

## From the Director's desk....



Sorghum production and utilization scenario has been dynamically changing in the country due to erratic rainfall and its distribution pattern. Due to the scanty rainfall and non-availability of water in the reservoirs, during this year more acreage under sorghum cultivation may be expected in both traditional sorghum growing areas as well as non-traditional areas such as rice-fallows in coastal Andhra Pradesh.

At DSR, this year has been a period of improved performance and good achievements. We have been able to meet the challenges to a large extent through the development of improved sorghum cultivars and production technologies. Four new cultivars developed under AIC-SIP were recommended by the Central Varietal Release Committee (CVRC) for notification. Two more cultivars have been identified by the Varietal Identification Committee during the sorghum annual group meetings and the release proposals are being submitted to the CVRC for release notification.

Our efforts to showcase the sorghum food processing technologies and processed nutritious foods have made dent with private entrepreneurs and niche food industries through popularization programmes in rural areas, urban food markets and media. The public-funded programmes on promotion of millet foods through INSIMP and millet cultivation through Tribal Sub Plan implemented by the Directorate have been received well by the target groups and won accolades from funding agencies.

We believe that cultivars and production technologies have to be consistently improvised to suit the diverse environmental conditions to meet the production targets. The efforts are underway in this direction.



(JV Patil)

## सारांश

- वर्षाकालीन ज्वार हेतु आशाजनक पुनः स्थापक: अनाजपैदावार के लिए चार नए पुनः स्थापक वंशक्रम - सीबी 458, सीबी 456, सीबी 306 तथा सीबी 457 अत्यधिक श्रेष्ठ थे। इन पुनः स्थापकों तथा विभिन्न विशेषताओं के लिए आशाजनक अन्य पुनः स्थापकों का खरीफ मौसम हेतु नए संकरों के विकास में उपयोग किया जा सकता है।
- ज्वार कृष्य किस्मों तथा पैतृक वंशक्रमों के पौष्टिक गुण : पचास श्रेष्ठ ज्वार जीनप्ररूपों में पॉलीफिनॉल, फेटेट, एंटी-ऑक्सिडेंट गतिविधि, सैनोंजेंस, ट्रिप्सिन इन्हिबिटर गतिविधि, रेशे, आयरन तथा जिंक सम्मिलित पोषण संबंधी सभी कारकों हेतु किए गए अध्ययन में उत्तम भिन्नताएं पाई गईं। अतः ज्वार के स्वास्थ्य तथा पोषण गुणवत्ता में सुधार हेतु प्रजनन हस्तक्षेप एक आशाजनक प्रक्रिया है।
- चारा पैदावार तथा गुणवत्ता प्राचलों हेतु एसएसजी 59-3 के उत्परिवर्तनीय यौगिकों (म्यूटाजेनिक डेरिवेटिव) में भिन्नताएं : कुछ उत्परिवर्तित वंशक्रमों में चेत तथा पैतृक एसएसजी 59-3 की अपेक्षा प्रोटीन की मात्रा तथा चारा पाच्यता ज्यादा थी। सभी वंशक्रमों में एचसीएन की मात्रा 200 पीपीएम की सुरक्षा सीमा के अंदर थी। उन्नत चारा गुणवत्ता युक्त वंशक्रमों का चारा ज्वार प्रजनन कार्यक्रम में प्रयोग किया जा सकता है।
- एसएसआर चिह्नों के प्रयोग द्वारा ज्वार जननद्रव्य प्रविष्टियों की आनुवंशिक विविधता का विश्लेषण : इस अध्ययन के परिणाम, आनुवंशिक विविधता के मूल्यांकन हेतु सिंपल सिक्वेस रिपिट (एसएसआर) चिह्नों की उच्च क्षमता की पुष्टि करते हैं। डेड्रोग्राम के विभिन्न समूहों से, वर्तमान प्रजनन कार्यक्रम में प्रयोग करने हेतु अपेक्षित लक्षणों पर आधारित आशाजनक जननद्रव्य वंशक्रमों का पता लगाया गया।
- धब्बेदार तना बेधक के प्रति प्रोटिएस इन्हिबिटर की जैविक क्रिया : सोयाबीन ट्रिप्सिन इन्हिबिटर युक्त कृत्रिम आहार तना बेधक में डिंबक भार कम करता है तथा उनकी वृद्धि व विकास को भी प्रभावित करता है। अतः तना बेधक से होने वाली क्षति को कम करने हेतु ज्वार के आनुवंशिक रूपांतरण में उपयोग करने के लिए इसे उम्मीदवार जीन माना जा सकता है।
- तना बेधक सह्यता हेतु दो बीटी जीनों युक्त पराजीनी मीठी ज्वार: उन्नीस पराजीनी घटनाओं में बीटी पराजीनों के स्थायी वशागती की पुष्टि की गई। संतती में स्वच्छ पर्ण ऊतकों में बीटी प्रोटीन 15-72.8 नैतो ग्रा / ग्रा. पर्ण ऊतक दर्ज किया गया।

सारांश - डॉ. महेश कुमार

## Research highlights

### Promising restorers for rainy season sorghum

The yield levels in kharif sorghum have reached a plateau and to break this plateau we need new improved parental lines to develop improved high yielding hybrids. In this study, diversified germplasm from world-wide collection were used to improve the available restorers of kharif hybrids. Sixty such restorers were evaluated for their performance for yield and yield related traits in randomized block design with three replications during kharif season for three years (2007, 2008 and 2010). The R line, C43 (male parent of CSH 16) was included as check in the experiment.

For grain yield, 20 lines yielded more than both the checks. Four lines, CB 458, CB 456, CB 306 and CB 457 were significantly supe-

rior for grain yield. Six restorers were having longer panicles compared to the check. The lines CB 446, CB 327 and CB 307 had panicle length more than 30 cm. Twenty lines had more number of primary branches than the check. CB 306 was having high number of primary branches followed by CB 81, CB 456 and CB 315. CB 462 and CB 457 were found to flower 3-5 days earlier to the check. These restorers promising for different traits can be used in developing new hybrids for growing in kharif.

