

**SYMPTOMOLOGY AND SERIODIAGNOSIS OF SOYBEAN FLORAL
DISTORTION OCCURRING IN CENTRAL INDIA**

**JADHAV P V¹, MANE S S², NANDANWAR R S³, KALE P⁴, DUDHARE MS⁵ &
KAWAR PG⁶**

^{1,4&5}*Biotechnology Centre, Panjabrao Deshmukh Krishi Vidyapeeth, Akola, MS, India- 444
104*

²*Department of Plant Pathology, Panjabrao Deshmukh Krishi Vidyapeeth, Akola, MS,
India- 444 104*

³*Department of Agricultural Botany Panjabrao Deshmukh Krishi Vidyapeeth, Akola, MS,
India- 444 104*

⁶*Division of Plant Molecular Biology and Genetic Engineering, Vasantdada Sugarcane
Institute, Pune, MS, India
E-mail: jpraveen26@yahoo.co.in*

The present investigation was undertaken to determine the causal agent/s associated with floral distortion symptoms in India where the failure of floral development in soybean was being observed. A comprehensive random roving survey was conducted at the crop's maturity stage (R7-R8) during *kharif* 2011-12. The samples collected showed no resemblance to any of the documented disorders of soybean and were used for studies at morphological, microscopic, serological and molecular levels. The symptoms recorded were abnormal plant height, mottling (ranging from mild to severe), chlorosis of leaves, necrosis of leaves and buds, twisting and thickening of stems, excess proliferation of sepals and distorted floral organs, i.e., petals, stamens, carpels and ovaries leading to failure of pod development on infected plants. Another conspicuous symptom was the delay in maturity in infected plants which remained green even after maturity of the crop. A PCR based diagnosis using universal primers was carried out. A total of 29 samples of different varieties collected from diverse locations were tested for detection of three suspected viruses, i.e., *Tobacco streak virus* (TSV), *Tobacco ring spot virus* (TRSV) and *Groundnut bud necrosis virus* (GBNV) using DAS- ELISA. All samples showed the presence of TSV whereas 19 samples showed mixed infections of TSV and GBNV. None of the samples were found to be positive for TRSV using DAS-ELISA.

Electron microscopy of these samples revealed spherical virus particles measuring a diameter of 28-30 nm (TSV) and 75-80nm (GBNV). SDS-PAGE analysis was performed to estimate molecular weight of the coat protein of purified viruses. A 30 kDa protein band corresponding to TSV coat protein was found in symptomatic soybean samples, whereas it was absent in the tissues of asymptomatic soybean plants. This confirms the presence of TSV associated with the disorder.



Symptomology and serodiagnosis of soybean 'Floral Bud Distortion' occurring in Central India

Pravin Jadhav, Ph. D.

Assistant Professor

Biotechnology Centre



DEPARTMENT OF AGRICULTURAL BOTANY

DR. PANJABRAO DESHMUKH AGRICULTURAL UNIVERSITY, AKOLA

MAHARASHTRA STATE, INDIA- 444 104

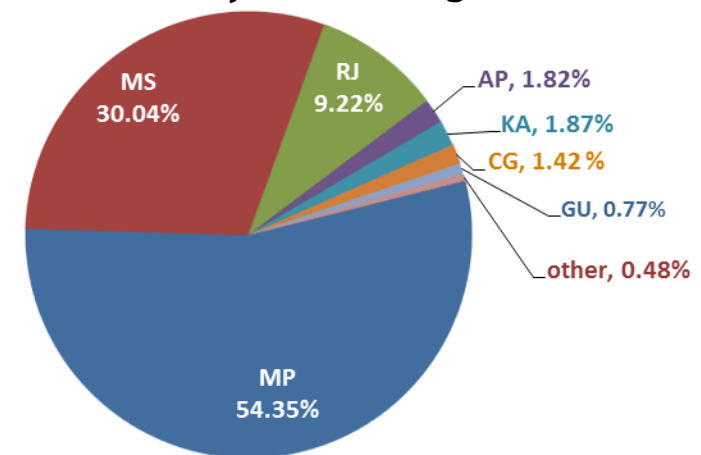
SOYBEAN : Prospects and Constraints

- Soybean is one of the major oilseed crops in the world
- Called as Golden Bean of 21st Century
- Unmatched composition of 40 percent protein and 20 percent oil with nutritional superiority
- Adaptability to varied agro-climatic conditions

INDIA

- Fifth largest producer
- The area under soybean increased from 3 Lakh ha (1970) to 106.95Lakh ha (2012)
- Yield stagnation is the major concern in spite of its high potential
- Biotic and abiotic factors proved to be major limiting factor to yield losses
- Emerging threat of 'Floral Bud Distortion' in soybean

Share of the major Producing States



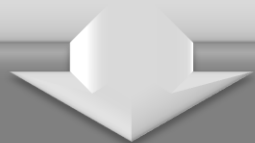
GENESIS

AWARENESS ABOUT THE CAUSE / FBD

No awareness about
Floral Bud Distortion



No any management
strategy



Various local names



MP:

- *BANZAWA*
- *BANZ POUDHA*
- *ROGI POUDH*

MS:

- *VANZ PATA*
- *TUREE*

KA:

- *BUTTI POUDHA*
- *JUDDI*

Floral Bud Distortion: Family Facts

- A peculiar disorder
- Symptomatic plant with prolonged vegetative phase
- Green staying plant even after maturity stage
- No-pod formation in symptomatic plants
- Increasing incidence of the disorder
- Neither farmers nor scientific/technical community are aware about the cause and control measure

➤ *This prompted us to make an attempt...*

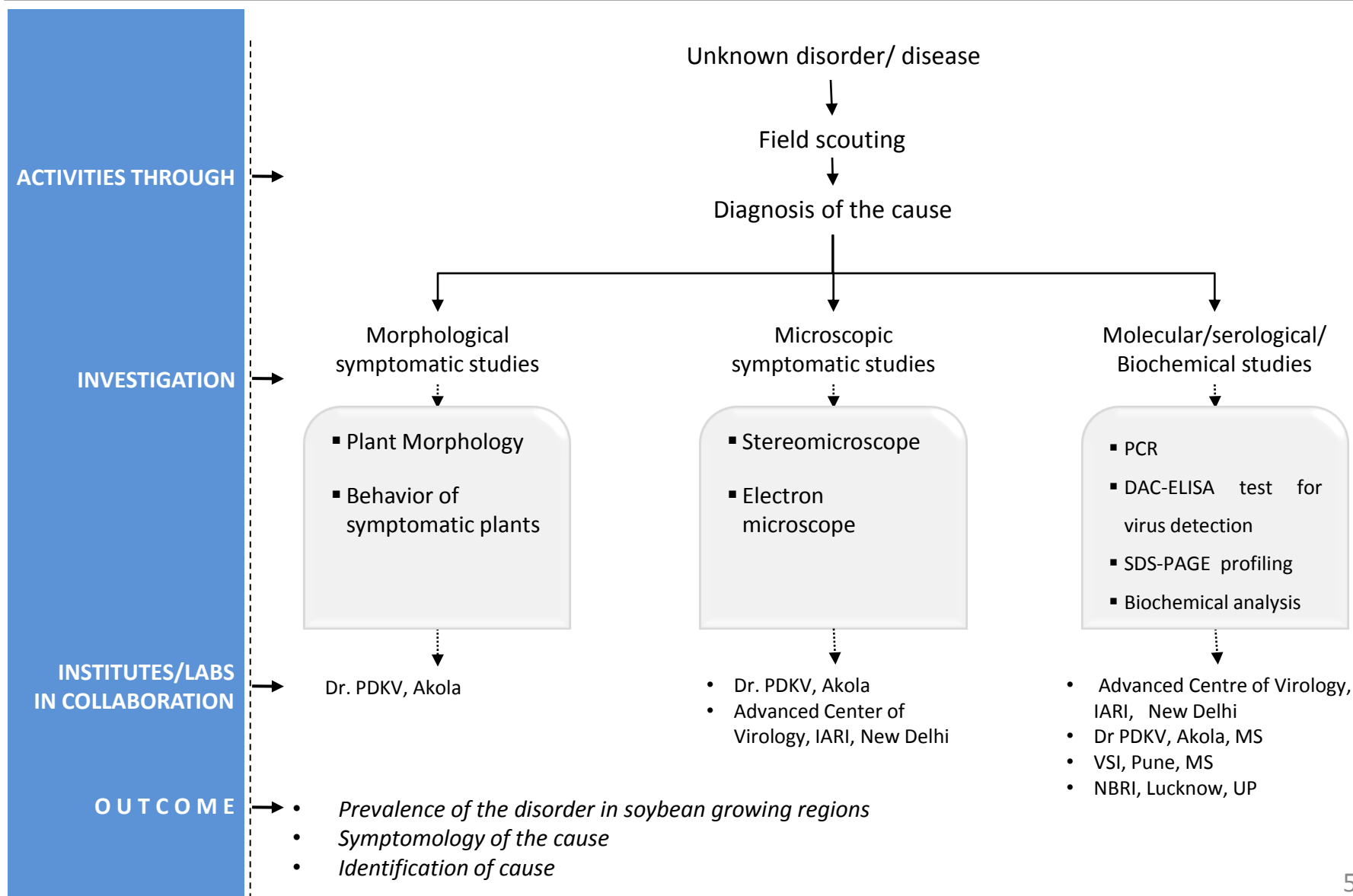
Objectives:

- ***To know the occurrence and understand symptoms of the disorder on farmer field***
- ***Diagnosis and identification of the causal agent of the disorder***



WORK PLAN

PHASE- I



ACTIVITY - I

Field scouting and Sample collection

Description of Survey

Survey Design : Extensive Random Roving survey [2000 Km]

Crop Season / Stage :
 • Kh- 2010-11 and 2011-12
 • Maturity, (R7-R8), 65-70 days old

Area Covered : MP, MS and adjoining soybean growing region of KA

Regions Madhya Pradesh : Burhanpur, Khandwa, Khirkiya, Harda, Hoshangabad, Pipriya, Rampur, Kareli, Narsimhapur, Chhindwara, Bispur, Tansara

Maharashtra : Akola, Amravati, Hadgaon, Latur, Murtijapur, Nandura, Nagpur, Udgir

