140	3	0.42
13.	ASSR-229	(TAAGGG)5	160	150-160	2	0.60
14.	ASSR-277	(TCCTGT)5	130	110-150	3	0.44
15.	ASSR-281	(CAAATG)6	220	200-250	2	0.70
16.	ASSR-304	(GTT)7	110	110-140	2	0.51
17.	ASSR-317	$(GAGCAT)_9$	150	130-170	2	0.46
18.	ASSR-352	$(TTTAA)_5$	130	100-130	3	0.48
19.	ASSR-363	$(GCATCA)_5$	190	190-210	2	0.76
20.	ASSR-366	$(CGT)_8$	140	140-180	3	0.70
21.	ASSR-379	$(TTCATG)_5$	140	140-170	3	0.62
22.	ASSR-390	$(GAGCAA)_6$	190	190-210	2	0.60
23.	ASSR-495	(CT)9	200	130-160	3	0.50
24.	ASSR-610	(GTG)6	150	140-150	2	0.49
		Average	**150**	**137-169**	**2.46**	**0.52**

These 24 polymorphic SSR markers yielded a total of 59 polymorphic bands, the number of polymorphic bands per primer ranged from 2 to 4, the average being 2.46 (Table 9). Markers ASSR-363, ASSR-281 and ASSR-366 were the most informative primers on the basis of highest polymorphic information content (PIC) of 0.76, 0.70 and 0.70, respectively. SSR marker ASSR-93 showed least PIC value of 0.30 (Table 9). Gel image obtained from banding profile of SSR marker ASSR-352 is presented in Figure 5. In the UPGMA dendrogram 36 pigeonpea genotypes were grouped into two main clusters consisted of 15 and 21 genotypes, respectively (Fig. 19). Cluster I was further divided into three sub-clusters *viz*., Ia (4 genotypes: Bahar, IPA-8F, JKM-7, MA PHT-2), Ib (10 genotypes: IPA-16F, MA-3, MAL-23, BWR-133, MAL-31, MAL-13, MA-6, MAL-34, MAL Deo-89 and ICP-11887) and Ic consisted of only one pigeonpea genotype i.e., IPA-9F. Cluster II was also divided into three sub-clusters, sub-cluster IIa consisted of 10 genotypes *viz*., IPA-204, KPL-43, BDN-2010, BDN-2004-1, ICP-9150, BDN-2001-9, NDA-1, Amar, ICP-2376, KPBR-80-2-1. While, sub-cluster IIb consisted of 8 genotypes (BDN-2029, BSMR-301, IPA-234, ICP-9174, BWR-23, BSMR-846, LRG-41 and ICP-7200) and sub-cluster IIc contains only three genotypes, namely, MAL-18, MAL-23 and ICP-8862 (Fig. 19).

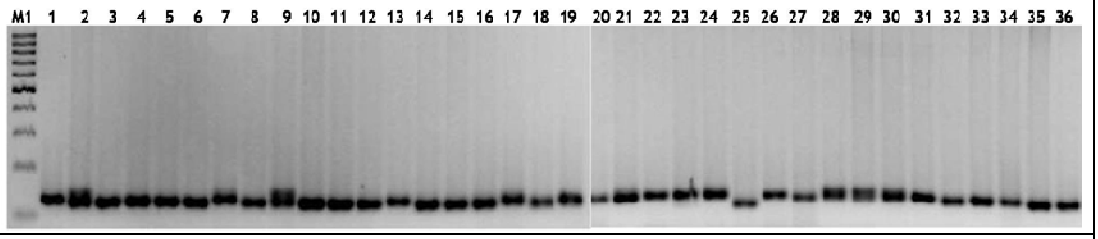


Fig.18. SSR banding profile obtained by ASSR-352 marker. 1-36 are different pigeonpea genotypes; M1= 100 bp DNA size marker.

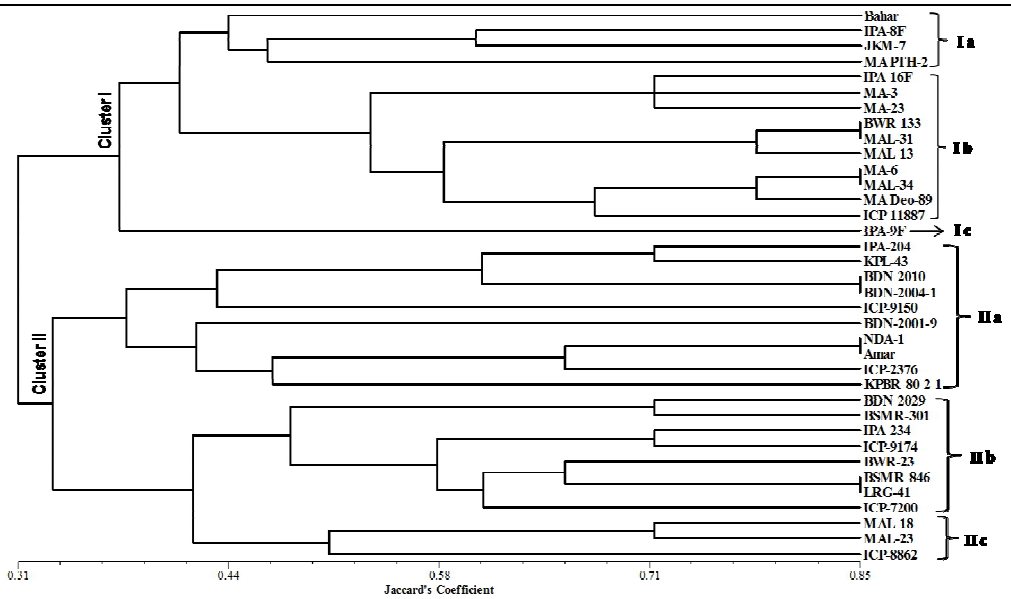


Fig. 19. Dendrogram of thirty six pigeonpea genotypes based on Jaccard's similarity coefficients of 24 polymorphic SSR markers.

SSR markers association with FW resistance

In order to determine the association of a particular SSR marker to the respective phenotype, the genotypic data generated by 24 polymorphic SSR markers were subjected to Kruskal-Wallis one-way analysis of variance (K-W ANOVA) by using the marker and the respective phenotypic data. K-W ANOVA detected the significant association of six SSR markers *viz*., ASSR-1, ASSR-23, ASSR-148, ASSR-229, ASSR-363 and ASSR-366 with *Fusarium* wilt resistance (Table 10). The same six markers also showed significant association in simple regression analysis owing to higher R^2 values and significant deviation of b value from zero. Among the six markers, ASSR-363 explained a maximum of 56.4% (*b* value = 1.86; $P< 0.01$) of phenotypic variation due to FW resistance. The phenotypic variation explained by these markers ranged from 23.7 to 56.4%. The markers identified through K-W ANOVA were also confirmed with simple regression analysis so,these markers could be utilized in the marker assisted breeding program for *Fusarium* wilt resistance in pigeonpea.

Table 10. Association of SSR markers with FW resistance based on Kruskal-Wallis one-way ANOVA and Simple regression analysis.

SSR markers	Kruskal-Wallis ANOVA		Simple Regression Analysis	
	HC value	p-value	R^2	b value
ASSR-1	8.19	0.042	37.5	1.47**
ASSR-23	8.11	0.044	34.3	1.28*
ASSR-148	7.87	0.50	23.7	1.12*
ASSR-229	9.91	0.98	35.2	1.30*
ASSR-363	11.54	0.991	56.4	1.86**
ASSR-366	10.75	0.99	41.1	1.58**

*, ** Significant at 5 and 1 % level, respectively.