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## Performance of promising rainy season restorers for grain yield and related traits (Average of 2007, 2008 and 2009)

R line	Plant height (cm)	Days to flower	No. of primary branches	Panicle length (cm)	Grain Yield/Plant (g)
CB 79	190.9	73	65.27	25.80	73.79
CB 81	199.6	73	71.44	26.98	66.67
CB 85	171.4	75	59.51	27.80	64.33
CB 435	163.3	73	68.87	26.99	65.11
CB 437	190.9	74	67.04	26.84	73.89
CB 446	167.5	72	57.96	30.37	65.11
CB 307	169.3	67	63.38	30.11	72.67
CB 452	166.5	72	57.20	26.01	69.44
CB 456	203.3	73	70.76	29.87	78.89
CB 306	193.5	73	76.09	26.45	77.00
CB 457	186.3	67	44.20	24.80	77.24
CB 458	194.7	69	55.49	28.43	83.33
CB 459	171.9	72	66.67	28.73	70.54
CB 460	187.5	75	56.31	28.29	74.80
CB 462	158.8	66	64.25	29.07	52.89
CB 308	171.8	73	55.24	21.65	71.56
CB 315	187.2	73	70.27	26.67	68.67
CB 316	158.6	76	58.39	29.23	61.33
CB 318	171.2	76	67.04	27.33	66.17
CB 321	170.6	71	54.00	26.12	64.44
CB 325	171.9	76	55.20	27.36	56.61
CB 327	166.3	68	63.35	30.15	53.32
C43	159.8	73	48.41	22.98	60.85
Mean	176.5	72.4	57.77	24.61	61.7
CD (5%)	17.62	2.3	7.69	7.44	17.54
CV (%)	6.18	5.0	18.23	4.96	17.51

## Nutritional property of sorghum cultivars and parental lines

Sorghum and other millets are considered as nutritious cereals. Still, the extent of research on the nutritional quality is meager. Extensive information is available on proximate composition of sorghum, but its phenolic content and associated antioxidant properties have not received the same interest.

Sorghum grains are known to contain good amount of polyphenols and other factors like fibre, phytate, tannin, etc. Sorghum is also reported to contain cyanogens and trypsin inhibitors. Trypsin inhibitors inhibit proteolytic enzymes. It is reported that the micronutrients content in sorghum grains is higher than that observed in other commonly consumed cereals. The protein and starch in grain sorghum are more slowly digested than other cereals, and slower rates of digestibility are particularly beneficial under some conditions.

Keeping the above in mind, a preliminary study was conducted to estimate the contents of nutritional/anti-nutritional factors in 50 sorghum genotypes comprising popular cultivars and parental lines of hybrids, and some germplasm accessions/breeding lines that are used in resistance breeding. Good variation was observed for all the factors studied like polyphenol, phytate, anti-oxidant activity (TEAC- Trolox equivalent antioxidant capac-

ity), cyanogens, TI (Trypsin inhibitor activity), fibre, iron and zinc during the year 2009. The variety SSG 59-3 had the highest polyphenol (1272 gallic eq mg/100g) followed by Urja (1135 gallic eq mg/100g), while the lowest was found in AKMS 14B (44 gallic eq mg/100g). The phytate content was the highest in DJ 6514 (3415 mg/100g) and the lowest was in POP 52 (720 mg/100g). The highest TEAC was shown by SSG 59-3 (2238 trolox eq mg/100g) followed by Urja, both having the dark coloured grains/glumes. But all coloured grains did not show higher TEAC. M 35-1, the most popular rabi cultivar exhibited lowest TEAC (33.4 trolox eq mg/100g). The fibre (NDF) content ranged between 5 (SLR 10) and 21% (AKR 150) with an average of 13%. Wide range was observed for cyanogens and TI. Parental lines like 2219B, 2077B and AKR 354, and varieties CSV 22 and CSV 14R had lower cyanogens and TI contents. The iron and zinc content also exhibited very wide range.

## Mean values for different nutritional factors

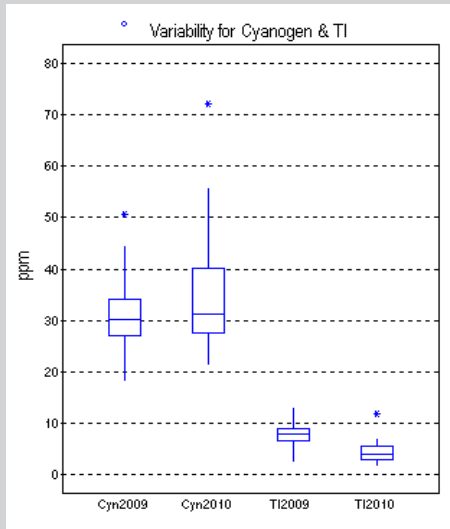
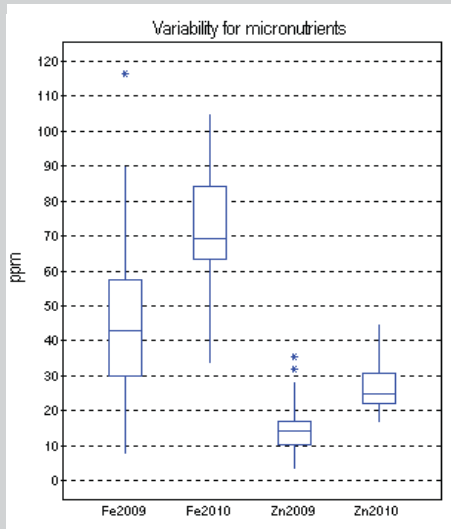
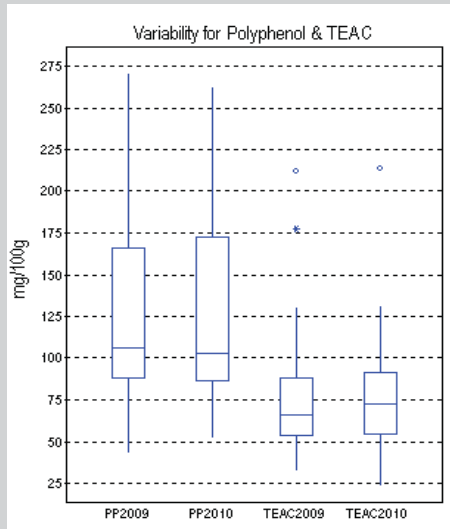
Nutritional factor	2009		2010		Correlation between years (r)
	Mean ± SE	CV (%)	Mean ± SE	CV (%)	
Polyphenol (mg/100g)	164.9 ± 10.8	9.25	124.0 ± 4.4	6.08	0.399
Phytate (mg/100g)	2434.5 ± 85.2	4.95	2362.2 ± 52.9	3.88	0.744**
TEAC (mg/100g)	146.4 ± 8.0	7.77	104.4 ± 9.9	13.37	0.982**
Cyanogen (ppm)	36.6 ± 1.9	7.24	40.9 ± 1.0	4.13	0.931**
Trypsin Inhibitor	7.6 ± 0.9	16.38	4.2 ± 0.2	5.95	0.167
NDF (%)	13.2 ± 1.3	14.14	-	-	-
Iron (ppm)	43.9 ± 5.1	16.36	70.7 ± 5.2	10.38	0.073
Zinc (ppm)	14.7 ± 2.4	22.74	26.3 ± 5.9	31.91	0.362