Karnataka : Hulsur, Bhalki, Bidar

No. of samples collected : ~ 178 per year

India: Soybeans

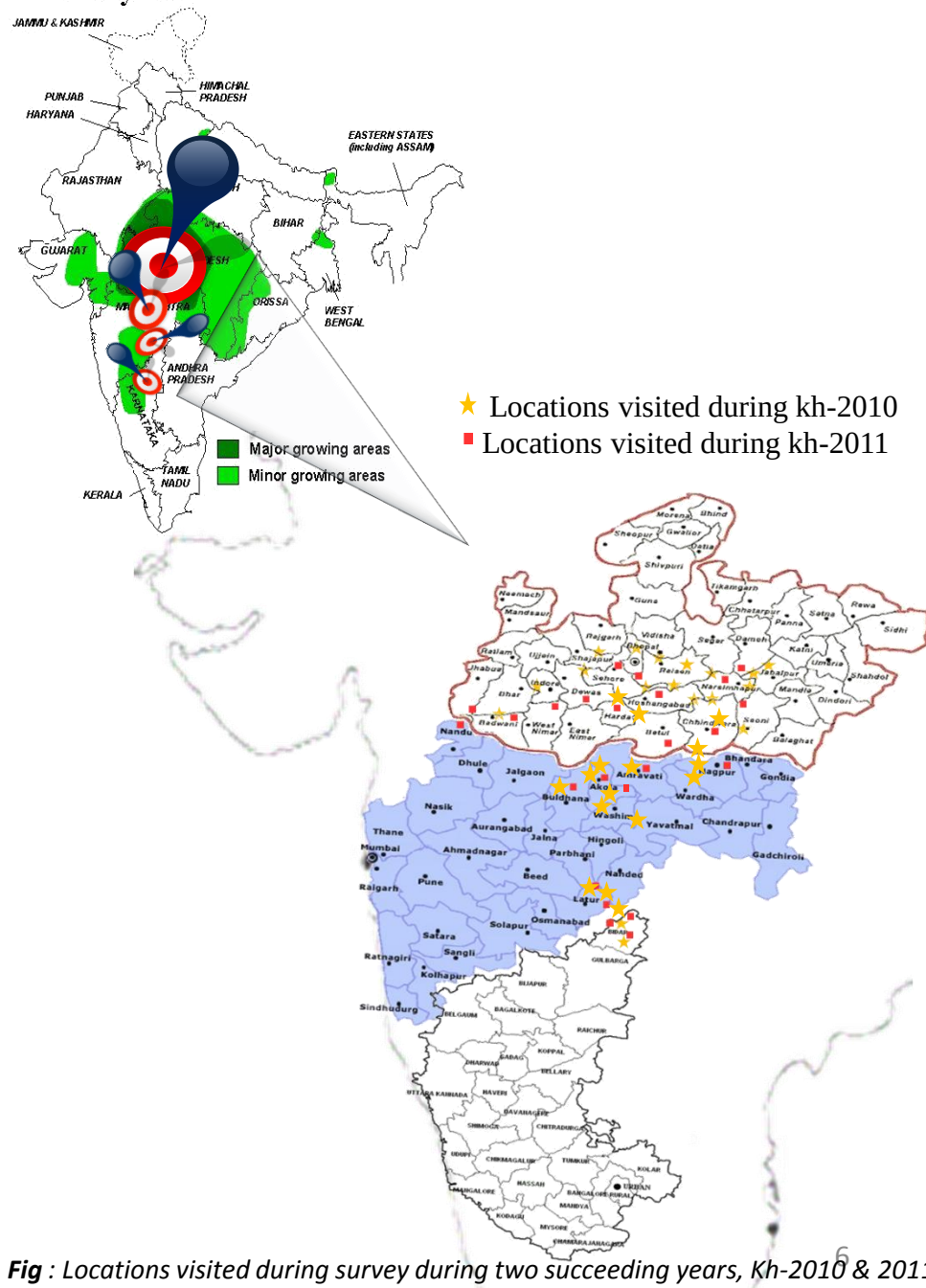


Fig : Locations visited during survey during two succeeding years, Kh-2010 & 2011

FIELD SCOUTING

Locations (Farmer field) visited during the survey, kh-2010-2011

SN	Name of Farmer	Place	Variety	Area (Ac)	Damage %
MADHYA PRADESH					
1.	Rajendra Parashar	Kanjai, Po. Gotegaon, Dist. Narsingpur	CO-2	8.0	5.0
2.	Dhanraj Singh Pawaiya	Kanjai, Po. Gotegaon, Dist. Narsingpur	CO-2	12.0	5.0
3.	Shankar Singh B. Patel	Kanjai, Po. Gotegaon, Dist. Narsingpur	CO-2	4.0	5.0
4.	Sunil Sharma	Kanjai, Po. Gotegaon, Dist. Narsingpur	JS 93-05	2.0	5.0
5.	Sunil Khadiya	Kanjai, Po. Gotegaon, Dist. Narsingpur	JS 73-22	2.0	5.0
6.	Mukesh Dube	Kanjai, Po. Gotegaon, Dist. Narsingpur	JS 73-22	12.0	5.0
7.	Ashish Jain	Gotegaon, Dist. Narsingpur	Co-2	10.0	50.0
8.	Sachin Santosh Chaurasiya	Simriya, Po. Gotegaon, Dist. Narsingpur	JS- 93-05	9.0	90.0
9.	Govind C. Choursiya	Simriya, Po. Gotegaon, Dist. Narsingpur	JS- 93-05	4.0	10.0
10.	Balgovind Pachani	Gotegaon, Dist. Narsingpur	JS 95-60	10.0	10.0
11.	Shailesh Jain	Gotegaon, Dist. Narsingpur	JS 95-60	20.0	5.0
12.	Ranjeet Patel	Karakbail, PO. Gotegaon, Dist. Narsingpur	JS 93-05	9.0	90.0
13.	Pramod Kumar B. Patel	Gondrai, Po. Gotegaon, Dist. Narsingpur	JS 93-05	15	10.0
14.	Santosh Kumar M. Patel	Gondrai, Po. Gotegaon, Dist. Narsingpur	JS 93-05	6.0	13.0
15.	Prabhudayal Patel	Gotegaon, Dist. Narsingpur	JS 93-05	3.0	5.0



FIELD SCOUTING

List of locations (Farmer field) visited during the survey, Kh-2010-11

SN	Name of Farmer	Place	Variety	Area (Ac)	Damage %
16.	Padmasingh S. Ahirwar	Burwani,Tq. Suhagpur	JS 93-05	2.0	10.0
17.	Pirhu S. Chaudhary	Burwani, Tq. Suhagpur	JS 10-44	2.0	15.0
18.	Daman Patel	Burwani, Tq. Suhagpur,	JS 93-05	6.0	10.0
19.	Bhagwan Singh Mehara	Barai, Tq. Bugroda, Dist. Raisen	Sonia Gold	4.0	10.0
20.	Premchand Mehara	Barai, Tq. Bugroda Dist. Raisen	Sonia Gold	12.0	10.0
21.	Omkar Darbar	Tornifata, TQ/Dist: Khandawa	JS 335	2.0	5.0
MAHARASHTRA					
22.	Dr. PDKV, Field	Dept. of Agril. Farm Akola	JS 335	1.0	5.0
23.	Vani Ambapur by S. P. Patil	Vani Ambapur	JS 335	15.0	5.0
24.	RRC, Amravati	RRC field, Amravati	AMS-MB-5-19	-	5.0
25.	RRC, Amravati	RRC field, Amravati	AMS-353 x EC-S	-	5.0
26.	RRC, Amravati	RRC field, Amravati	JS-335	-	5.0
27.	RRC, Amravati	RRC field, Amravati	Bragg	-	5.0
28.	Swapanrao Jadhav	Mogha, Tq. Udgir Dist. Latur	JS 335	18.0	15.0
29.	Narsighrao Jadhav	Udgir Dist. Latur	JS 335	8.0	20.0
30.	Shivajirao J. Patil	Udgir Dist. Latur	JS 335	10.0	15.0
31.	Sunil V. Patil	Udgir Dist. Latur	Sonia Gold	13.0	15.0
KARNATAKA					
30.	Vithalrao Patil	Hulsur, Tq. Bhalki Dist. Bidar	JS 335	10.0	15.0
31.	Rajkumar Biradar	Bhalki, Dist. Bidar	Sonia Gold	10.0	20.0
32.	Tryambakrao Patil	Bidar	Sonia Gold	12.0	15.0



FIELD SCOUTING

Farmer fields visited during survey in Madhya Pradesh, Kh- 2011-12

SN	Place	Farmers Name/ Contact Number	Variety grown	Area (ac)	Incidence (%)
Madhya Pradesh					
1	Dahinala, Tq. Burhanpur	Sitaram Charan	JS 335	4.0	10.0
2	Dahinala, Tq. Burhanpur	Nahnu Charan	JS 335	2.0	5.0
3	Dahinala, Tq. Burhanpur	Raj Omkar	JS 335	3.0	5.0
4	Borgaon Bujurg, Tq. Pandana,	Gulam Shaikh	JS 335	8.0	5.0
5	Borgaon Bujurg, Tq. Pandana	Balabhai Dhana Charan	Samrat (Early)	7.5	30.0
6	Borgaon Bujurg, Tq. Pandana	Charan	JS 93-05	6.0	10.0
7	Kumthi, Tq. Pandana	Dilip Gujjar	JS 75-06	6.0	5.0
8	Borgaon Khurd	Dashrath Gopalji	JS 75-06	3.0	5.0
9	Khandawa	-	JS-335	3.0	5.0
10	Khedi, Tq. Harsud	Kadwaji	JS-335	2.0	5.0
11	Ashapur	-	JS 75-06	3.0	5.0
12	Bhediakhal	-	JS 10-44	4.0	5.0
13	Bothiya	-	JS 10-44	2.0	5.0
14	Chipwad, Tq. Khirkhiya	Dhiraj Singh	JS 93-05	12.0	15.0
15	Chipwad, Tq. Khirkhiya	Dayaram Rajput	JS 93-05	10.0	20.0
16	Pipriya	-	JS 75-06	5.0	30.0
17	Rampur	-	JS 75-06	4.0	20.0
18	Bachwani	Ghoraka Patel	JS-335	5.0	20.0
19	Belkhedi	Rajkumar Patel	JS 10-44	3.0	5.0
20	Dumah	Jagdish Badku Domar	JS 335	6.0	80.0
21	Manakwara	-	JS 93-05	6.0	20.0
22	Kareli	-	JS 93-05	5.0	20.0
23	Kalyanpur	Tigaddaji	JS-335	5.0	20.0
24	Jungawani	Manhar Sahu	JS 75-06	2.0	15.0
25	Bisapur	Narendra Patel	JS 93-05	5.0	5.0
26	Tansara maal	-	Js-335	1.5	5.0
27	Gotegaon, Dist. Narsingpur	Rajendra Chaudhary	JS 335	16.0	5.0
			JS 94-52	13.0	10.0