Genetics of Fusarium wilt (FW) resistance

Genetics of FW resistance in pigeonpea is still unclear as reports varied from multiple factors to involvement of a single dominant gene to two complementary genes. A thorough knowledge of the genetics of FW resistance will be useful in initiating an effective resistant breeding programme to develop FW resistant pigeonpea cultivars.Genetics of FW resistance was studied using nine F_2 populations of pigeonpea developed from crosses between three susceptible genotypes MA-6, MAL-13 and MAL-18 with three resistant genotypes BSMR-846, BWR-23 and BDN-2029. Inheritance study showed digenic (one dominant and one recessive gene), trigenic dominant and monogenic dominant control of resistance in different crosses as the 2 analysis of the different segregating F_2 populations did not deviate significantly from the expected ratios of 13:3, 63:1 and 3:1, respectively (Table 11). Resistant parent BSMR-846 possessed one dominant

and one recessive gene for FW resistance. Whereas, resistant parents BWR-23 and BDN-2029 possessed three and one dominant genes, respectively for FW resistance.

Table 11. FW reaction of nine F_2 populations of pigeonpea derived from crosses between three resistant and three susceptible genotypes.

Cross	F_1	No. of F_2 plants		Total	Expected ratio (R:S)	χ^2 value	P-value
		R	S				
MA-6 × BSMR-846	R	136	40	176	13:3	1.83	0.176
MAL-13 × BSMR-846	R	185	30	215	13:3	3.07	0.080
MAL-18 × BSMR-846	R	145	27	172	13:3	0.96	0.327
MA-6 × BWR-23	R	181	07	188	63:1	5.42*	0.020
MAL-13 × BWR-23	R	148	5	153	63:1	1.36	0.244
MAL-18 × BWR-23	R	132	5	137	63:1	0.60	0.438
MA-6 × BDN-2029	R	67	27	94	3:1	0.69	0.409
MAL-13 × BDN-2029	R	141	56	197	3:1	1.33	0.248
MAL-18 × BDN-2029	R	98	34	132	3:1	0.04	0.841

Involvement of one to three major genes governing FW resistance suggested the use of these resistant sources in breeding programs. Three parental genotype combinations *viz.*, Bahar × BSMR-846, MA-6 × BDN-2029 and MAL-18 × BDN-2029 (F_2 population) are selected for mapping of the FW resistance with the available pigeonpea SSR markers. Dutta et al. (2012) revealed that random sets of genic and genomic SSR markers have shown very low (usually less than 5%) level of polymorphism among the pigeonpea varieties in molecular diversity and trait mapping studies. They suggested a set of 370 validated genomic HASSR markers, which will be very useful for genetic diversity analysis, DNA fingerprinting and QTL mapping studies in pigeonpea due to their high level of polymorphism.

(2013-14)

Targets

1) Marker assisted selection in advanced backcross populations (BC_3F_1) of wheat for spot blotch resistance.
2) Phenotypic evaluation of individual BC_3F_3 plants obtained from crosses involving HUW-234 and HUW-468 as recipient parents.
3) Phenotyping of the F_2 and backcross population of pigeonpea against *Fusarium* wilt.
4) Validation of SSR markers associated with *Fusarium* wilt resistance.

Achievements

(A) WHEAT

1. In foreground analysis, individual BC_2F_1 plants (backcrossing done on selected individual BC_1F_1 plants in previous year i.e., 2012-13) of the following crosses (Table 12) were first tested with marker *Xgwm111* associated with QTL *QSb.bhu-7D* and +ve plants were identified. After that, these +ve plants were tested with marker *Xgwm148* associated with QTL *QSb.bhu-2B*.Plants heterozygous (+ve) for both the SSR markers viz., *Xgwm111* and *Xgwm148* confirmed to possess both the QTLs *QSb.bhu-7D* andQTL *QSb.bhu-2B*. Foreground analysis with SSR marker *Xgwm148* in BC_2F_1 of the crosses NW-2036 × HU 33) × NW-2036 and (DL 803-3 × HU 33) × DL 803-3 has been shown in figure 20 and figure 21, respectively.After morphological comparison of selected plants with their respective recurrent parents and background analysis, finally selected plants from BC_2F_1populations of the eight crosses were further backcrossed to increase the recovery of recurrent parent genome having resistance to spot blotch. At present, BC_3F_1 seeds of these crosses are in hand for future work.

Table 12. Marker assisted backcross breeding for introgression of spot blotch resistance in wheat genptypes DBW-14, NW-2036 and DL 803-3.

S. No.	Name of backcross population	Code name of the BC population	No. of BC_2F_1 plants selected on the basis of *xgwm111*	No. of BC_2F_1 plants selected on the basis of *xgwm148*	No. of BC_2F_1 plants finally selected[#]
1.	(DBW-14 × HU 42) × DBW-14	WB 1	36/84	14/36	08
2.	(DBW-14 × HU 84) × DBW-14	WB 2	68/145	33/68	21
3.	(NW-2036 × HU 42) × NW-2036	WB 3	81/147	24/81	17
4.	(NW-2036 × HU 84) × NW-2036	WB 4	44/106	18/44	12
5.	(DL 803-3 × HU 42) × DL 803-3	WB 5	121/301	45/121	18
6.	(DL 803-3 × HU 84) × DL 803-3	WB 6	65/115	21/65	15
7.	(NW-2036 × HU 33) × NW-2036	WB 9	187/385	68/187	21
8.	(DL 803-3 × HU 33) × DL 803-3	WB 12	175/340	51/175	14

[#]selected on the basis of background selection and phenotypic comparison with recurrent parent.

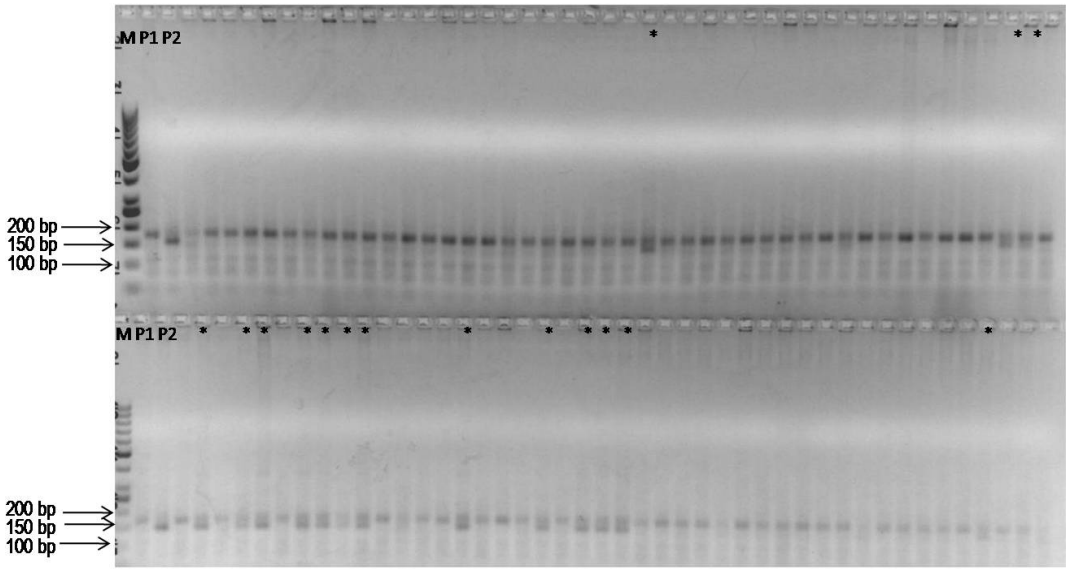


Fig. 20. Foreground analysis of BC_2F_1 population derived from the cross [(NW-2036 × HU 33) × NW-2036] with SSR marker *Xgwm148* associated with spot blotch resistance QTL *QSb.bhu-2B*. Lane M =50 bp DNA size marker; P_1 = NW-2036 (recurrent parent); P_2= HU 33 (donor parent) and rest are individual BC_2F_1plants. * indicates heterozygous BC_2F_1 individual plants selected through foreground selection.