\*\* Highly significant (p<0.001)

In order to confirm the results, about 25 genotypes including those that had extreme values were reanalyzed during 2010. The ranges obtained were almost within the range obtained previously. The chemical analysis in SSG 59-3 was performed without glumes which lead to lower polyphenol content but TEAC was the highest again. Correlation between old and new values was calculated to find the consistency of mean values. The correlation was significant for polyphenol, phytate, TEAC and cyanogens. In case of polyphenol exclusion of one value (SSG 59-3) lead to rise in the correlation from 0.4 to 0.86 (p< 0.001). From the results we can assume that the mean values obtained are repetitive for these four factors.

In case of TI the correlation was non-significant between old and new values. Similar is the case with micronutrients where most of the individual mean values did not match with old result, and the overall mean was higher. This shows that the results for iron and zinc are not repetitive. The inconsistency in the results may be due to environmental influence as samples analysed were from different seasons or due to analytical errors. Hence, repeated analysis preferably from different seasons and locations is must before identifying suitable donor parents for micronutrient improvement. For other nutritional factors sufficient variability has been observed among the cultivars and parental lines, and therefore, breeding interventions to improve the health and nutritional quality of sorghum holds promise.

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Variability for nutritional factors in sorghum during 2009 and 2010

## Sorghum transgenic plants for salinity tolerance

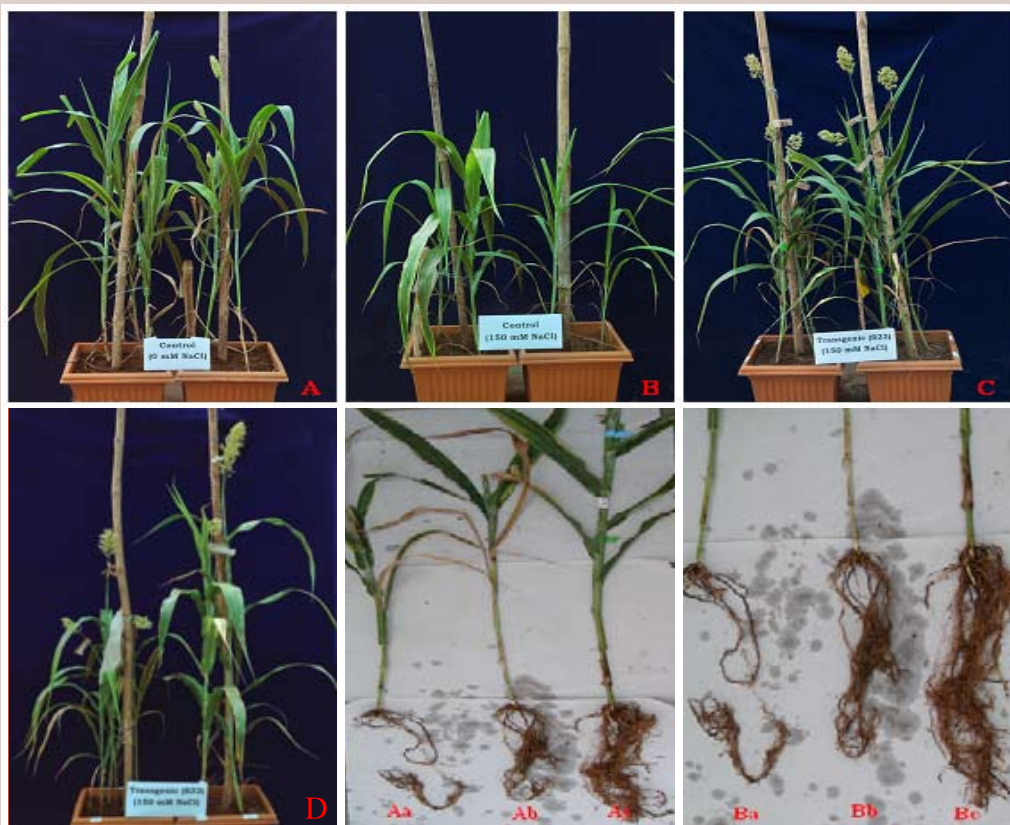
Sorghum is one of the main dry-land grain crop in India and worldwide. Salinity is one of the main limiting factors in agriculture in several parts of the semi-arid and arid regions. So far, attempts to improve the salt tolerance in sorghum through conventional breeding programmes have met with very limited success. Application of molecular genetics and plant transformation techniques may form very potential tools for the development of salt tolerant crops. Sorghum transgenic plants with serine-rich protein gene, *PcSrp* (isolated from *Porteracea coarctata*, a wild halophytic grass) controlled by rice actin (*ACT1*) promoter was successfully produced via particle bombardment using shoot tip explants of sorghum genotype

M35-1. Ten sorghum transgenic events carrying *PcSrp* gene were selected and screened for salinity tolerance in  $T_2$  generation. The transgenic along non-transgenic control (M35-1) plants were grown in pots saturated with 150 mM NaCl till the maturity in glass house condition. In this condition, the four transgenic events (S23, S27, S31 and S32) out of ten tested reached normal development and set seed where as the control plants grown with and without NaCl delayed flowering indicated that these four events were tolerant to salinity. The development of good roots system in the promising four transgenic events was not affected by salinity when compared with the control plants grown with and without NaCl.