24



5



8



15



16



23

FIELD SCOUTING

Farmer fields visited during survey in Maharashtra & Karnataka State, India, Kh-2011-12

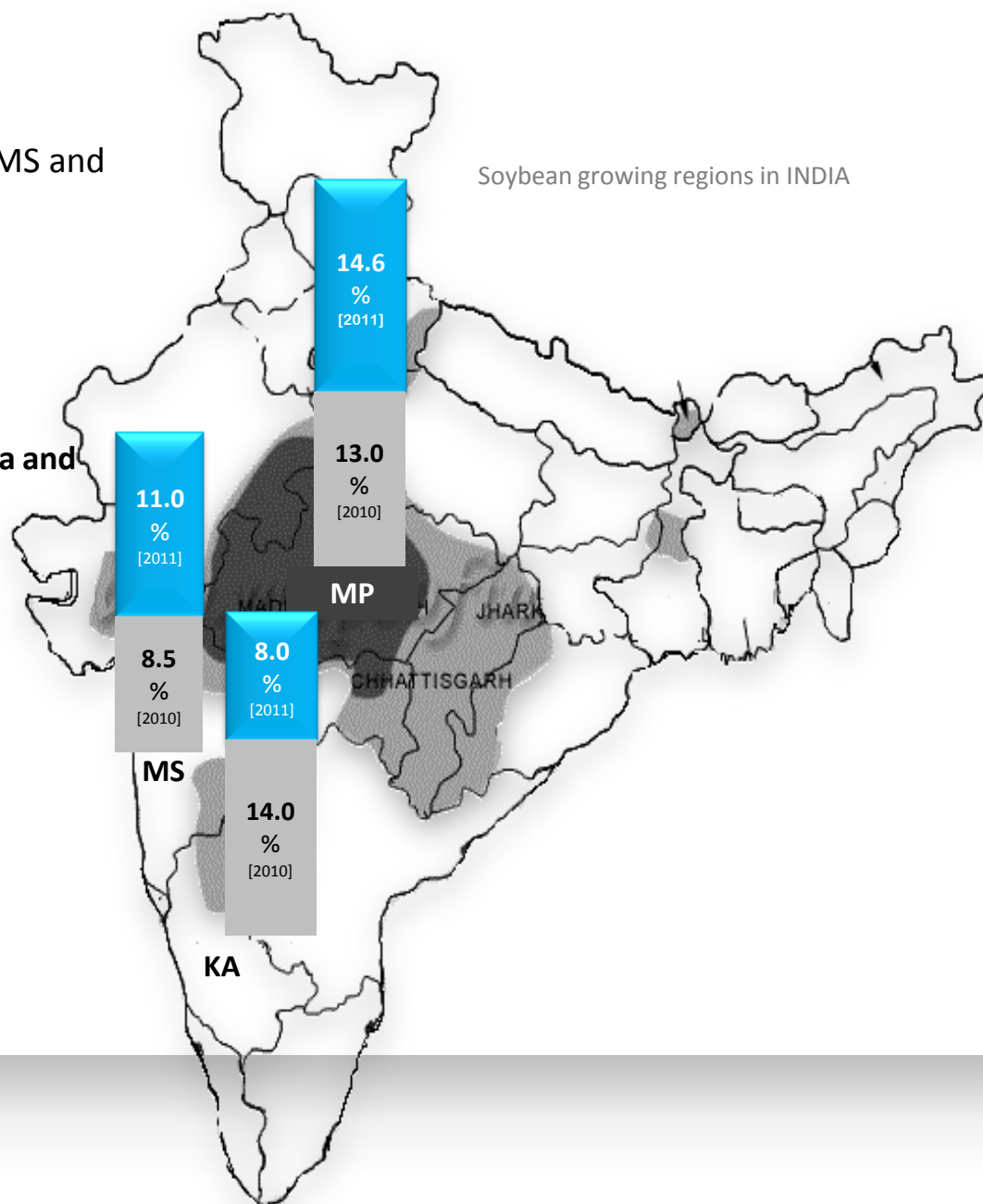
Sr. No	Place	Farmers Name/ Contact Number	Variety grown	Area (ac)	Incidence (%)
Maharashtra					
28	Nandura	-	JS 335	4.0	5.0
29	Hadgaon, tq. Murtijapur Survey No. 7980	Prabhakar Jogale	JS 335	1.0	90.0
30	Mogha, Tq. Udgir	J. Manikrao	JS 335	8.0	10.0
31	Udgir Dist. Latur	S. Patil	JS 335	10.0	10.0
32	PKV, Akola	Beside Poly house	JS 335	-	2.0
34	PKV, Akola	Plot No.69 beside Satpuda Hostel	JS 335	1.0	2.0
35	COA field, Nagpur	IVT Trial, COA, Nagpur			10.0
36	RRC Amravati	H-Block, RRC, Amravati	NRC-37	-	3.0
37	Wanirambapur	Rambhapur Block Field No- 3 BREEDER SEED	JS 335	9.0	5.0
38	Wanirambapur	Pailpada Block, Field No- 12, BREEDER SEED	JS 335	13.40	5.0
39	Wanirambapur	Darasingh Rathor ANAWI Block, Field No- 5, BREEDER SEED	JS 335	1.70 Ha	5.0
Karnataka					
40	Hulsur, Tq. Bhalki	Vittalrao Patil	JS 335	6.0	5.0
41	Hulsur, Tq. Bhalki	Abhangrao Patil	JS 335	4.0	4.0
42	Bhalki, Dist. Bidar	Tanaji Sawale	JS 335	7.0	15.0



FIELD SCOUTING

PREVALENCE of Floral Bud Distortion in MP, MS and KA during Kh-2010 and 2011

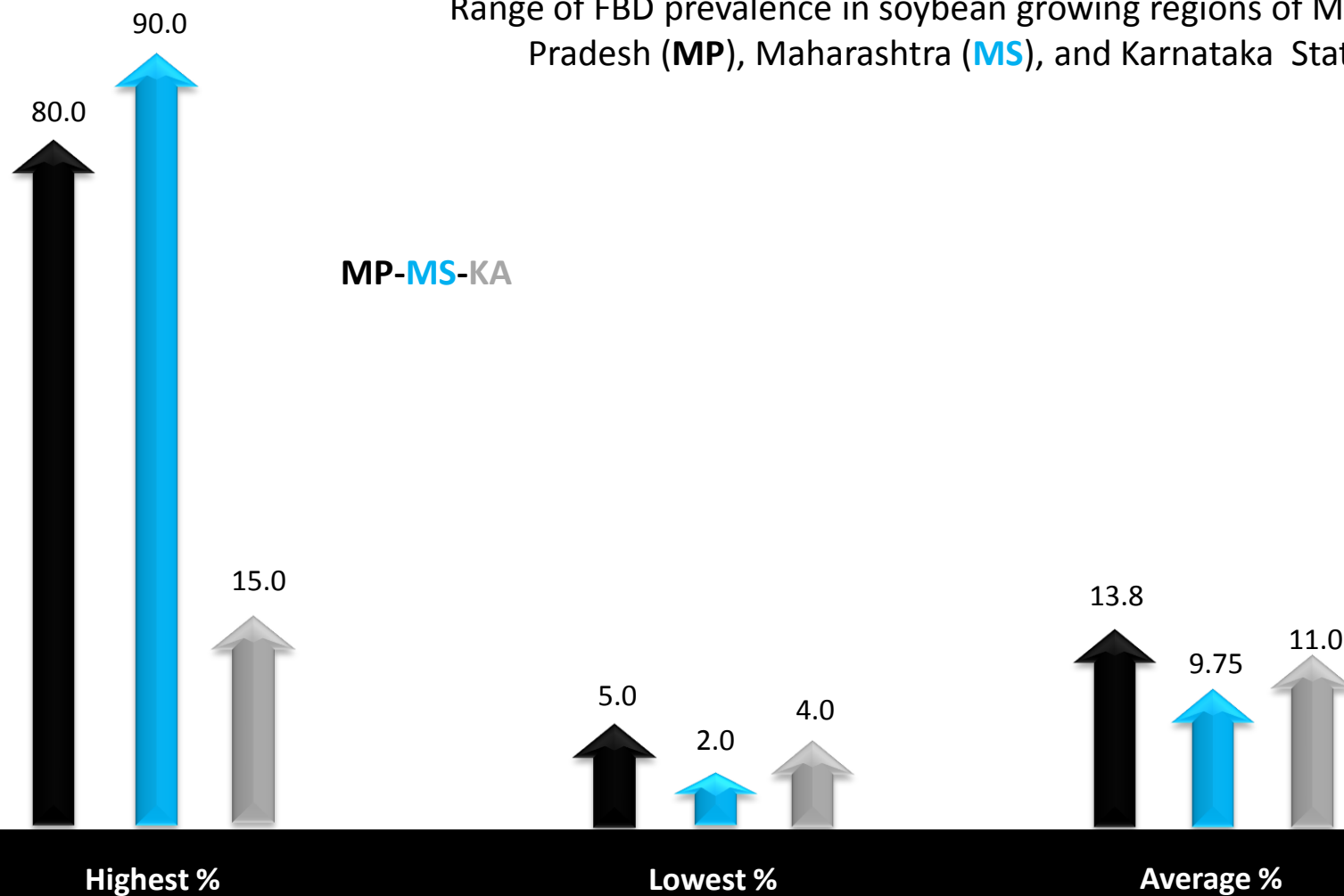
- Recorded increased infestation in Maharashtra and Madhya Pradesh
- Decreased infestation in KA