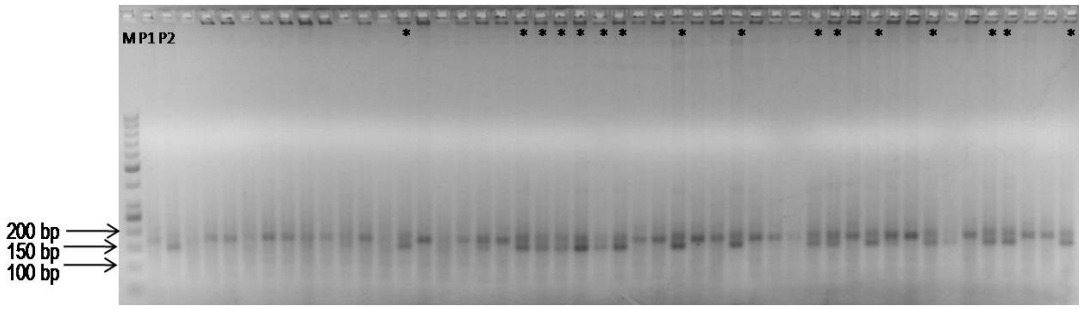


Fig. 21. Foreground analysis of BC_2F_1 population derived from the cross [(DL 803-3 × HU 33) × DL 803-3] with SSR marker *Xgwm148* associated with spot blotch resistance QTL *QSb.bhu-2B*. Lane M =50 bp DNA size marker; P_1 = DL 803-3 (recurrent parent); P_2= HU 33 (donor parent) and rest are individual BC_2F_1plants. * indicates heterozygous BC_2F_1 individual plants selected through foreground selection.

2. During 2012-13, eight and six individual BC_2F_1 plants were selected (on the basis of FG, BG, artificial disease screening and morphological traits) from (HUW 234 × CSISA 6713) × HUW 234 and (HUW 468 × CSISA 6705) × HUW 468 crosses, respectively. Seeds from each of the selected plants were grown at the Wheat research station, Wellington, Tamil Nadu for generation advancement to obtain BC_2F_2 plants. Individual ($BC_2F_{2:3}$) plants were harvested separately. A total of 147 BC_2F_3 progenies were grown at main centre BHU during Rabi season 2013-14. Data were collected on 24 morphological traits mentioned under wheat DUS testing along with spot blotch disease screening. Some of the BC_2F_3 progenies showed segregation for the trait of interest. 32 and 41 progenies were selected from the two BC populations having similarity to their respective recurrent parents i.e., HUW 234 and HUW 468, respectively (Table 13 and 14). These selected plants will be subjected to background profiling and further selection.

Numerical coding of the different trait measured in the present study

Trait	Numerical Code				
	1	**2**	**3**	**4**	**5**
EGH	Erect	Semi erect	Intermediate	Semi-spread	Spread
AP	Dark purple	Purple	Green	-	-
APub	Strong	Moderate	Absent	-	-
FLA	Erect	Semi erect	Semi drooping	drooping	-
WLS	Absent	weak	medium	strong	very strong
WLB	Absent	weak	medium	strong	very strong
WP	Absent	weak	medium	strong	very strong
WE	Absent	weak	medium	strong	very strong
FC	Dark green	green	pale green	-	-
PT	Solid	medium	hollow	-	-
POG	Absent	medium	strong	very strong	
DM	Very early	Early	Medium	Late	Very late
EC	White	brown	dark brown	-	-
ED	Very lax	lax	medium	long	very long
AL	Very short	Short	Medium	Long	Very long
AC	White	Brown	Dark brown	Black	-

Table 13. Morphological and protein content of selected BC_2F_3 progenies developed from the marker assisted backcross breeding for spot blotch resistance using HUW 234 as recurrent parent.

S. No.	Line	EGH	AP	APub	FLA	FLL	FLB	DH	WLS	WLB	WP	WE	FC	PT	POG	DM	EC	ED	SL	SPE	AL	AC	PH	Protein
1.	HUW 234-8-16-1-1	4	3	3	3	22.33	2.12	4	4	2	4	1	2	1	2	4	2	3	7	19	4	3	74	13.8
2.	HUW 234-8-16-2-1	1	3	3	3	22.01	1.80	2	3	3	3	1	1	1	2	3	2	4	10	18	5	3	66	13.5
3.	HUW 234-8-16-2-2	2	3	3	3	20.11	2.00	3	3	4	3	1	2	1	2	3	2	4	8	17	5	3	67	13.6
4.	HUW 234-8-16-4-1	3	3	2	2	20.17	1.67	3	3	2	3	2	3	2	3	2	3	2	9	17	4	2	78	13.4
5.	HUW 234-8-16-7-1	1	3	2	1	20.00	2.00	1	2	2	2	2	2	3	2	2	2	3	8	19	5	2	71	12.0
6.	HUW 234-8-16-7-2	1	3	1	2	19.00	2.00	1	3	2	2	2	2	1	3	1	3	4	9	21	5	1	70	12.8
7.	HUW 234-8-16-9-1	2	3	3	2	19.50	2.00	1	3	2	3	2	1	1	3	1	2	3	9	19	4	2	69	12.3
8.	HUW 234-8-16-9-2	1	3	2	2	20.00	2.00	1	3	2	2	2	1	1	2	1	2	3	1	21	4	2	68	12.1
9.	HUW 234-8-16-10-1	3	3	2	3	19.00	1.80	2	4	2	3	2	2	3	2	1	2	4	10	18	5	2	84	12.8
10.	HUW 234-8-16-10-2	3	3	2	3	20.00	2.00	2	3	2	2	1	2	3	2	1	2	4	10	19	5	2	82	12.3
11.	HUW 234-8-16-11-1	1	3	2	2	25.00	2.20	3	4	4	3	2	1	2	3	2	2	4	9	17	4	2	88	13.5
12.	HUW 234-8-16-11-2	2	3	2	3	23.12	2.10	3	4	3	3	2	1	2	2	2	3	4	10	17	4	2	88	13.0
13.	HUW 234-8-16-11-3	2	3	2	3	23.33	2.10	3	3	2	2	2	1	2	2	2	2	4	10	19	4	2	89	12.9
14.	HUW 234-8-16-12-1	4	3	2	3	20.00	2.00	2	3	2	3	2	1	2	2	1	2	4	9	20	4	1	84	12.9
15.	HUW 234-8-16-12-2	5	3	2	2	20.00	2.10	1	3	2	2	1	1	2	2	1	3	4	10	21	5	1	82	13.5
16.	HUW 234-8-16-13-1	1	3	2	2	20.60	1.90	2	2	2	2	2	1	1	4	1	3	4	10	18	3	1	71	12.7
17.	HUW 234-8-16-13-2	1	3	2	2	20.00	1.67	2	2	1	2	1	1	1	3	1	3	4	10	19	3	1	72	12.4
18.	HUW 234-8-16-14-1	4	3	2	2	20.00	1.53	1	3	2	2	2	3	1	2	2	1	3	10	17	4	2	87	11.9
19.	HUW 234-8-16-14-2	5	3	2	2	18.16	1.67	1	3	2	2	2	3	2	2	2	1	4	9	19	4	2	88	12.3
20.	HUW 234-8-16-15-1	3	3	2	3	18.00	1.71	4	3	2	3	1	3	1	3	1	3	4	9	19	4	3	63	13.4
21.	HUW 234-8-16-15-2	3	3	2	3	19.17	1.86	4	3	2	3	2	3	1	2	1	3	4	10	21	4	3	62	12.6
22.	HUW 234-8-16-17-1	3	3	2	2	18.50	1.50	1	2	1	2	2	2	3	2	2	2	4	10	17	3	2	92	12.8
23.	HUW 234-8-16-17-2	2	3	2	2	20.33	1.63	1	2	1	2	1	2	3	2	2	2	3	11	19	3	2	91	13.3
24.	HUW 234-8-16-18-1	2	3	2	2	19.00	2.00	1	3	2	2	1	2	1	2	2	2	4	11	17	3	1	84	12.8