### Screening of $T_2$ generation sorghum transgenic events for salinity tolerance in 150 mM NaCl

A-Non-transformed control plants grown in 0 mM NaCl; B-Non-transformed control plants grown in 150 mM NaCl; C-Transgenic event S23 plants grown in 150 mM NaCl; D-Transgenic event S32 plants grown in 150 mM NaCl;

Aa & Ba-Non-transformed control plants grown in 150 mM NaCl; Ab & Bb-Non-transformed control plants grown in 0 mM NaCl; Ac & Bc-Transgenic event S32 plants grown in 150 mM NaCl.





## Performance of SSG 59-3 mutant derivatives for fodder yield and morphological traits over two years (2009 and 2010)

Mutant	GFY (q/ha)	DFY (q/ha)	Plant height (cm)	Days to flower	Leaf no.	Leaf length (cm)	Leaf breadth (cm)	Early vigour	Stem girth (cm)	Tiller no.	Leaf-stem ratio
SSG 224	664.0	169.7	273.8	75.4	12.4	78.1	4.9	3.33	2.51	3.45	0.28
SSG 225	651.9	132.2	253.1	71.4	11.0	78.0	5.2	3.50	2.22	4.18	0.25
SSG 226	677.4	143.8	268.0	72.6	11.0	72.7	4.8	3.67	2.12	4.00	0.39
SSG 227	718.1	152.5	267.4	76.3	11.9	80.2	5.0	3.00	2.42	3.99	0.33
SSG 231	716.0	163.3	260.0	76.8	11.8	81.2	5.7	3.50	2.60	3.23	0.27
SSG 232	611.6	111.3	261.6	67.5	10.7	78.6	5.3	3.17	2.81	4.45	0.26
SSG 233	498.9	91.4	244.6	67.2	13.0	84.0	4.9	3.00	2.66	4.25	0.29
SSG 234	616.6	128.0	251.2	72.9	10.5	76.9	4.7	3.33	2.25	4.80	0.24
SSG 236	663.9	144.9	266.1	76.4	12.1	79.9	5.3	3.17	2.14	4.58	0.30
SSG 237	440.5	106.3	254.9	60.7	10.5	74.8	4.9	4.00	1.95	4.07	0.32
SSG 241	533.1	125.6	263.5	69.4	11.8	76.5	5.4	2.83	2.54	4.13	0.28
SSG 244	576.0	137.6	273.9	68.9	11.9	81.6	5.5	2.67	2.55	4.43	0.25
SSG 253	602.4	127.9	262.0	66.9	11.9	76.1	5.3	3.67	2.34	3.97	0.23
SSG 256	721.4	159.1	261.7	78.6	11.3	81.2	5.3	3.50	2.29	3.88	0.31
SSG 260	498.5	103.7	247.3	70.4	11.3	77.7	6.0	3.00	1.96	3.80	0.26
SSG 59-3	815.6	181.0	280.9	70.3	12.4	80.3	4.8	4.33	2.11	5.30	0.24
Mean	634.9	167.5	261.9	71.3	11.6	78.6	5.2	3.35	2.34	4.16	0.28
C.V.	12.9	19.2	6.4	2.1	9.1	5.9	10.4	23.00	32.51	21.25	12.63
C.D. 5%	81.5	22.8	18.8	2.5	1.6	7.3	0.8	1.24	0.45	1.23	0.07

## Variation among the mutagenic derivatives of SSG 59-3 for fodder yield and quality parameters

The popular multicut forage sorghum variety, SSG59-3 was released in 1974. It was obtained from an advanced-generation selection of a cross between non-sweet Sudan grass and the sweet sorghum variety JS 263. It has high tillering capacity and can give three cuts. Till today it is being used as a check variety in AICSIP forage sorghum trials.

Efforts were made to create variability in SSG 59-3 for different traits through mutagenesis at Hisar. The variety SSG 59-3 was planted after treating with the combined treatment of gamma rays doses viz., 30, 40 and 50 kR + 0.2 % EMS treatment. Not even a single plant germinated at 40 and 50 kR + 0.2 % EMS treatment. Forty nine plants germinated after treatment with 30 kR + 0.2% in M1 generation were raised in kharif 2003 and selfed for getting M2. Their M2 generation was evaluated for variation in kharif 2004. These plants have been selfed since then and selections have been made for different traits.

Fifteen such mutagenic derivatives of SSG 59-3 were evaluated for morphological and quality traits at 3 centres (Hisar, Pantnagar and DSR) during 2009 and at 5 centres (Hisar, Pantnagar, Udaipur, Ludhiana and DSR) during 2010. The results over two years indicated that eventhough there were no lines with better fodder yield over SSG 59-3, lot of variation was observed for earliness, leaf parameters and fodder quality. Four lines flowered earlier to SSG 59-3 of which SSG 237 was the earliest (10 days earlier to SSG 59-3). SSG 237 had narrow leaves with high leaf/stem ratio compared to SSG 59-3. The SSG 233 had longer leaves with more leaf stem ratio compared to SSG 59-3. High leaf stem ratio was observed in SSG 226, SSG 227 and SSG 256 also.