FIELD SCOUTING

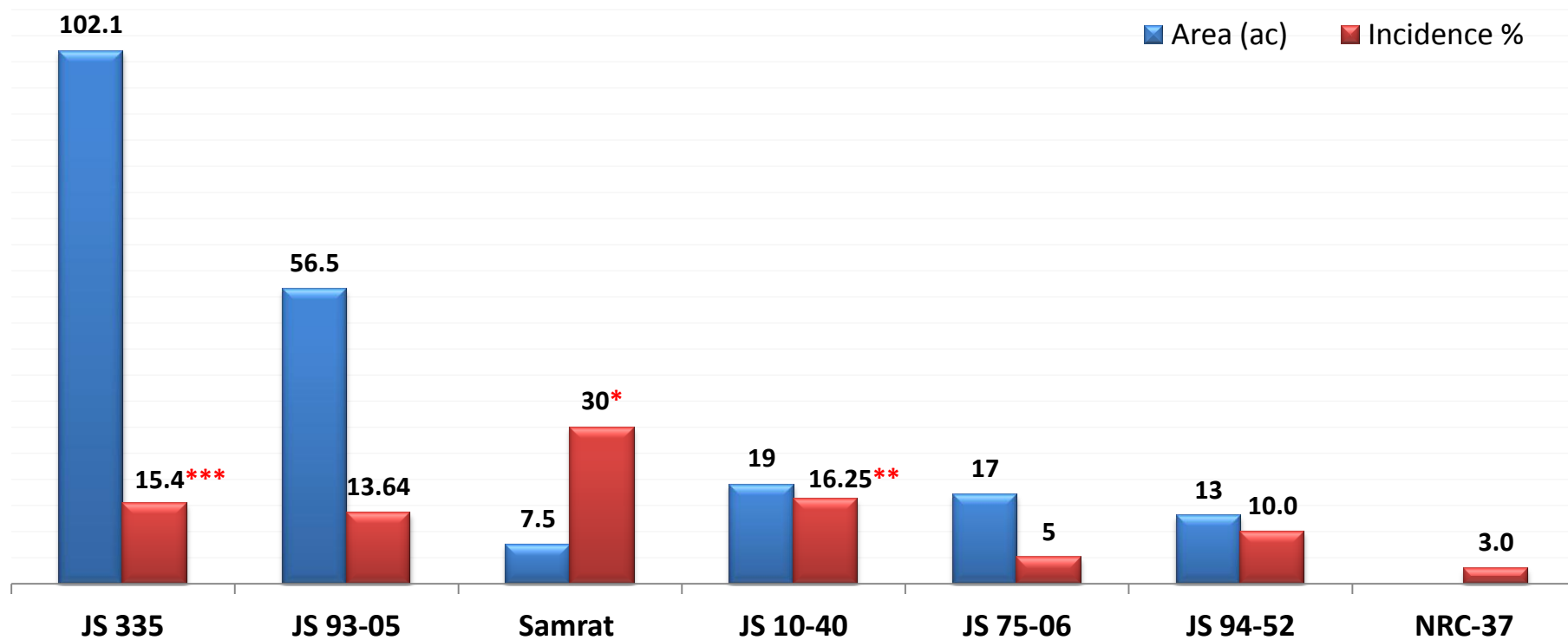
Kh-2010 & 2011

Range of FBD prevalence in soybean growing regions of Madhya Pradesh (**MP**), Maharashtra (**MS**), and Karnataka State(**KA**)



FIELD SCOUTING

VARIETIES Affected by FBD during Kh-2011



Almost all grown soybean varieties (7) affected by FBD

ACTIVITY – II

Morphological symptomatic studies

- Morphological symptoms of symptomatic plants
- Microscopic studies
- Critical observation on yield loss in symptomatic plants



JS 335,
Wanirambapur,
MS



JS 93-05
Borgaon Bujurg, MP

FIELD VIEW OF FBD INCIDENCE



JS 335, Akola

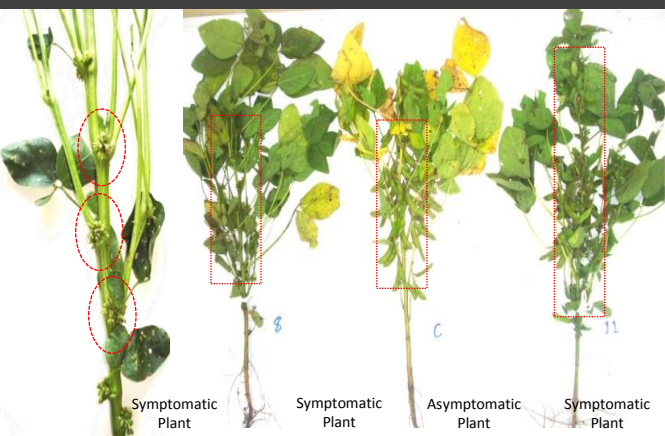


JS 335
Bhalki, KA



Appearance of Symptomatic plants in soybean field, Burwani, Dist: Hoshangabad

PRINCIPLE symptoms of 'Floral Bud Distortion'



1

- Different symptoms on soybean plants affected by Floral bud distortion with asymptomatic plant



2

- Green staying symptomatic soybean plant at maturity stage

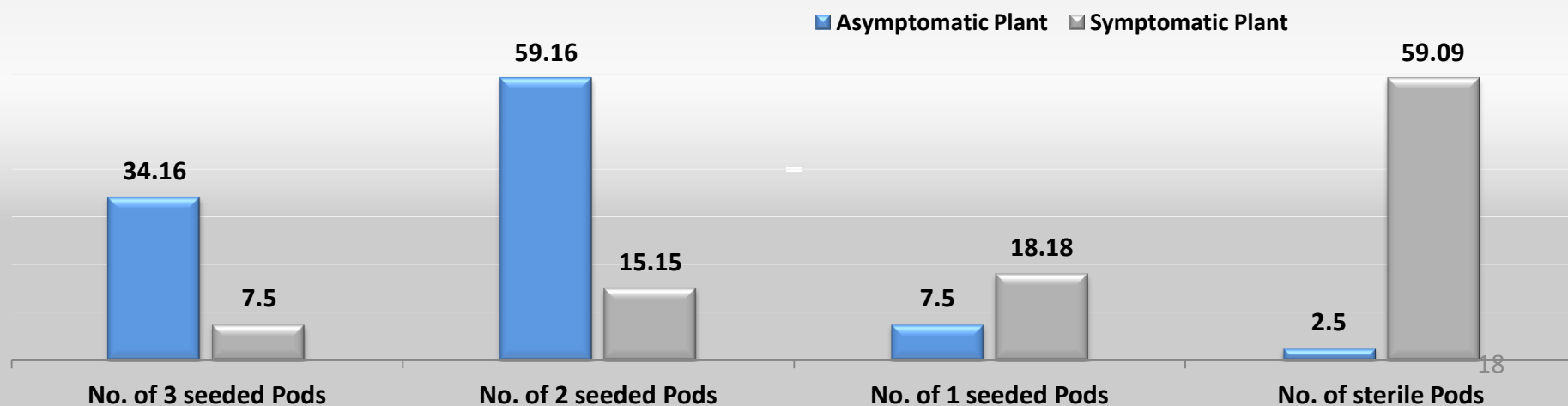
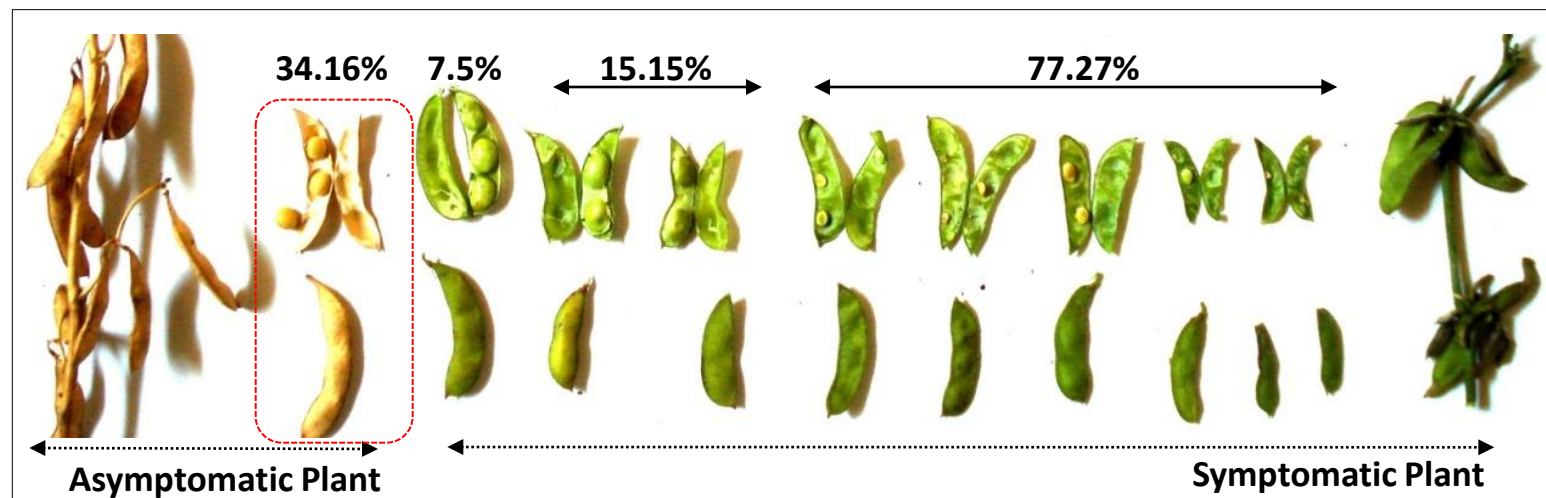


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- Thickening and Twisting of stem in symptomatic soybean plant
- A: Thicken stem (S) at maturity stage; B: Normal Stem (As); C & D : Twisting of stem (S) [Grau et al., 2000]



Percent number of pods in symptomatic (affected by NPS) and asymptomatic soybean plants



ACTIVITY – III

Diagnosis of symptomatic soybean plants

- PCR based screening of soybean samples for presence of the phytoplasma
- Online survey for critical assumptions from eminent soybean scientists
- ELISA- based diagnosis for the presence of different viruses
- Electron Microscopy
- Biochemical studies on symptomatic and asymptomatic soybean plants

ACTIVITY - III

PCR screening for the presence of phytoplasma

Test Sample: 29 symptomatic soybean samples and control (+ve & -ve)

Methods:

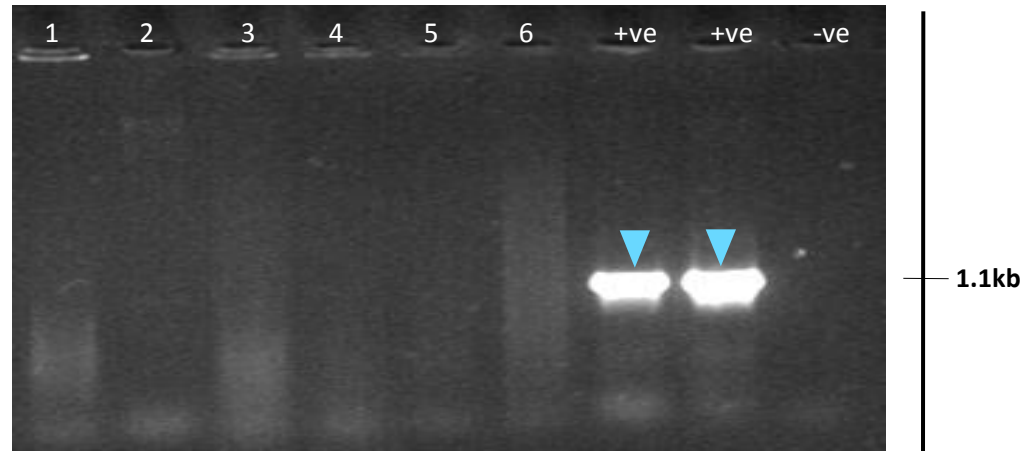
DNA Extraction and PCR

- DNA isolation using modified Procedure from Zhang et al., 1998; Palmano, 2001 and Lee et al., 2012 using stem and leaf tissues of symptomatic and asymptomatic soybean plants
- PCR using 16Sr RNA based “universal” phytoplasma specific PCR primer sets