S. No.	Line	EGH	AP	APub	FLA	FLL	FLB	DH	WLS	WLB	WP	WE	FC	PT	POG	DM	EC	ED	SL	SPE	AL	AC	PH	Protein
25.	HUW 234-8-16-18-2	3	3	2	2	23.00	2.10	1	3	3	3	2	2	1	2	2	2	4	11	17	3	1	83	12.9
26.	HUW 234-8-16-19-1	2	3	2	2	19.87	1.90	3	3	2	3	1	1	1	4	2	2	4	10	19	4	2	8	12.5
27.	HUW 234-8-16-19-2	2	3	2	2	18.67	1.90	3	2	2	2	2	1	1	3	1	1	4	11	19	4	2	79	13.3
28.	HUW 234-8-16-19-3	1	3	2	2	20.00	1.90	3	2	2	2	1	2	2	2	1	1	3	10	17	4	2	81	12.9
29.	HUW 234-8-16-20-1	2	3	1	2	17.50	1.80	2	3	3	2	1	3	2	4	2	3	4	11	21	3	2	80	13.3
30.	HUW 234-8-16-20-2	2	3	2	2	19.33	1.72	2	3	2	2	1	2	2	3	2	3	4	10	19	3	2	82	14.1
31.	HUW 234-8-16-21-1	4	3	2	2	22.50	1.80	2	3	2	2	1	3	2	2	2	3	4	10	19	3	2	73	13.9
32.	HUW 234-8-16-21-2	4	3	2	3	19.00	1.77	3	3	1	2	1	3	2	2	2	3	4	10	19	3	2	72	13.2
	HUW 234	2	3	2	3	21.67	2.05	1	3	2	2	2	3	2	2	2	2	4	10	19	4	2	88	

EGH = Early growth habit; AP = Auricle pigmentation; APub = Auricle pubescence; FLA = Flag leaf attitude; FLL = Flag leaf length; FLB = Flag leaf breadth; DH = Days to heading; WLS = Waxiness of leaf sheath; WLB = Waxiness of leaf blade; WP = Waxiness of peduncle; WE = Waxiness of ear; FC = Foliage colour; PT = Pith; POG = Pubescence of outer glume; DM = Days to maturity; EC = Ear colour; ED = Ear density; SL = spike length(cm); SPE = No. of spikelets per ear; AL = Awn length; AC = Awn colour and PH = Plant height (cm).

Table 14. Morphological and protein content of selected BC_2F_3 progenies developed from the marker assisted backcross breeding for spot blotch resistance using HUW 468 as recurrent parent.

S. No.	Line	EGH	AP	APub	FLA	FLL	FLB	DH	WLS	WLB	WP	WE	FC	PT	POG	DM	EC	ED	SL	SPE	AL	AC	PH	Protein
1.	HUW 468-16-22-1-1	2	3	2	3	23.50	1.50	4	2	3	1	1	2	2	2	3	2	3	8	17	4	2	81	13.8
2.	HUW 468-16-22-1-2	1	3	2	3	21.33	1.53	3	2	3	2	1	1	1	2	3	2	3	9	19	4	2	79	14.0
3.	HUW 468-16-22-2-1	2	3	3	3	20.10	1.53	3	3	3	2	1	1	1	2	2	1	4	10	17	3	1	90	13.6
4.	HUW 468-16-22-2-2	3	3	3	3	2t6.50	1.55	3	3	3	2	2	2	2	2	2	1	4	10	17	3	1	89	12.7
5.	HUW 468-16-22-5-1	3	3	2	2	21.50	1.50	3	3	3	2	2	1	1	3	1	2	4	11	19	4	2	88	14.6
6.	HUW 468-16-22-5-2	2	3	3	2	19.01	1.53	3	2	3	2	1	1	2	3	1	2	4	11	19	4	2	86	15.4
7.	HUW 468-16-22-5-3	1	3	3	3	23.50	1.50	2	2	3	2	1	1	2	3	1	2	3	11	21	4	2	88	14.3
8.	HUW 468-16-22-5-4	1	3	2	3	19.87	1.58	1	3	3	2	2	2	1	3	1	2	4	10	17	3	2	90	14.0
9.	HUW 468-16-22-14-1	1	3	1	1	22.33	1.68	1	3	3	2	1	2	2	2	1	2	3	11	19	2	3	82	13.5
10.	HUW 468-16-25-1-1	3	3	2	4	21.00	1.50	5	3	2	3	2	2	1	4	5	2	4	9	19	5	2	81	13.9
11.	HUW 468-16-25-1-2	4	3	2	3	22.50	1.62	4	4	2	3	1	1	1	4	5	2	4	10	19	5	1	80	13.4
12.	HUW 468-16-25-3-2	2	3	2	2	18.25	1.67	1	3	2	2	2	1	2	2	3	2	4	10	19	3	1	82	13.3
13.	HUW 468-16-25-4-1	2	3	2	1	20.33	1.50	3	3	1	1	2	1	2	2	3	1	4	10	19	4	2	89	12.9
14.	HUW 468-16-25-4-2	2	3	3	1	20.50	1.73	1	3	1	1	2	1	1	3	2	1	4	10	19	4	2	87	13.3
15.	HUW 468-16-25-6a-1	2	3	2	2	18.00	1.58	1	4	3	2	2	3	3	2	1	2	3	10	19	3	2	81	14.2
16.	HUW 468-16-25-6a-2	1	3	2	1	17.32	1.52	1	3	3	2	2	2	1	3	1	2	3	10	19	3	2	82	13.4
17.	HUW 468-16-25-6a-3	2	3	2	2	18.00	1.55	2	3	2	2	1	1	2	2	1	2	3	11	21	2	2	80	13.6
18.	HUW 468-16-25-6B-1	2	3	2	3	20.72	1.62	3	3	3	2	1	1	1	2	3	2	4	10	17	5	2	79	12.8
19.	HUW 468-16-25-6B-2	3	3	2	3	19.85	1.67	3	3	3	2	1	1	1	2	2	2	4	11	19	5	2	80	13.7
20.	HUW 468-16-25-6B-3	4	3	2	3	18.12	1.72	3	3	3	2	2	1	2	2	3	2	3	9	17	4	2	77	13.1
21.	HUW 468-16-25-6B-4	4	3	2	4	21.92	1.71	3	3	2	2	2	1	2	2	3	1	4	11	19	4	2	76	13.6
22.	HUW 468-16-25-7-1	5	3	1	4	23.00	1.73	4	2	2	2	1	3	2	2	4	1	4	9	19	5	2	81	12.8
23.	HUW 468-16-25-7-2	5	3	2	3	21.00	1.67	3	2	3	2	1	2	1	2	3	1	3	10	19	5	2	80	13.4
24.	HUW 468-16-25-8-1	5	3	2	2	21.83	1.83	4	1	1	1	1	3	1	3	4	1	5	11	17	4	2	89	13.4
25.	HUW 468-16-25-8-2	5	3	2	3	22.91	1.93	3	1	1	1	1	2	3	2	3	1	5	11	18	4	2	91	13.4