The lines SSG 231, SSG 260, SSG 232 and SSG 237 had high protein percentage and IVDMD values compared to SSG 59-3. High IVDMD of 53% was observed in SSG 226 whereas SSG 59-3 recorded 47% IVDMD. All the lines had HCN content within the safe limit of 200ppm, with least being in SSG 226 (67 ppm), followed by SSG 232 (71 ppm). The lines with improved fodder quality (high protein and IVDMD) can be used in the breeding program.

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**Simarjit Kaur, PAU, Ludhiana, BR Ranwah, MPUA&T, Udaipur**  
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## Performance of SSG 59-3 mutant derivatives for fodder quality over two years (2009 and 2010)

Genotype	Protein (%)	IVDMD (%)	HCN (ppm)
SSG 224	9.29	49.80	79.48
SSG 225	9.09	49.51	86.78
SSG 226	8.91	53.10	66.63
SSG 227	8.87	49.65	72.01
SSG 231	9.60	51.03	73.92
SSG 232	9.45	50.06	70.56
SSG 233	7.50	50.46	85.95
SSG 234	7.76	48.85	112.04
SSG 236	8.31	50.29	83.76
SSG 237	9.42	51.53	87.95
SSG 241	8.44	48.12	74.84
SSG 244	8.04	48.56	83.12
SSG 253	9.08	49.91	91.47
SSG 256	8.84	51.05	99.39
SSG 260	9.51	51.63	73.83
SSG 59-3	9.05	47.12	82.89
Mean	8.92	49.97	82.79
C.V.	5.50	4.54	11.94
C.D. 5%	0.75	2.98	16.58

## Genetic diversity analysis of selected sorghum germplasm accessions using simple sequence repeat (SSR) markers

Genetic diversity analysis of selected sorghum germplasm accessions was carried out using simple sequence repeat (SSR) markers. This study includes 96 local germplasm accessions collected from different states of the India and the World of different races were maintained at DSR germplasm collection unit. These germplasm lines had been phenotyped (passport data) for a number of traits includes pest resistance (shoot fly and stem borer), disease resistance (grain mold, downy mildew and charcoal rot), drought (terminal drought and stay green trait), fodder and roti making quality. This investigation was an attempt to analyze the genetic diversity in these germplasm lines and explore the possibility of using SSR markers to group these lines for different agronomic traits.

A total of 104 alleles generated from 20 sorghum Xtxp SSR markers detected by silver-stained non denaturing PAGE gels. The average number of alleles amplified per locus was 5.2 ranged from 2 to 8 alleles. Maximum number of alleles (8) per locus was observed with the Xtxp 286 followed by 7 alleles (Xtxp 12, Xtxp159, and Xtxp 141), 6 alleles (Xtxp 319, Xtxp 58, Xtxp 100, Xtxp 258, Xtxp 20 and Xtxp 18), 5 alleles (Xtxp 38, Xtxp 289, Xtxp 210 and Xtxp 15), 4 alleles (Xtxp 177 and Xtxp 65), 3 alleles (Xtxp 168, Xtxp 57 and Xtxp 17) and with Xtxp 59 only 2 alleles were observed. The observed level of SSR polymorphism was found to be sufficient to distinguish precisely in all the 96 germplasm accessions included in this study from each other.

The genetic diversity between genotypes was assessed on the basis of similarity coefficients and complemented with UPGMA cluster analysis. The correlation coefficients between the co-phenetic matrix computed from the dendrogram and the original similarity matrices were  $r=0.76$  ( $t=18.94$ ,  $p=1.00$ ) for the PAGE-generated marker data set. The coefficients of similarity ranged from 0.15 to 0.90 and these results suggested a good fit of the dendrogram generated from the rough data values. The dendrogram grouped the 96 sorghum germplasm lines into 9 major groups.

The results of this exploratory study confirmed the high potential of SSR markers for the assessment of genetic diversity. The efficiency of identification of sorghum genotypes possible using these genetic markers is indicated by the clear distinctiveness of all 96 genotypes used in this study using just 20 marker loci were distributed throughout the 10 sorghum chromosome pairs. The 96 germplasm lines used in this study are of shoot fly and stem borer resistant, stay green trait lines, drought tolerant lines and lines for fodder and roti making quality. Based on their positions in the dendrogram, few promising germplasm lines based on their trait of interest (shoot fly resistant germplasm line E77, stay green lines SEVS3 and SEVS26) from different cluster in the dendrogram were identified for current breeding programmes, development of mapping populations and line improvement programmes.

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## Grouping of genotypes based on dendrogram analysis based on Jaccard's similarity coefficient