Primer	Location	Oligonucleotide sequence
P1	16S	5'AAGAGTTTGATCCTGGCTCAGGATT3'
P3	16S	5'GGATGGATCACCTCCTT3'
P7	23S	5'CGTCCTTCATCGGCTCTT3'
<i>Universal primer set</i>		
P1	:	5'-AAG AGT TTG ATC CTG GCT CAG GAT T-3'
16S-Sr	:	5'-GGT CTG TCA AAA CTG AAG ATG-3'
<i>Nested PCR</i>		
R16F2n	:	5'-GAA ACG ACT GCT AAG ACT GG-3'
R16R2n	:	5'-TGA CGG GCG GTG TGT ACA AAC-3'

PCR Reaction

2 min at 94⁰C, 1 cycle; 1 min at 94⁰C, 2 min at 52- 60⁰C, 3 min at 72⁰C, 35 cycles; and 10 min at 72⁰C, 1 cycle

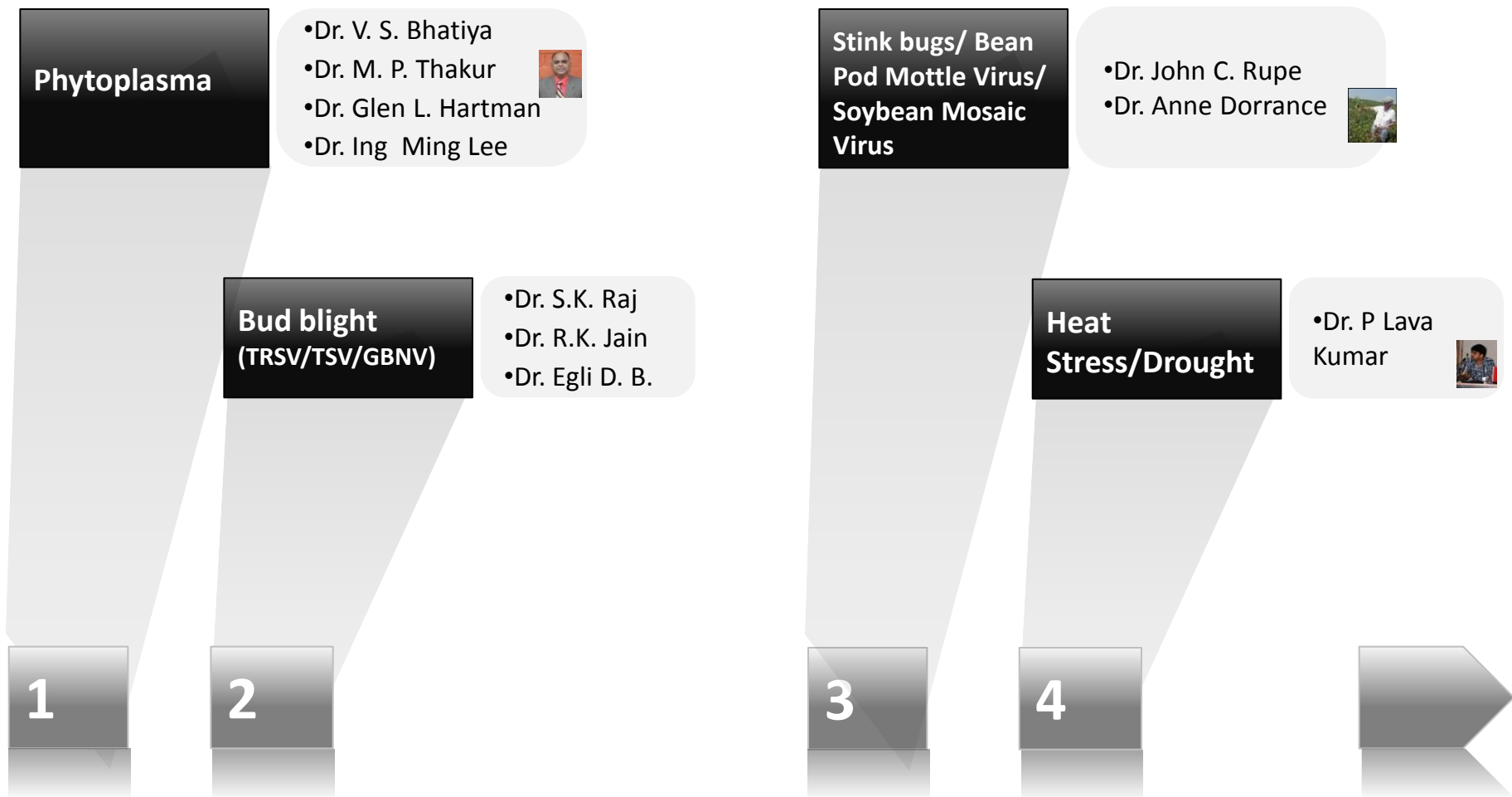


PCR product of 1.1kb size in positive samples and absent in symptomatic soybean samples screened using 16S based universal PCR primers P1-P7

- 1 to 5 : Soybean samples (Test Samples)**
6 : Sugarcane Healthy leaf (-ve control)
+ve : SCGS samples
-ve : Healthy sample

Not found POSITIVE RESULTS for phytoplasma

CRITICAL ASSUMPTIONS



Dr. R.K. Jain
Head,
Division of Plant Pathology
Indian Agricultural Research
Institute (IARI) , New Delhi- 110
012 rakeshjain56@yahoo.co.in

Dr. Egli D. B.
Professor,
University of Kentucky, Lexington,
KY; (859) 257-5020 ext. 80753
degli@uky.edu

Dr. John C. Rupe
Professor,
University of Arkansas ,
Fayetteville, AR 72701 , Phone:
(479) 575-2778, E-mail-
jrupe@uark.edu

Dr. Glen L. Hartman
Professor, Soybean Pathology,
USDA, [National Soybean Research
Center](http://www.nsr.usda.gov), MC 637, 1101 W Peabody
Dr Urbana, IL 61801 ; E-mail:
ghartman@illinois.edu

Dr. V. S. Bhatiya
Principal Scientist (Plant
Physiology)
Directorate of Soybean
Research, Indore, MP,
[bhatia_virender_singh@ho
tmail.com](mailto:bhatia_virender_singh@hotmail.com)

SEROLOGICAL STUDIES of symptomatic soybean plants

DAC-ELISA based diagnosis of soybean sample

Sample	Location	Morphological symptoms	GBNV	TSV
Healthy			0.18 (-ve)	0.18 (-ve)
Positive			2.74 (+ve)	0.66 (+ve)
1	Tornafata Dist. Khandwa, MP	Stay green, dwarf, bushy plant, bud proliferation	0.28 -ve	1.13 +ve
2	Barai, Dist. Raisen, MP	Stay green, aborted bud	0.34 -ve	0.94 +ve
3	Karakbail, Dist. Narsimhapur, MP	Bud proliferation, bud proliferation	0.21 -ve	0.87 +ve
4	Burwani, Dist. Shajpur, MP	Bud proliferation, stay green	0.26 -ve	0.93 +ve
5	Wanirambapur, Dist. Akola, MS	Bud proliferation, stay green	0.28 -ve	1.31 +ve
6	Udgir, Dist. Latur, MS	Bud proliferation, stay green	0.88 +ve	1.18 +ve
7	Bhalki, Dist. Bidar, KA	Bud proliferation, stay green	0.76 +ve	1.06 +ve
8	Khed Hulsur, Dist. Bidar, KA	Bud proliferation, stay green	0.83 +ve	0.87 +ve
9Madhya Pradesh				
9	Dahinala, Dist. Khanwa MP	Reduced leaves and phyllody appearance	1.15 (+ve)	0.85 (+ve)
10	Bachwani, MP	Reduced pods, stay green, brown-black spots on stems	0.47 (-ve)	0.88 (+ve)
11	Chipwada, Dist. Harda, MP	Short plant, stay green	1.5 (+ve)	0.98 (+ve)
12	Pipriya, MP	Expanded height, bud proliferation, stay green	0.34 (-ve)	0.92 (+ve)
13	Dahinala, Dist. Khanwa MP	Bud proliferation, stay green, brown-black spots on stem	1.12 (+ve)	0.93 (+ve)
14	Bisapur, MP	Short plant, bud proliferation, stay green	0.92 (+ve)	0.75 (+ve)

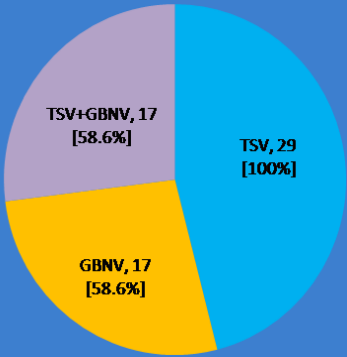
- All Plants positive for *Tobacco streak virus (TSV)*
- 7 plants showed mixed infection of TSV and GBNV

Diagnosis of symptomatic soybean samples collected from diverse locations of Madhya Pradesh using DAC-ELISA