S. No.	Line	EGH	AP	APub	FLA	FLL	FLB	DH	WLS	WLB	WP	WE	FC	PT	POG	DM	EC	ED	SL	SPE	AL	AC	PH	Protein
26.	HUW 468-16-25-8-3	5	3	2	2	22.00	1.87	4	1	1	1	1	3	3	2	4	1	4	10	19	3	2	88	14.0
27.	HUW 468-16-25-8-4	3	3	2	3	20.50	1.50	3	2	2	1	1	2	3	2	2	1	4	9	17	4	1	86	13.6
28.	HUW 468-16-25-9-1	5	3	3	3	24.72	1.82	4	3	3	2	1	1	2	3	5	2	4	10	23	4	1	89	13.2
29.	HUW 468-16-25-9-2	4	3	3	3	23.83	1.73	4	3	3	2	1	1	2	3	5	2	4	9	21	4	1	90	13.8
30.	HUW 468-16-25-9-3	4	3	3	3	26.00	2.00	4	3	3	2	1	2	3	3	5	2	3	10	23	4	2	91	13.3
31.	HUW 468-16-25-16-1	2	3	1	3	26.33	2.00	5	3	2	2	2	1	1	2	4	2	4	10	21	4	2	82	12.8
32.	HUW 468-16-25-16-2	2	3	2	3	28.00	1.93	5	3	2	2	2	1	1	2	4	2	3	10	21	4	2	81	13.0
33.	HUW 468-16-25-17-1	3	3	2	2	23.00	1.50	5	3	3	2	2	2	2	2	5	1	4	10	19	3	2	77	13.9
34.	HUW 468-16-25-17-2	5	3	2	2	23.91	1.73	5	3	3	2	2	1	1	2	4	1	4	10	19	3	2	74	14.9
35.	HUW 468-16-25-19-1	3	3	2	2	22.00	1.62	2	2	3	2	2	2	3	2	3	2	4	9	21	4	2	81	13.4
36.	HUW 468-16-25-19-2	4	3	2	2	19.00	1.61	4	2	3	2	1	1	1	2	3	2	4	8	17	4	1	82	14.2
37.	HUW 468-16-25-20-1	2	3	1	3	24.00	1.62	3	3	3	2	1	2	1	2	3	1	4	11	19	5	2	82	12.6
38.	HUW 468-16-25-20-2	2	3	2	3	17.50	1.40	3	3	3	2	1	2	1	2	2	1	4	10	19	5	2	79	13.4
39.	HUW 468-16-25-20-3	5	3	2	3	21.00	1.50	3	3	3	2	2	1	1	2	2	2	4	11	21	4	2	78	13.7
40.	HUW 468-16-25-21-1	5	3	2	3	21.6	1.33	4	4	3	2	2	1	1	4	4	1	3	9	19	5	1	90	13.8
41.	HUW 468-16-25-21-2	2	3	2	3	24.35	1.67	3	3	3	3	3	1	1	4	3	2	3	9	19	5	1	89	13.1
	HUW 468	1	3	2	2	21.91	1.69	1	3	3	3	1	2	2	3	2	2	4	10	19	4	2	79.8	

(B) PIGEONPEA

Genetics of *Fusarium* wilt resistance in long duration pigeonpea

A thorough knowledge of the inheritance of FW resistance in pigeonpea is very much needed to initiate an effective breeding programme. So, we have attempted to understand the genetics of FW resistance involving diverse susceptible/ resistant genotypes of mostly long duration pigeonpea. Four long duration FW susceptible pigeonpea genotypes viz., BAHAR, MA-6, MAL-13 and MAL-18 and four FW resistant genotypes viz., BDN-2004-1, BDN-2001-9, BWR-133 and IPA-234 were selected for the present study. All the genotypes were sown in crossing blocks at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India during *Kharif* 2011-12. The different cross combinations were made to obtain twelve F_1 hybrids as presented in table 15. The twelve F_1 along with parents were grown in crossing blocks during *Kharif* 2012-2013 and each F_1 plant was allowed selfing in a mosquito-net protected field to avoid out crossing simultaneously fresh F_1s were also made to grow parents, F_1s and their respective F_2 populations in the same year i.e., *Kharif* 2013-2014.

All the F_1 plants exhibited resistant reaction to FW indicating dominance of resistance over susceptibility (Table 15). The F_2 population of the crosses BAHAR × BDN-2004-1 (χ^2 = 1.81; P = 0.179), MA-6 × BDN-2004-1 (χ^2 = 3.49; P = 0.062), MAL-13 × BDN-2004-1 (χ^2 = 0.15; P = 0.700) and MAL-18 × BSMR-846 (χ^2 = 2.59; P = 0.107) segregated into 3R:1S genetic ratio indicating monogenic (one dominant) control of FW resistance in these crosses. These results further substantiated that the resistant parent BDN-2004-1 possessed one major dominant for resistance. Similarly, three crosses viz., BAHAR × BDN-2001-9, MA-6 × BDN-2001-9 and MAL-13 × BDN-2001-9, involving the resistant parent BDN-2001-9 was studied (Table 15). Their F_2 populations also segregated into 3R:1S (χ^2 = 1.72-2.58; P = 0.108-0.190) and, thus exhibited monogenic control of resistance.

The dominant nature of inheritance will offer ease in incorporation of FW resistance from resistant to susceptible cultivars with any selection method but resistance based on single dominant gene is generally considered to be vulnerable to genetic changes in pathogen virulence. The F_2 population of the crosses BAHAR × BWR-133 (χ^2 = 2.22; P = 0.136), MA-6 × BWR-133 (χ^2 = 0.1.91; P = 0.167) and MAL-13 × BWR-133 (χ^2 = 0.39; P = 0.532) segregated with a good fit to 15R:1S. It confirmed that two duplicate dominant resistance genes governed the resistance in these three crosses (Table 15). Thus, it is evident that resistant parent BWR-133 possessed two duplicate dominant genes for FW resistance. The 15R:1S ratio suggested that two independent dominant genes with equal effects confer resistance to FW. The F_2 population of the crosses BAHAR × IPA-234 (χ^2 = 1.45; P = 0.0.229 and MAL-13 × IPA-234 (χ^2 = 0.50; P = 0.480) segregated with a good fit to 9R:7S. It confirmed that two complementary dominant resistance

genes governed the resistance in these two crosses. It is concluded that in IPA-234 two pairs of dominant genes governed the FW resistance.

Molecular markers now served several functions in pigeonpea including, genetic diversity analysis, characterization of hybrid parents, purity assessment, mapping for various traits viz., drought tolerance, determinacy, sterility mosaic disease, fertility restoration etc. So, it is possible to validate the present results at molecular level through QTL mapping of the gene (s)/QTL (s) associated with FW resistance in the segregating F_2 populations used in this investigation.

Table 15. FW reaction of twelve F_2 populations of pigeonpea derived from crosses between four resistant and four susceptible genotypes.