Genotypes	Trait	Geographical origin
<b>Group 1 (16 genotypes)</b>		
IS 2123	shoot fly and stem borer resistant	USA
IS 1055	shoot fly resistant	India
IS 1082	shoot fly, stem borer, midge resistant	India
EP 112 (IC 345201), EP 114 (IC 345203), EP 117 (IC 345206) and EP 120 (IC 345209)	potential stay green local germplasm	Karnataka
EP 124 (IC 420939) and EP 127 (IC 420942)	potential stay green local germplasm	Maharashtra
Hathikunta and Tandur local	land races	Andhra Pradesh
Swathi, SPV 462, M35-1 and CSV14R	improved rabi variety	India
DJ6514	susceptible shoot fly genotype	India
E 77 (IC 333435)	promising shoot fly resistant germplasm	UP
<b>Group 2 (12 genotypes)</b>		
EP 37 (IC 305918), EP 42 (IC 305923), EP 45 (IC 305926), EP 46 (IC 305927) and EP 52 (IC 305933)	promising stay green germplasm	Karnataka
EP 41 (IC 305922)	durra bicolor intermediate germplasm	Karnataka
EP 115 (IC 345204)	Land race used for papad preparation	Karnataka
EP 105 (IC345194)	pop sorghum	Karnataka
EP 106 (IC 345195) and EP 107 (IC 345196)	durra promising stay green	Karnataka
EP 123 (IC 420938)	fodder quality germplasm	Maharashtra
EP 128 (IC 420943)	roti making quality germplasm	Maharashtra
<b>Group 3 (9 genotypes)</b>		
EP 88 (IC 343587), EP 90 (IC 343589), EP 91 (IC 343590), EP 92 (IC 343591), EP 93 (IC 343592), EP 94 (IC 343593), EP 95 (IC 343594)	promising drought tolerant lines	Maharashtra
EP 82 (IC 343581) and EP 55 (IC 343554)	promising stay green lines	Maharashtra
<b>Group 4 (20 genotypes)</b>		
296B	terminal drought resistant B-line	India
EP 58 (IC 343557) and EP 59 (IC 343558)	fodder quality lines	Karnataka
EP25 (IC 305906)	promising stay green line	Karnataka
EP 83 (IC 343582), EP 84 (IC 343583), EP 86 (IC 343585) and EP 87 (IC 343586)	promising drought tolerant lines	Maharashtra
EP 22 (IC 305903), EP 23 (IC 305904), EP 57 (IC 343556), EP 24 (IC 305905), EP 64 (IC 343563), EP 65 (IC 343564), EP 68 (IC 343567), EP 73 (IC 343572), EP 78 (IC 343577), EP 79 (IC 343578), EP 80 (IC 343579) and EP 81 (IC 343580)	promising stay green germplasm	Maharashtra

## Development of transgenic sweet sorghum for tolerance to stem borer

Sweet sorghum cultivars are highly susceptible to yield losses due to damage by the stem borer (*Chilo partellus*) insect pest. Introduction of Bt genes *cry1Aa*, *cry1Ac*, *cry1B* and *vip* through genetic transformation for imparting resistance to stem borer is a viable option. At DSR, Hyderabad, Bt transgenic plants were generated in sweet sorghum genotypes RSSV 9 and SSV 84 and characterized for transgenic expression.

Transgenic plants carrying Bt genes (*1Aa* or *cry 1B*) were recovered after particle bombardment and Agrobacterium method in both the genotypes. A total of 196 T<sub>0</sub> plants were generated, among which 61 were confirmed as transformants by PCR analyses. After advancement to T<sub>2</sub> generation, 64 of 189 T<sub>2</sub> progeny plants were confirmed by PCR, some of which were verified for transgene integration by Southern analysis. Transgenic expression through Western blotting was confirmed in six events in RSSV9 (2 with *cry1Aa* and 4 with *cry1B*) and one event in SSV84 (with *cry1B*) were confirmed so far. Bt protein in fresh leaf tissues of the progeny plants ranged from 15-72.8 ng/g leaf tissue. Seed set in confirmed plants was low.

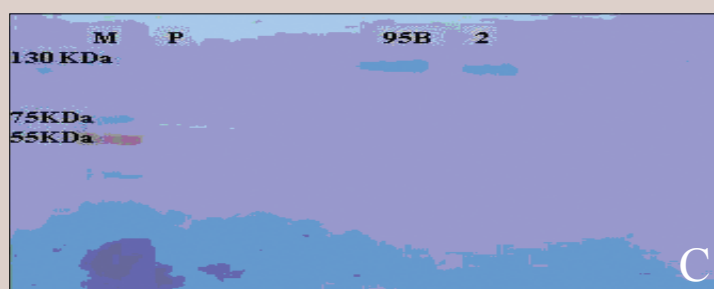
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Group 5 (3 genotypes)		
EP 102 (IC 345191) and EP 103 (IC 345192)	promising stay green germplasm	Karnataka
EP 97 (IC 345186)	promising stay green line	Karnataka
Group 6 (20 genotypes)		
EC 1 (IC 345703)	durra caudatum intermediate stay green line	
EC 34 (IC 345736)	durra promising stay green germplasm	
PEC 26 (IC 392149) and PEC 22 (IC 392145)	promising stay green germplasm	Andhra Pradesh
EA 6 (IC 345248)	guinea caudatum promising stay green line	India
EA 10 (IC 345252)	bicolor promising stay green germplasm	India
EA 11 (IC 345253)	guinea promising stay green germplasm	India
PEC 2 (IC 392125)	guinea promising stay green germplasm	Maharashtra
PEC 5 (IC 392128) and PEC 7 (IC 392130)	promising drought tolerant germplasm	Maharashtra
EP 1 (IC 305882), EP 60 (IC 343559), EP 9 (IC 305890), EP 13 (IC 305894), EP 14 (IC 305895), EP 16 (IC 305897) and EP 17 (IC 305898)	promising staygreen germplasm	Maharashtra
PEC 15 (IC 392138)	stay green germplasm	Karnataka
EP 104 (IC 345193)	durra bicolor intermediate promising stay green line	Karnataka
IS 18551	Shoot fly, stem borer, downy mildew resistant	Ethiopia
Group 7 (5 genotypes)		
EC 11 (IC 345713), EC 12 (IC 345714) and EC 20 (IC 345722)	promising stay green germplasm	Andhra Pradesh
EA 2 (IC 345244)	durra promising stay green line	India
EA 4 (IC 345246)	bicolor promising stay green line	Andhra Pradesh
Group 8 (5 genotypes)		
EP 133 (IC 420948)	promising stay green germplasm line	Maharashtra
SEVS 2 (IC 347568)	guinea fodder quality /stay green germplasm	Andhra Pradesh
SEVS 3 (IC 347569), SEVS 8 (IC 347574), SEVS 18 (IC 347584), SEVS 20 (IC 347586) and SEVS 26 (IC 347592)	durra staygreen lines	Andhra Pradesh
SEVS 17 (IC 347583)	guinea late flowering germplasm	Andhra Pradesh
Group 9 (2 genotypes)		
EC 21 (IC 345723) and EC 33 (IC 345735)	promising stay green lines	Andhra Pradesh



**Genetic transformation and molecular characterization of Bt transgenic sweet sorghum**

A. PCR analysis of *cry1B* transgenic DNA samples with bar primers. B. Southern blot analysis of *cry1B* transgenic DNA and plasmid samples were digested with the *Hind III* and probed with PCR amplified *cry1B* fragment. C. Western blot analysis with pure protein and transgenic plant protein were blot transferred and treated with *Cry1B* antibody.