Sample	Location	Morphological symptoms	GBNV	TSV
15	Borgaon Bujrug, Tq. Pandana	Reduced podding, no pod filling, stay green, brown-black spots on pods and stem, thickening of stem	0.76 (+ve)	1.3 (+ve)
16	Kalyanpur, MP	Short plant, stay green, brown-black spots on stem, thickening of stem	0.75 (+ve)	1.06 (+ve)
17	Jungwani, Dist. Chindwada, MP	Short plant, bud proliferation, brown spots on stem	2.78 (+ve)	1.22 (+ve)
18	Dahinala, Dist. Khanwa MP	Bud proliferation	1.61 (+ve)	1.05 (+ve)
Maharashtra State				
19	Hadgaon, Dist Akola, MS	Bud proliferation, stay green	0.88 (+ve)	0.78 (+ve)
20	Dr. PDKV, Akola, MS	Expanded height, floral bud proliferation, stay green	0.43 (-ve)	2.54 (+ve)
21	Dr. PDKV, Akola, MS	Extended height, few pods, stay green, thickening of stem	0.37 (-ve)	1.03 (+ve)
22	Dr. PDKV, Akola, MS	Chlorotic leaves, bud proliferation	2.51 (+ve)	1.59 (+ve)
23	Waniorambhapur, Dist. Akola, MS	Expanded height, bud proliferation, stay green	0.48 (-ve)	0.94 (+ve)
24	Amrawati, MS	Shot plant, bud proliferation, stay green, thickening of stem	1.4 (+ve)	1.99 (+ve)
25	Buldhana, MS	Bud proliferation, stay green	0.86 (+ve)	0.79 (+ve)
26	Udgir, Dist. Latur, MS	Reduced podding, no pod filling	0.35 (-ve)	0.92 (+ve)
27	Nandura, Dist. Buldhana, MS	Reduced podding, no pod filling, stay green, brown-black spots on pods and stem	0.61 (+ve)	1.33 (+ve)
Karnataka State				
28	Bhalki, KA	Reduced podding, no pod filling	0.29 (-ve)	0.80 (+ve)
29	Hulsur, Dist. Bidar, KA	Bud proliferation, stay green	0.94 (+ve)	0.83 (+ve)



**All samples (15) were positive for tobacco streak virus (TSV)
10 samples showed mixed infection of TSV and GBNV**



TSV, 29 [100%]

GBNV, 17 [58.6%]

TSV+GBNV, 17 [58.6%]

- Negative reaction

+ Positive Reaction [0.5 -1.0]

++ Strong reaction [1.1-1.50]

+++ Very strong reaction [>1.51]

Sample	Location	Genotype	GBNV	TSV	TRSV	Phytoplasma
Madhya Pradesh						
1	Dahinala, Dist. Khanwa MP	JS335	++	+	-	-
2	Bachwani, MP	JS335	-	+	-	-
3	Chipwada, Dist. Harda, MP	JS 93-05	++	+	-	-
4	Pipriya, MP	JS 75-06	-	+	-	-
5	Dahinala, Dist. Khanwa MP	JS 335	++	+	-	-
6	Bisapur, MP	JS 93-05	+	+	-	-
7	Borgaon Bujrug, Tq. Pandana	Samrat	+	++	-	-
8	Kalyanpur, MP	JS 335	+	++	-	-
9	Jungwani, Dist. Chindwada, MP	JS 75-06	+++	++	-	-
10	Ashapur	JS 10-44	+	++	-	-
11	Gotegaon, Dist. Narsimhapur, MP	CO-2	-	+	-	-
12	Barai, Dist. Raisen, MP	Sonia Gold	-	+	-	-
13	Gotegaon, Dist.Narsingpur	JS 95-60	-	+	-	-
14	Burwani, Dist. Shajpur, MP	JS 10-44	-	+	-	-
Maharashtra State						
15	Hadgaon, Dist Akola, MS	JS 335	+	+	-	-
16	Dr. PDKV, Akola, MS	JS 335	-	+++	-	-
17	Dr. PDKV, Akola, MS	JS 335	-	++	-	-
18	Dr. PDKV, Akola, MS	JS 335	+++	+++	-	-
19	Wanirambhapur, Dist. Akola, MS	JS 335	-	++	-	-
20	RRC, Amrawati, MS	NRC 37	++	+++	-	-
21	Buldhana, MS	JS 335	+	+	-	-
22	Udgir, Dist. Latur, MS	JS 335	-	+	-	-
23	Nandura, Dist. Buldhana, MS	JS 335	+	++	-	-
24	Wanirambapur, Dist. Akola, MS	JS 335	-	+	-	-
25	Udgir, Dist. Latur, MS	JS 335	+	+	-	-
Karnataka State						
26	Bhalki, KA	JS 335	-	+	-	-
27	Hulsur, Dist. Bidar, KA	JS 335	+	+	-	-
28	Bhalki, Dist. Bidar, KA	JS 335	+	+	-	-
29	Khed Hulsur, Dist. Bidar, KA	Sonia Gold	+	+	-	24-

LINK between cause and symptoms

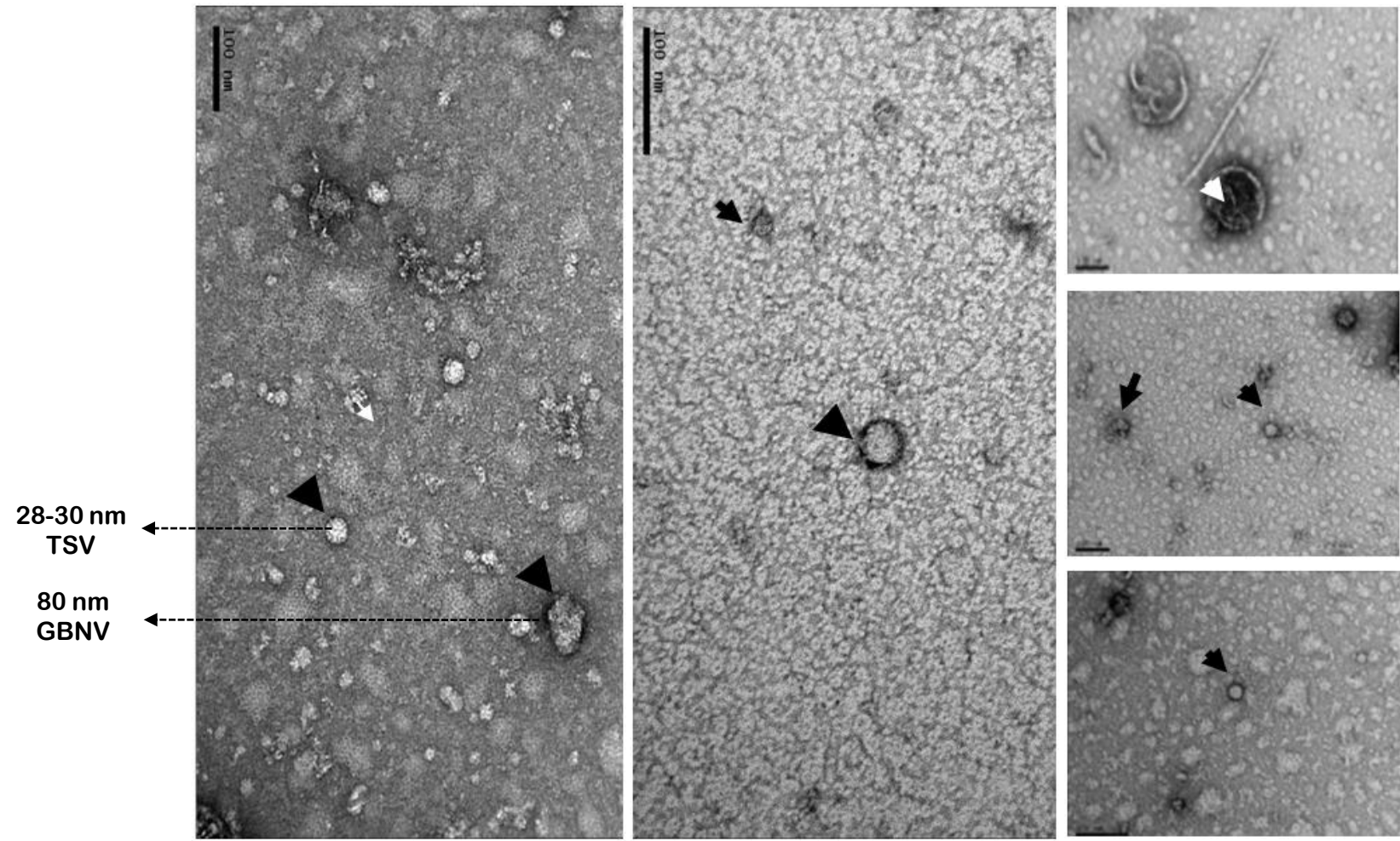
CAUSE & EFFECT

*Presence of virus
and symptoms*

- **Thicken green stem** appearance **without pod(s)** may be due to severe infection of TSV⁺⁺⁺
- **Reduced growth** along with necrotic pods found in plants positive for GBNV⁺⁺⁺
- **Reduced growth with thicken green stem** appearance is due to mixed infection of the both viruses



ELECTRON MICRORAPHS OF SOYBEAN SAMPLES



Electron Micrograph of purified virus particles of TSV and GBNV in symptomatic soybean plants [Barr= 100nm]

BIOCHEMICAL ANALYSIS

SDS-PAGE profiling of partially purified virus coat proteins in soybean

[Almeida et al., 2005; Bhat et al., 2001; Kannan 2004]

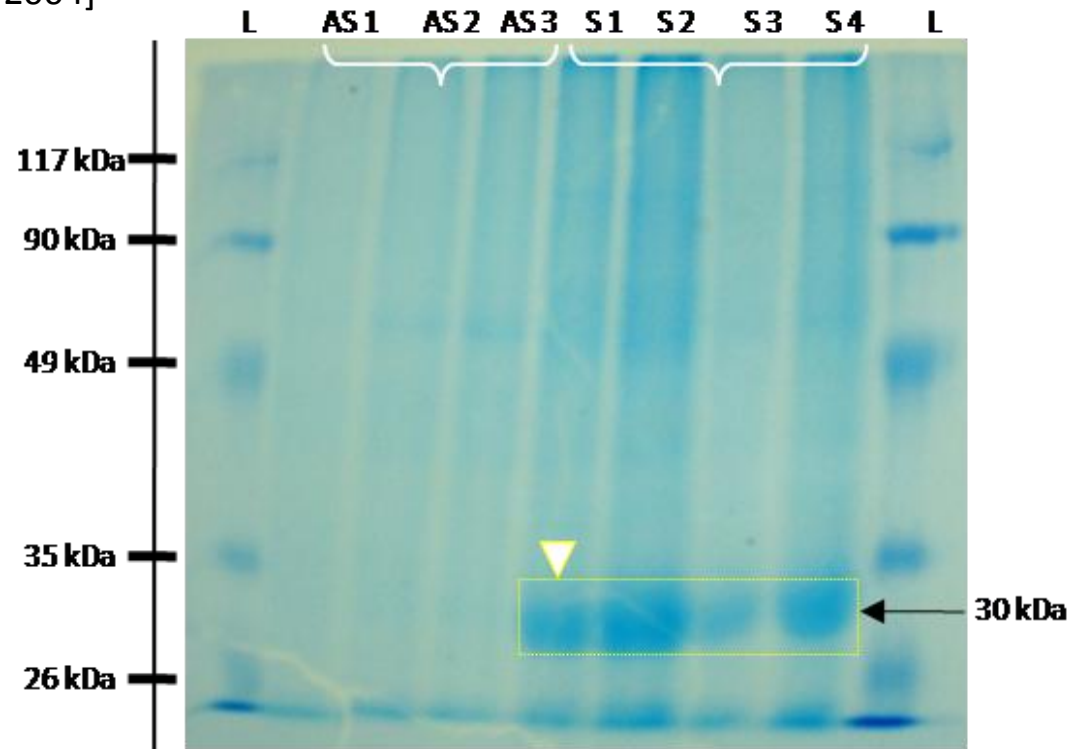
A. Isolation of purified viral protein from **nodal portion** of symptomatic and asymptomatic plants