Cross	F_1	No. of F_2 plants		Total	Expected ratio (R:S)	χ^2 value	P-value
		R	S				
BAHAR × BDN-2004-1	R	150	39	189	3:1	1.81	0.179
MA-6 × BDN-2004-1	R	178	43	221	3:1	3.49	0.062
MAL-13 × BDN-2004-1	R	104	37	141	3:1	0.15	0.700
MAL-18 ×BDN-2004-1	R	107	25	132	3:1	2.59	0.107
BAHAR × BDN-2001-9	R	108	25	133	3:1	2.58	0.108
MA-6 × BDN-2001-9	R	76	18	94	3:1	1.72	0.190
MAL-13 × BDN-2001-9	R	116	29	145	3:1	1.81	0.179
BAHAR × BWR-133	R	181	17	198	15:1	2.22	0.136
MA-6 × BWR-133	R	78	8	86	15:1	1.91	0.167
MAL-13 × BWR-133	R	163	9	172	15:1	0.39	0.532
BAHAR × IPA-234	R	44	26	70	9:7	1.45	0.229
MAL-13 × IPA-234	R	69	61	130	9:7	0.50	0.480

Validation of SSR markers associated with FW resistance

In order to determine the utility of molecular markers associated with the FW resistance, four each of the FW resistant and susceptible pigeonpea genotypes were screened with six SSR markers reported by Singh et al. (2013). SSR marker ASSR 1 amplified a fragment of 120 bp in 'BAHAR' and other three FW susceptible genotypes viz., MA-6, MAL-13 and MAL-18 (Table 16, Fig. 24). Whereas, an amplification product of 100 bp was found in three of the four FW resistant genotypes except, BWR-133. Similarly, marker ASSR 148 amplified a 100 bp fragment in all FW susceptible genotypes except, MAL-18 and 110 bp amplification product in all FW

resistant genotypes except, BWR-133. An amplification product of 150 bp was produced by marker ASSR 229 in FW susceptible genotypes but 'BAHAR' unable to amplify it and produced a fragment of 135 bp (Table 16; Fig. 25). It was interesting to note that marker ASSR 366 uniformly produced a band of 120 bp in all the FW susceptible and resistant pigeonpea genotypes except, two resistant genotypes (BDN-2001-9 and IPA-234).

Table 16. Size of amplification product in eight pigeonpea genotypes using SSR markers associated with Fusarium wilt (FW) resistance.

Genotype	**Approximate size of amplification product (bp)**					
	ASSR 1	**ASSR 23**	**ASSR 148**	**ASSR 229**	**ASSR 363**	**ASSR 366**
BAHAR	120	150	110	150	200	135
MA-6	120	135	110	135	200	135
MAL-13	120	135	110	135	170	135
MAL-18	120	150	100	135	200	135
BDN-2004-1	100	135	100	150	170	135
BDN-2001-9	100	150	100	135	170	120
BWR-133	120	135	110	150	170	135
IPA-234	100	135	100	150	200	120

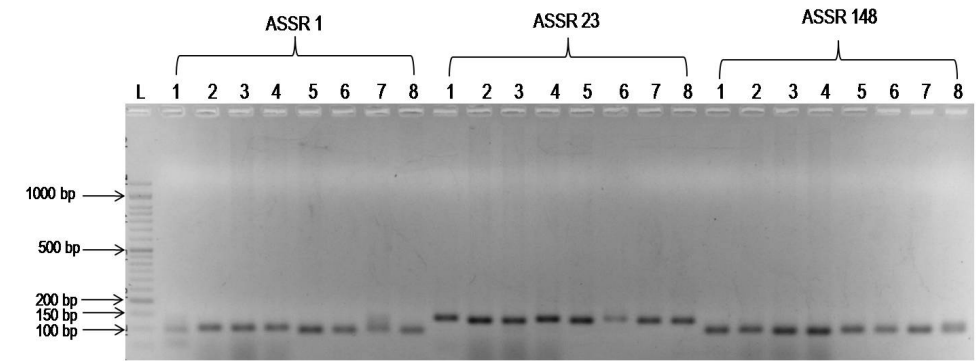


Fig. 24. PCR banding pattern of the SSR markers ASSR 1, ASSR 23 and ASSR 148 associated with FW resistance. L = 50 bp DNA ladder; 1-8 (pigeonpea genotypes as listed in table 15).

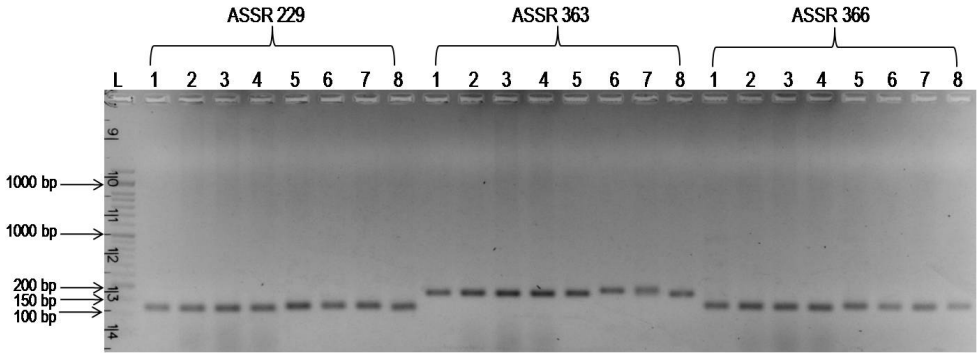


Fig. 25. PCR banding pattern of the SSR markers ASSR 229, ASSR 363 and ASSR 366 associated with FW resistance. L = 50 bp DNA ladder; 1-8 (pigeonpea genotypes as listed in table 15).

Marker ASSR 1 was able to identify 9 out of 12 cross combinations made in the present study. Similarly, parents involved in 8 cross combination will be distinguished by three of the SSR markers used in the present study i.e., ASSR 23, ASSR 148 and ASSR363 (Table 17). While, ASSR 366 was only identify five crosses out of a total of 12 cross combinations made in the present investigation. On the basis of differential amplification of six SSR markers used in the present study, markers ASSR 1, ASSR 23 and ASSR 148 were found to be the most efficient in parental polymorphism screening of the crosses made between diverse FW susceptible and resistant pigeonpea genotypes. These markers can be used in pre-screening of the initial pigeonpea accessions that will be used as parents for future breeding work in pigeonpea with increased *Fusarium* wilt resistance.

Table17.Validation of SSR markers associated with Fusarium wilt (FW) resistance in pigeonpea.

Genotype	FW reaction	ASSR 1	ASSR 23	ASSR 148	ASSR 229	ASSR 363	ASSR 366
BAHAR	S	+	+	–	–	–	–
MA-6	S	+	–	–	+	–	–
MAL-13	S	+	–	–	+	+	–
MAL-18	S	+	+	+	+	–	–
BDN-2004-1	R	–	–	+	–	+	–
BDN-2001-9	R	–	+	+	+	+	+
BWR-133	R	+	–	–	–	+	–
IPA-234	R	–	–	+	–	–	+

+ indicates presence of a band specific to Fusarium wilt susceptible check 'BAHAR' and – indicates presence of a band at different position than in 'BAHAR'.

11. Major equipments/facilities generated:

I. Facilities:

1. A well established molecular breeding laboratory.
2. A wilt -sick field plot for screening of pigeonpea *Fusarium* wilt under field conditions.
3. A polyhouse facility for screening of disease resistance under controlled conditions (temperature, light and humidity).