## Biological activity of protease inhibitor against spotted stem borer, *Chilo partellus*

Stem borer (*Chilo partellus* Swinhoe) is the most destructive pest of sorghum throughout the crop growth and development, both in Asia and Africa. Considerable progress has been made in developing transgenic plants with toxin genes from *Bacillus thuringiensis* (Bt) for resistance to stem borer. However, there are distinct possibilities of development of resistance to Bt and hence, the need to identify alternative genes such as protease inhibitors, for deployment through transgenic crops to control this pest. Hence we studied the biological effects of soybean trypsin inhibitor on the growth and development of *C. partellus*, to identify candidate genes for deployment through transgenic plants for controlling this pest.

**Biological activity of soybean trypsin inhibitor (SBTI) through surface treatment of artificial diet:** SBTI was tested at 0.5% and 1.0% concentration through surface treatment into the artificial diet. The larvae were released on the artificial diet. Ten neonate larvae were released in each replication. There were five replications for each treatment in a completely randomized design. The vials were kept in the insect rearing laboratory at 27±1°C, 65% relative humidity, and 12 h photoperiod. Larval weights and mortality were recorded on tenth day after initiating the experiment. Haemolymph was collected from the few surviving larvae of SBTI treated and control insects and centrifuged in refrigerated centrifuge at 10,000 rpm for 10 min at 4°C to sediment the haemocytes and to get clear plasma. Protein content of haemolymph was estimated according to Lowry et al. (1951), using bovine serum albumin as the standard.

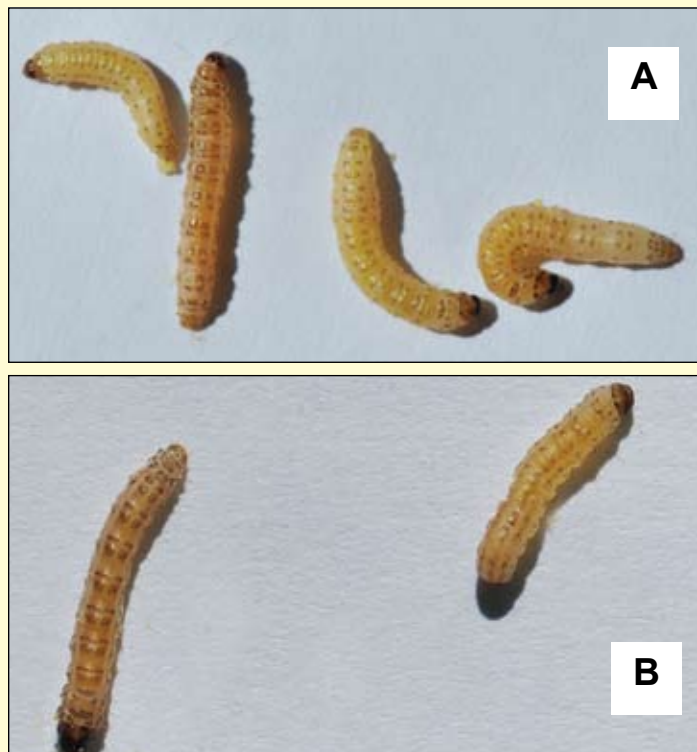
Fewer larvae survived (13-23%) when fed on diet treated with SBTI when compared to the larvae fed on the control diet (90%) at 10 days after initiating the experiment. There was a significant reduction in larval weights in diet having trypsin inhibitor (54.0 and 70.9 mg at 1 and 0.5%, respectively) as compared to the untreated control diet (91.8 mg). Post-embryonic development period was prolonged in larvae fed on artificial diets containing trypsin inhibitor.

### Effect of soybean trypsin inhibitor on larval weight of *Chilo partellus*

One of the most striking features of the development of holometabolous insects is the synthesis of a quantitatively significant class of polypeptides known as storage proteins. These proteins comprise the bulk of the polypeptides in larval haemolymph. Quantitative differences between SBTI

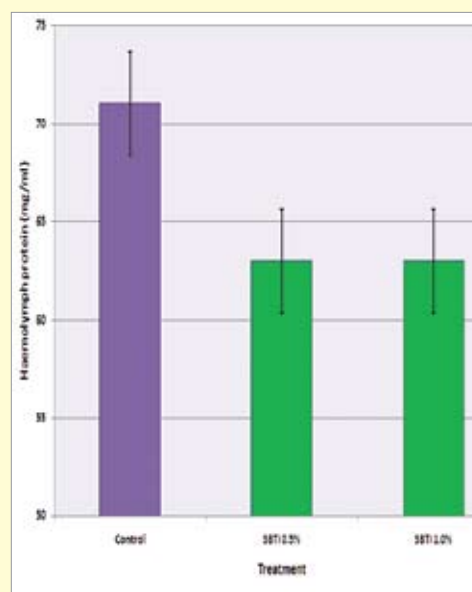
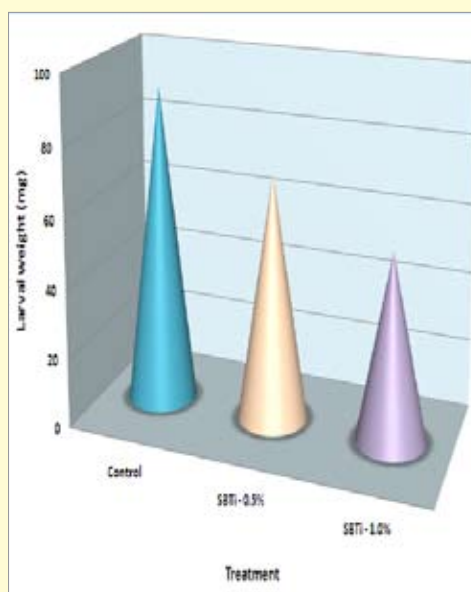
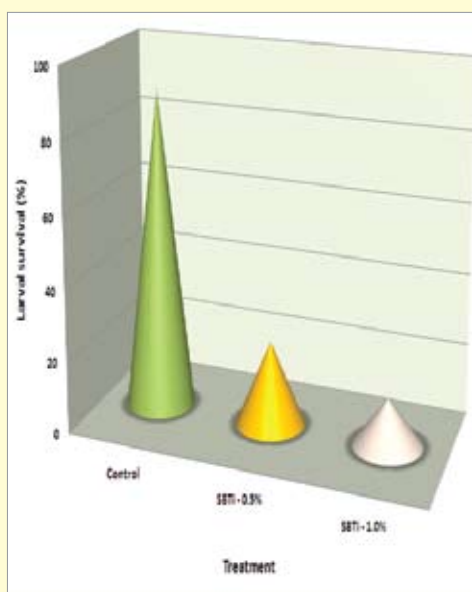
treated and control insects in haemolymph protein concentration were observed.