B. Analysis of partial purified viral protein from **different tissues** of soybean plant

A. Isolation of purified viral protein from nodal portion of symptomatic and asymptomatic plants

▪ Symptomatic plant: S1 – S4

▪ Asymptomatic Plant : AS 1 – AS3

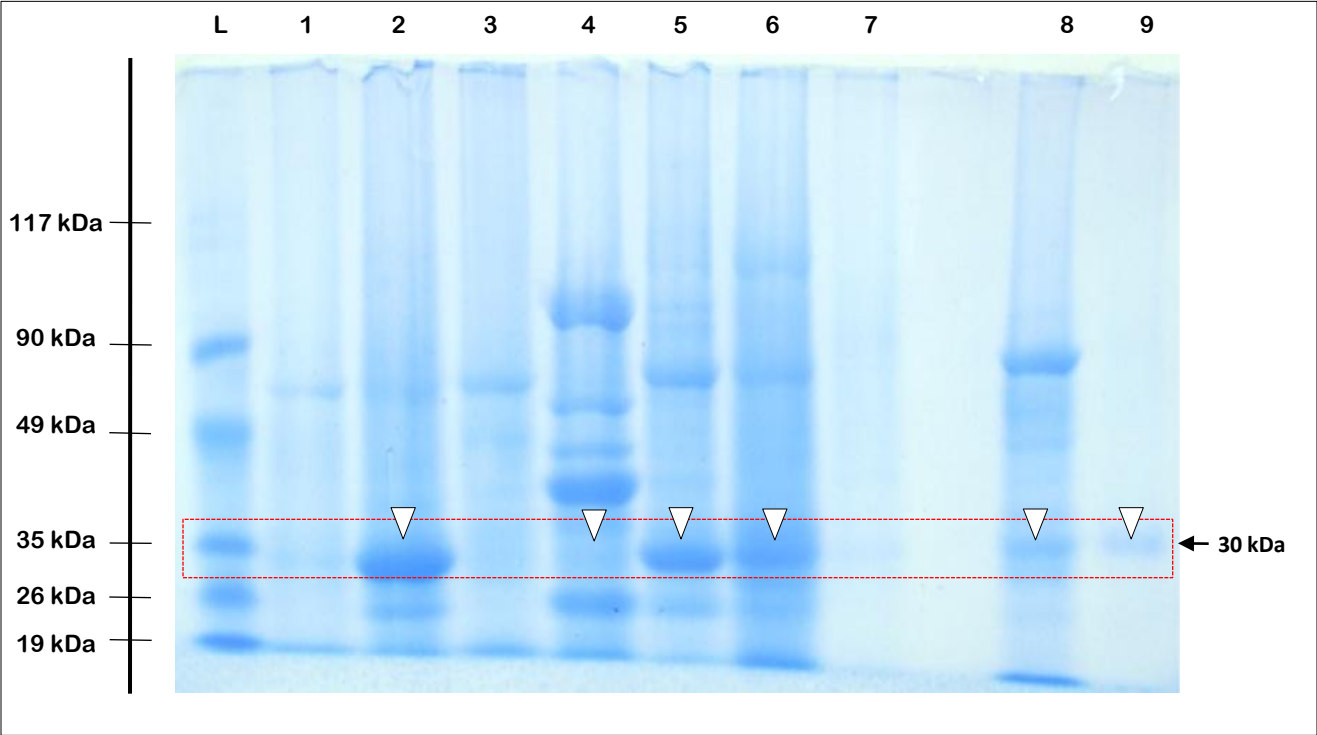


It reveals presence of the *Tobacco streak virus* in symptomatic soybean plants (S1 – S4)
Absent in asymptomatic soybean plant (AS1-AS3)

SDS-PAGE analysis of partial purified viral protein from different tissues of soybean plant

B. Analysis of partial purified viral protein from **different tissues** of soybean plant

- L:** Ladder
- 1:** Nodal portion (As)
- 2:** Nodal portion (S)
- 3:** Leaves (As)
- 4:** Pod (S)
- 5:** Leaf stalk (S)
- 6:** Leaves (s)
- 7:** Leaf stalk (As)
- 8 & 9:** Bud (S)



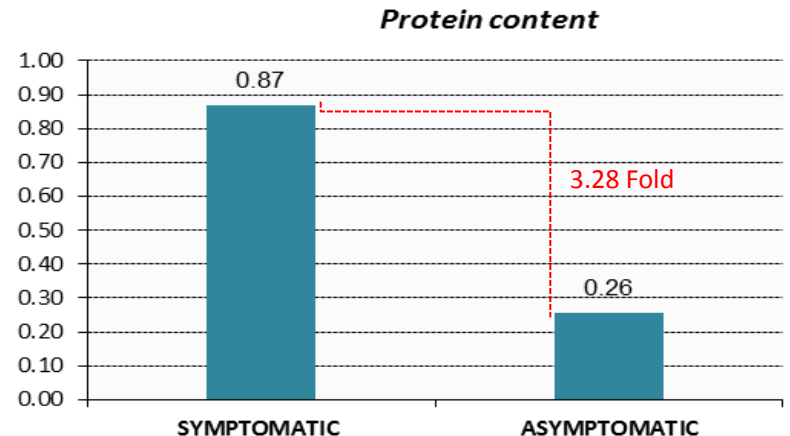
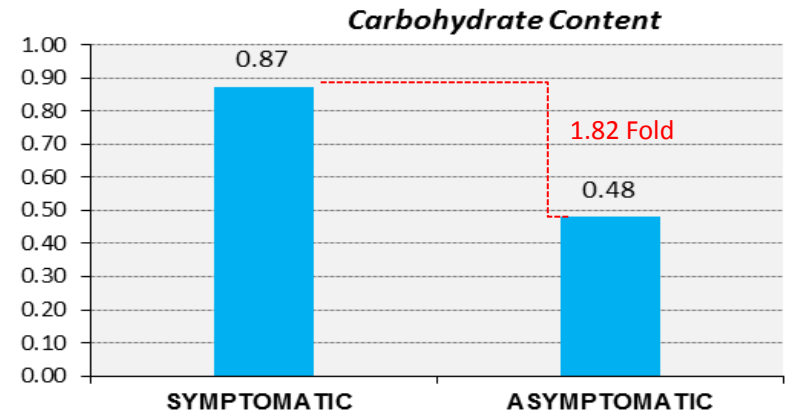
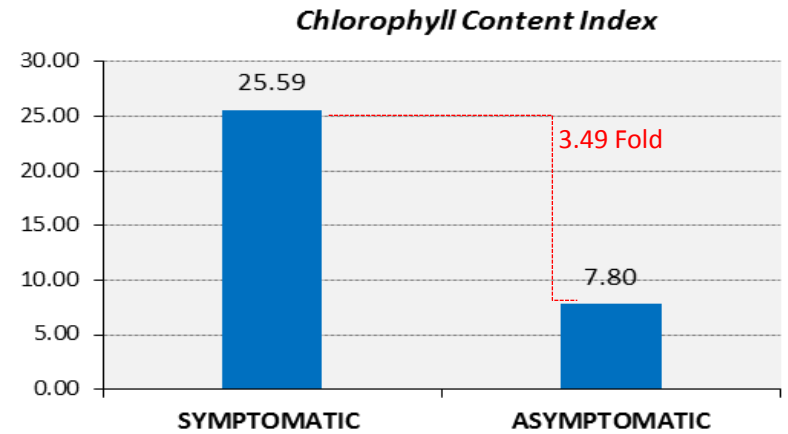
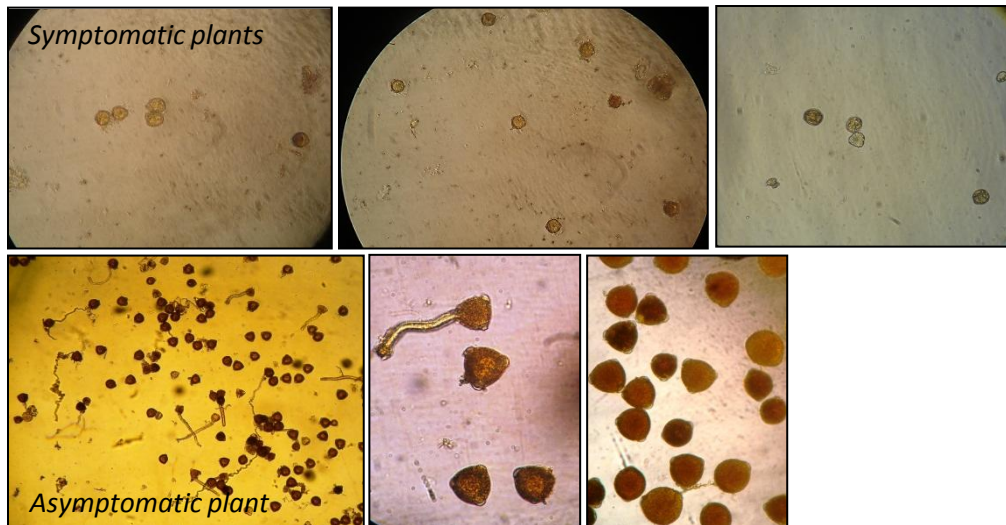
▶ It reveals presence of the *Tobacco streak virus* in symptomatic soybean plants

Biochemical studies in symptomatic and asymptomatic soybean plants

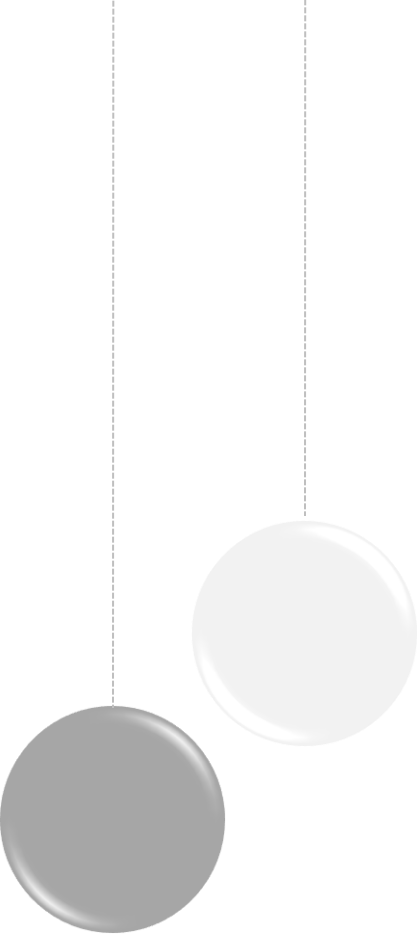
- Chlorophyll content
- Carbohydrate content
- Protein content

Microscopy of pollens from symptomatic and asymptomatic soybean plants

- Reduced number of pollens
- Non-functional pollens in symptomatic plants



ACHIEVEMENTS

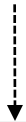
A decorative graphic on the left side of the slide. It features two overlapping circles: a dark gray circle in the foreground and a light gray circle behind it. Two vertical dashed lines extend upwards from the top of each circle towards the top of the slide.