II. Equipments:

S. No.	Instrument	Make
1.	Deep freezer (-80ºC)	New Brunswick Scientific
2.	Refrigerated Microcentrifuge	Eppendorf
3.	Gradient thermalcycler	Eppendorf
4.	Gel Documentation System	Biorad
5.	Gel electrophoresis unit with power supply	Biorad
6.	Tissue Lyser	Quiagen
8.	Biophotometer	Eppendorf
9.	UV/Vis spectrophotometer	PerkinElmer
10.	Incubator	NSW
11.	Lypholizer	Eppendorf
12.	Laminar Air-flow cabinet	Klenzoids
13.	Ice flaking machine	Brema Ice maker, Italy
14.	Autoclave	NSW, New delhi
15.	pH meter	Cyberscan
16.	Electrical balance	Sartorious
17.	Gel rocker	Banglore Genei
18.	Vortex	Spinix
19.	Micropipette set	Eppendorf
20.	Multicoloured xerox cum printer	Sharp

12. Budgetary requirements for the next financial year, if any with full justifications: NIL

S. No.	Particulars Amount	(Rs. Lakhs)
-	-	-

13. Achievements:

(i) Research Programmes completed

(ii) Publications:

a. Research papers

1. Kumar U, Joshi AK, Kumar S, Chand R and Röder MS (2010). Quantitative trait loci for resistance to spot blotch caused by *Bipolarissorokiniana* in wheat (*T.aestivum* L.) lines 'Ning 8201' and 'Chirya 3'. **Mol. Breed**.26: 477-491.
2. Kumar U, Joshi AK, Kumari M, Paliwal R, Kumar S and Röder MS (2010).Identification of QTLs for stay green trait in wheat (*Triticum aestivum* L.) in the Chirya 3 × Sonalika population. **Euphytica**174: 437-445.

3. Bashyal BM, Chand R, KushwahaC, Joshi AK and Kumar S (2011). *Bipolarissorokiniana* of Barley: Infection behavior in different member of poaceae. **Indian Phytopathol**. 64: 28-30.

4. Joshi AK and Chand R (2012). Progress of researches done to understand host-pathogen relationship for spot blotch pathogen of wheat. J of Wheat Res. 3: 1-7.

5. Lillemo M,. Joshi A.K, Prasad R, Chand R,.Singh , R.P. 2012 Association of *Lr34* and *Lr46* with spot blotch resistance in wheat **Theoretical and Applied Genetics** 126: 711- 726.

6. Eisa M, Chand R and Joshi A.K. 2013 Biochemical and histochemical factors associated with slow blighting to spot blotch (*Bipolaris sorokiniana*(Sacc.) Shoem.) in wheat (*Triticum* spp.) **Zemdirbyste-Agriculture** 100:191–198

7. Eisa M, Chand R and Joshi A.K. 2013 Biochemical and histochemical traits: a promising way to screen resistance against spot blotch (*Bipolaris sorokiniana)* of wheat. European J of **Plant Pathology** 137: 805-820.

8. Singh AK, Rai VP, Chand R, Singh RP and SinghMN (2013). Genetic diversity studies and identification of SSR markers associated with *Fusarium* wilt (*Fusarium udum*) resistance in cultivated pigeonpea (*Cajanus cajan*). **J. Genet.**92 (2): 273-280.

9. TiwariC, WallworkH, Kumar U, Dhari R, Arun B, Mishra VK, Reynolds M and JoshiAK (2013). Molecular mapping of heat tolerance traits in spring wheat (*Triticum aestivum* L.) under hot humid environment of eastern Gangetic plains of India. **Field Crop Res**. 154: 201-210.

10. Meena N, Mishra VK, Baranwal DK, Singh AK, Rai VP, Prasad R, Arun B and Chand R (2014). Genetic evaluation of spring wheat (*Triticum aestivum* L.) recombinant inbred lines for spot blotch (*Bipolaris Sorokiniana*)resistance and yield components under natural conditions for south asia. **J. Agr. Sci. Tech**. 16:1429-1440

11. Srinivasa J, Arun B, Mishra VK, Chand R, Sharma D, Bhardwaj SC and Joshi AK (2014). Accessing the spelt gene pool to develop well-adapted bread wheat lines with increased grain zinc and iron. **Crop Sci** 54: 1-11

12. Singh P.K; Yong He, Z. X.o,. Singh,.R.P, Chand R.,. Mishra, V. K. Malaker P. K., Reza, M. A., Rahman, M. Islam R,. Chowdhury A. K,. Bhattacharya P.M, Kalappanavar, I K. and Arun K. Joshi2015.Development and characterization of the 4th CSISA-spot blotch nursery of bread wheat **European J of Plant Pathology** 143: 595-605

13. Srinivasa J, Arun B, Mishra VK, Singh GP, Velu G, Babu R, Vasistha NK and Joshi AK (2014). Zinc and iron concentration QTL mapped in a *Triticum spelta* × *T. aestivum* cross.**Theor Appl Genet**127: 1643-1651..

14. SarojSandeep K., Mahendra. N. Singh·Tejveer Singh·Vinod K. Mishra. (2015). Identification of stable restorers and genetics of fertility restoration in late maturing pigeonpea [*Cajanus cajan* (L.) Millspaugh]., **Plant Breed**. 09/2015; DOI:10.1111/pbr.12309

15. Vasistha Neeraj K., Arun Balasubramaniam, Vinod K. Mishra, Ramesh Chand, Jayasudha Srinivasa, Punam S. Yadav, Arun K. Joshi. 2015. Enhancing spot

blotch resistance in wheat by marker-aided backcross breeding. **Euphytica** DOI 10.1007/s10681-015-1548-3

16. Sahu, Ranabir; Sharaff, Murali; Pradhan, Maitree; Sethi, Avinash; Bandopadhyay, Tirthankar; Mishra, Vinod; Chand, Ramesh; Chowdhury, Apurba; Joshi, Arun; Pandey, Shree (2016). Elucidation of defense-related signaling responses to spot blotch infection in bread wheat (*Triticum aestivum* L.). **The Plant Journal**. 86(1):35-49.
17. Yusuf Comfort S.; Ramesh Chand; Vinod K.Mishra and Arun K. Joshi 2016.The association between leaf malondialdehyde and lignin content and resistance to spot blotch in wheat. **Journal of Phytopathology** (DOI: 10.1111-jph.12509).
18. Pandey Anju, Md. Shamshul Qumor Ansari, Sudhir Navathe, Ramesh Chand, Vinod Kumar Mishra and Arun Kumar Joshi2016. Association of lesion mimic trait with spot blotch resistance in wheat. **Tropical Plant Pathology**, Volume 41(6) pp 406–414 (10.1007/s40858-016-0115-3).
19. Shahu Ranabir; Kundu Pritha, Sharaff, Murali; Pradhan Maitree, Sethi Avinash, Mishra Vinod Kumar, Chand Ramesh, Joshi Arun Kumar, Kumar Aundy and Pandey Shree (2016). Understanding the defence-related mechanism during the wheat's interaction with fungal pathogens. **Indian Phytopath.** 69(3): 260-265.
20. Suneel Kumar,S., Röder, M.S. Singh, R.P., Chand, R., Joshi, A.K., Kumar,U. 2016 Mapping of spot blotch disease resistance using NDVI as substitute to visual observation in wheat (*Triticumaestivum, L.*)**Molecular Breeding** 36: DOI 10.1007/s11032-016-0515-6
21. Pandey, A.K. Mishra,V.K. Chand, R and Singh, R.K. 2016 Spot blotch lines of wheat (Triticum aestivum) for spot blotch resistance and yield trait.**Bangladesh J. Bot.** 45(5): 1187-1195

b.Review papers: - Nil

c. Popular articles: - Nil

d.Scientific bulletins: - Nil

e. Popular bulletins: - Nil

f. Chapters in books: - Nil

g. Books: - Nil

(iii)Patents filed: - Nil

(ii) Success stories developed: -Nil

(iii)Technologies generated/transferred:

- Techniques for artificial screening of spot blotch resistance in wheat and *Fusarium* wilt resistance in pigeonpea were developed and standardized.

- Two major QTLs, *QSB.bhu2B* and *QSB.bhu7D* associated with spot blotch resistance were successfully validated in a RIL population Yangmai 6 x Sonalika as well as in 310 wheat germplasm lines. SSR markers flanking these QTLs are being used for marker assisted selection.
- Some pigeonpea SSR markers namely, ASSR-1, ASSR-23, ASSR-148, ASSR-229, ASSR-363 and ASSR-366 were identified as tightly linked markers with *Fusarium* wilt resistance.

(iv) Resources generated (in form of sequenced data):

- Spot blotch fungal pathogen (*Bipolaris sorokiniana*): DNA binding protein MAT-1 and MAT-2 weresequenced and submitted to NCBI with accession no. JN128872, JN128873, JN128874, JN128875, JN128876, JN128877, JN128878, JN128879, JN128880, JN128881, JN128882 and JN128883.
- *Fusarium* wilt pathogen (*Fusarium udum*): Internal transcribed spacer 1 region of Faizabad and Ghazipur isolates were sequenced and submitted to NCBI with accession no. KC859449 and KC859450.

(v) Students completed M.Sc. /M. V. Sc. /Ph. D degree under the programme:

Particular	**Number of Students under the NAE programme**		
	Masters	**Ph. D.**	**Others (specify)**
Degree Awarded	22	07	Many students of Genetics and Plant Breeding, Plant Pathology and Plant Physiology disciplines have taken the help of the facilities available in Niche Area lab.

(vi) Linkages established within the country and abroad with various agencies (to be named with complete address)

a) International Maize and Wheat Improvement Center (CIMMYT), Mexico.

b) Directorate of Wheat Research, Karnal- 132 001, India.

c) Indian Institute of Pulses Research, Kalyanpur, Kanpur- 208 024, India.

(vii) Three related photographs (with date) showing the important activities in lab, field as applicable

Niche Area of Excellence Funded well equiped lab

Students interacting with Dr. Pawan Kumar Singh, Scientist, CIMMYT, Mexico in wheat fields during crop season Rabi 2013-14 at BHU centre.

Students acquiring the knowledge about real time PCR during a summer training organized in June, 2014 in Niche Area of Excellence laboratory, BHU, Varanasi.

(x) PPP developed, if any: -

(xi) No. of trainings organized with No. of participants:

Title of the Training/duration	**Number of participants**		
	Faculty	**Farmers***	**Students/Others**
Year round (2009-10)**Laboratory constructed**	-	-	-
Year round (2010-11)	06	-	-
Year round (2011-12)	04	-	-
Year round (2012-13)	04	-	03 (M.Sc./Ph.D.)
Year round (2013-14)	01		17 (M.Sc./Ph.D.)
"**Summer Training on Techniques and Tools of Plant Biotechnology - 2013**"in NAE Laboratory organized from June 8 to 28, 2013	-	-	09 (Students across the India)
"**Summer Training on Techniques and Tools of Plant Biotechnology - 2014**"in NAE Laboratory organized from June 6 to 26, 2014	-	-	10 (Students across the India)

*Training to farmers is not an objective of NAE project at our centre.

List of faculty trained under NAE project (year wise):

S. No.	**Name of trainee**	**Year**	**Designation**	**Present address**
1.	Dr. Ramesh Chand	2010-11	Professor	Deptt. of Mycology and Plant Pathology, I.Ag.Sc, BHU, Varanasi
2.	Dr. (Mrs.) Vineeta Singh	"	Asstt. Prof.	"
3.	Dr. B. Arun	"	Asstt. Prof.	Deptt. of Genetics and Plant Breeding, I.Ag.Sc, BHU, Varanasi
4.	Dr. P.K. Singh	"	Assoc. Prof.	"
5.	Dr. V.K. Mishra	"	Professor	"
6.	Dr. M.N. Singh	"	Professor	"
7.	Dr. (Mrs.) Chanda Kushwaha	2011-12	Asstt. Prof.	Deptt. of Plant Pathology, BAU, Sabour, Bihar
8.	Mr. Prabhat Kumar	"	Asstt. Prof.	"
9.	Mr. Deepak Baranwal	"	Asstt. Prof.	Deptt. of Plant Breeding and Genetics, BAU, Sabour, Bihar
10.	Mr. Sudhir Kumar	"	Asstt. Prof.	"
11.	Dr. (Mrs.) Vijya P.	2012-13	Asstt. Prof.	Deptt. of Plant Physiology,

				I.Ag.Sc, BHU, Varanasi
12.	Dr. S.P. Pandey	”	Asstt. Prof.	Life Sciences, IISER, Kolkata
13.	Dr. Dirk B. Hays	”	Assoc. Prof.	Plant Molecular Biology, University of California Davis
14.	Essa Nazir Mosaid	,,	Scientist	Yamen
15.	Comfort Yusuf Sonken	2013-14	Scientist	Nigeria

14. Outcome for the stake holders:

For the country: Introgression of spot blotch resistance *loci* in two popular wheat varieties of Eastern Indo-Gangetic plain (HUW-234 and HUW-510) and newly identified promising wheat lines (PBW-154, HUW-510, DBW 14, NW 2036 and DL 803-3) is in advanced breeding stage. The improved varieties would be able to give maximum yield in spot blotch disease prone areas which would ultimately enhance the production of wheat in the country. Mapping of *Fusarium* wilt resistance locus (loci) is also in advanced stage. Mapping of *Fusarium* wilt resistance would help in transfer of gene into high yielding otherwise wilt susceptible pigeonpea varieties. The development of improved genetic material would be available to the breeder throughout the country.

(b) For the state: Farmers of Bihar, Bengal and Easter Uttar Prdesh would be benefitted from the research outcome of this project. As many agronomically adopted cultivars become susceptible appropriate resistance sources identified in this progrmme would be used by conventional breeder in their crossing programme. As qualified centre of molecular breeding for major crops with state of art facilities would facilitate, nearby Universities and institutes working on the spot blotch of wheat and pigeonpea to use these facilities.

(c) For the region: Around a dozen advanced superior lines of wheat and pigeonpea for traits of importance developed; As a qualified centre of advanced research in wheat and pigeonpea would maintain a repository of immortal mapping populations particularly for spot blotch resistance in wheat and *Fusarium* wilt resistance in pigeonpea. Segregating populations for spot blotch and wilt resistance materials would be shared with other research institutions.

(d) For the University: Materials developed in this programme is now being utilized by various research groups i.e. molecular biologists, biochemists, plant pathologists, geneticists and plant breeders. This has enhanced the capacity of research and training for students and faculty in molecular breeding.

(e) For the students: More than 100 graduate and post graduate students trained on different aspects of molecular marker analysis e.g., Genomic DNA isolation, PCR amplification, visualization of amplified products through gel electrophoresis etc. In addition to this, M.Sc. and

Ph.D. students from different departments are getting time to time help as and when required both in terms of infrastructural facilities and technical guidance for their thesis work.

(f) For the farmers: Some of the advanced materials of wheat obtained from elsewhere were tested here for their performance of yield and spot blotch resistance are under farmers participatory research and likely to be promoted under national testing. Few lines of pigeonpea are also in advanced breeding stage.

(g) For agribusiness management/industries: Linkage with around a dozen agri-businessman/industries/NGOs/Cooperatives established for genetic insulation of major crops against important biotic and abiotic stresses to bring about concomitant increase in production and productivity and also reduction in coat of cultivation.

Authentication:

(Head) 14/06/2017

HEAD
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