- ▶ **Understanding of prevalence of soybean Floral Bud Distortion in Central India**
- ▶ **Understanding principle symptoms associated with FBD**
- ▶ **Detection of Causal agent(s) i.e. TSV and GBNV in soybean**
- ▶ **Biochemical differences in symptomatic and asymptomatic soybean plants**

PHASE - II

FUTURISTIC STRATEGY

Comparative tissue proteomic analysis in symptomatic and asymptomatic soybean plant



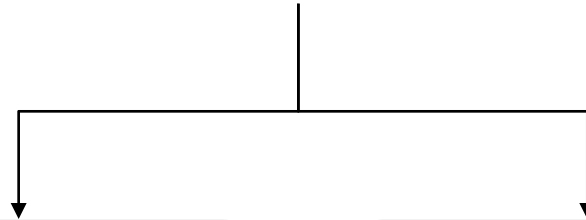
Identification of differentially expressed proteins in symptomatic and asymptomatic plant tissue



Highlight putative function of respective protein and interference between male and female floral organs

- *Understanding of putative functions of respective proteins expressed in floral organs at flowering stage*

Differential molecular analysis of affected and healthy soybean plant



Quantitative RT-PCR analysis using genes targeting to siphonogamy

Differential expression using Suppression Subtractive cDNA hybridization

- *Identification of differentially expressed genes in floral organs of symptomatic and asymptomatic plant*

ACKNOWLEDGEMENT

- Dr. R. K. Jain**

Head, Division of Plant Pathology, IARI, New Delhi

- Dr. V. K. Baranwal**

Principal Scientist/In-charge, Advanced center for Plant Virology, IARI, NEW DELHI

- Dr. Prashant Kavar**

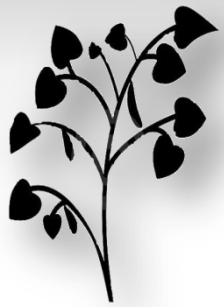
Scientist, Molecular Biology and Genetic Engineering Division, Vasantdada Sugarcane Institute, PUNE

- Dr. S.K. Raj**

Scientist-G & Head, Plant Molecular Virology Lab, Center for Plant Molecular Biology (CPMB), National Botanical Research Institute (NBRI), LUCKNOW



Thank You!

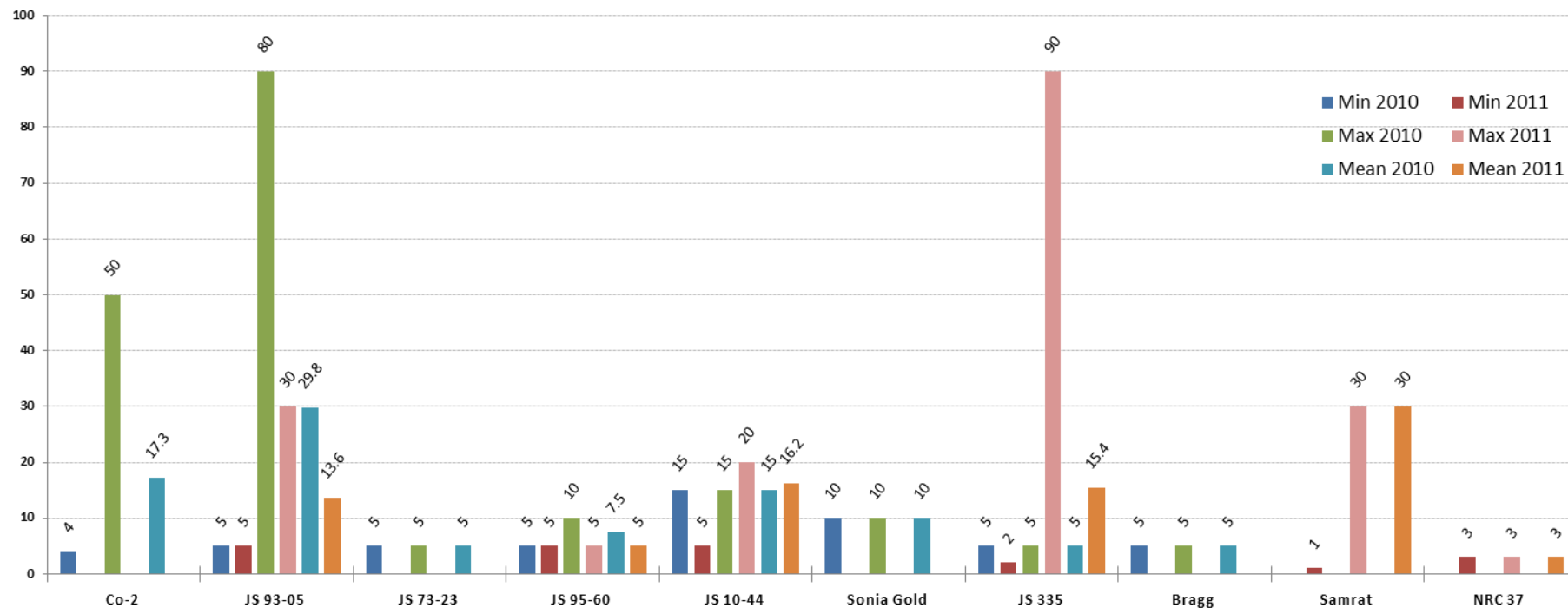


e-mail: jpraveen26@yahoo.co.in

Symptomes	:	Bud Blight	Green Bud Syndrome	Floral Bud Distortion
Yield loss	:			
Cause	:	TRSV, SMV	Stink bug, budworm	TSV, GBNV and any other unknown
Occurrence	:	All seasons	individual plants , small patches or areas <80 acres in size	Individual plant, randomly scattered in field
Vectors (if any)	:	Thrips, grasshoppers, dagger nematodes	-	Thrips
Seed born	:	Possible	-	-
Plant height	:	Stunted		
Bud/stem characteristics	:	- Bending of terminal bud to form characteristic hook - Browning, necrosis of bud, brown discoloration of pith	Uneven maturity of pods on individual plant	Distorted green bud Plant fails to develop plant
Pods	:	Plant may produce pods Pods may have brown patches	Plant may develop pods but remain green at maturity stage	Plant fails to develop even structural pod(s)
Leaf	:	leaflets may be smaller than normal and may deform by cupping		
Maturity	:	Delayed	Delayed	Delayed
Growth of N-fixing nodules	:	Suppressed	-	Normally grown
Conditions Favoring Disease	:	Fields next to clovers, pastures, and weedy areas		
Management	:	Plant virus-free seed Resistant variety		
Reference	:	http://cropdisease.crops.cornell.edu/soybeans/bud-blight.html		

FIELD SCOUTING

VARIETIES Affected by FBD during Kh-2011



Almost all grown soybean varieties (7) affected by FBD



FIELD SCOUTING

Location and genotype wise prevalence of Floral Bud Distortion in MP, MS and KA during Kh-2010-11 and 2011-12

SN	Soybean genotypes																		
		MP						MS						KA					
		Min		Max		Mean		Min		Max		Mean		Min		Max		Mean	
		Kh-10	Kh-11	Kh-10	Kh-11	Kh- 10	Kh- 11	Kh-10	Kh-11	Kh-10	Kh-11	Kh- 10	Kh- 11	Kh-10	Kh-11	Kh-10	Kh-11	Kh- 10	Kh- 11
1.	Co-2	4.0	-	50.0	-	17.3 (L-4/32ac)	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	JS 93-05	5.0	5.0	80.0	30.0	24.8 (L-10/58ac)	13.6 (L-11/56.5ac)	-	-	-	-	-	-	-	-	-	-	-	-
3.	JS 73-23	5.0	-	5.0	-	5.0 (L-2/14ac)	-	-	-	-	-	-	-	-	-	-	-	-	-
4.	JS 95-60	5.0	-	10.0	-	7.5 (L-2/30 ac)	-	-	-	-	-	-	-	-	-	-	-	-	-
5.	JS 10-40/44	15.0	5.0	15.0	20.0	15.0 (L-1/2ac)	16.3 (L-4/19ac)	-	-	-	-	-	-	-	-	-	-	-	-
6.	Sonia Gold	10.0	-	10.0	-	10.0 (L-2/14ac)	-	15.0	-	15.0	-	15.0 (L-3/35ac)	-	15.0	-	20.0	-	17.5 (L-3/35ac)	-
7.	JS 335	5.0	5.0	5.0	80.0	5.0 (L-1/2ac)	21.0 (L-5/23ac)	5.0	2.0	20.0	90.0	12.5 (L-6/52ac)	11.6 (L-15/66ac)	15.0	4.0	15.0	8.0	12.5 (L-1/13ac)	7.5 (L-3/17ac)
8.	AMS-MB-5-19	-	-	-	-	-	-	5.0	-	5.0	-	5.0 (L-1)	-	-	-	-	-	-	-
9.	AMS 353 x EC-5	-	-	-	-	-	-	5.0	-	5.0	-	5.0 (L-1)	-	-	-	-	-	-	-
10.	Bragg	-	-	-	-	-	-	5.0	-	5.0	-	5.0 (L-1)	-	-	-	-	-	-	-
11.	Samrat	-	-	-	30.0	-	30.0 (L-1/7.5ac)	-	-	-	-	-	-	-	-	-	-	-	-
12.	NRC- 37	-	-	-	-	-	-	-	-	-	2.0	2.0 (L-1)	-	-	-	-	-	-	-
13.	JS 94-52	-	-	-	10.0	-	10 (13 ac)	-	-	-	-	-	-	-	-	-	-	-	-