In control larvae, there was an increase in protein concentration (71 mg/ml) as they would be synthesized *de novo* from the nutrients derived from food. In SBTI treated larvae conspicuously lower protein concentration (63 mg/ml) compared to control larvae may be attributed to reduced feeding activity.



### Variation in the larval growth of *Chilo partellus*

A: untreated control; B: soybean trypsin inhibitor treated larvae



### Effect of soybean trypsin inhibitor on haemolymph protein of *Chilo partellus*

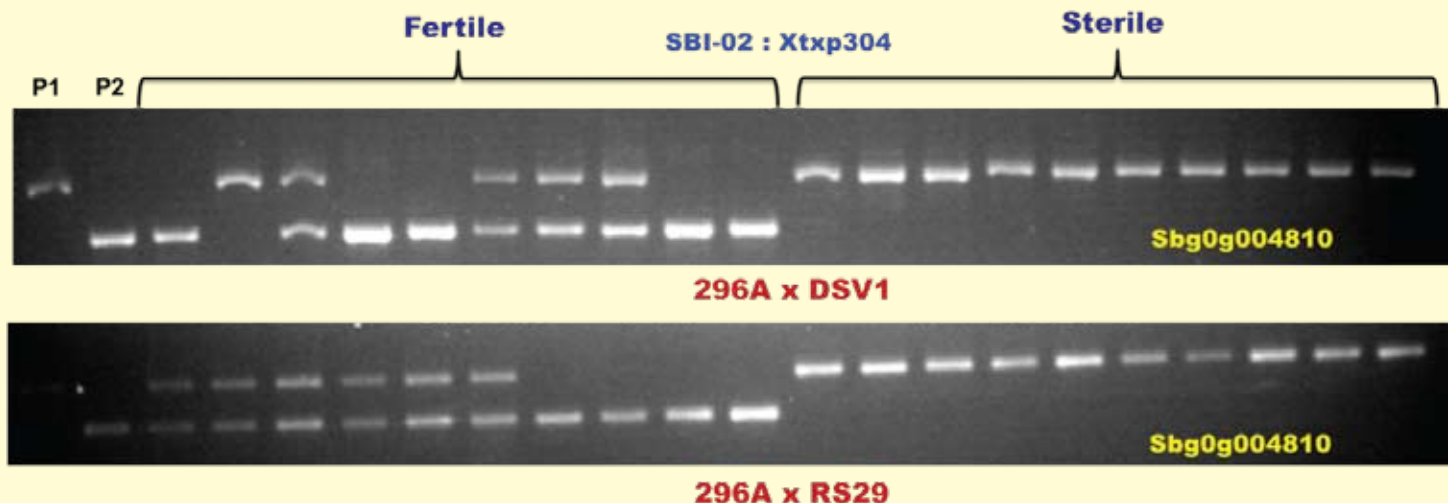
Soybean trypsin inhibitor have been shown to reduce larval weights of *C. partellus* in artificial diet and also affected the growth and development. Hence this can be considered as candidate genes for use in genetic transformation of sorghum for minimizing the losses due to *C. partellus*.

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## **Mapping of Rf gene using two sorghum genetic crosses**

**S**orghum is one of the pioneering crops where cytoplasmic male sterility (CMS) system was successfully exploited for mass production of  $F_1$  hybrid and its commercial utility. Sorghum  $F_1$  hybrids are superior by 50-60% in their grain yield compared to the traditional landraces. The commercial success of any hybrid depends mainly on its grain yield superiority and its fertility restoration. Of the available sorghum sterility systems, only the A1(milo) CMS system is commercially exploited where in pollen fertility restoration has been reported to be controlled by one or two major genes with several modifiers. The operation of Rf gene was found to be different with different genetic backgrounds. In this connection, a study was undertaken to map Rf gene loci using 296A CMS line and two sorghum inbred restorer lines, RS29 and DSV1.  $F_1$ s were developed between 296A x RS29 (=CSH13), and 296A x DSV1. Both the  $F_1$ s were fertile indicating dominance of fertility restoration over sterility maintenance. The  $F_1$ s were selfed and the selfed seeds of  $F_1$ s were used to grow two  $F_2$  populations separately during the kharif season of 2012 at the experimental fields of the

Directorate of Sorghum Research, Rajendranagar, Hyderabad. In each  $F_2$  population, every  $F_2$  plant was labeled, and genomic DNA was extracted. After 25 days after anthesis, the seed set data were recorded on a binary scale, as "fertile" (if seed set was observed) and "sterile" (no seed set). Using the selective genotyping approach, genotyping was done on 10 random plants selected each from fertile and sterile groups. Of the several markers tested, the SSR marker, Xtxp304 present on sorghum chromosome 2 was found to be tightly linked with fertility restoration. Pentatricopeptide repeat (PPR) genes, through their RNA editing ability are known to be involved in fertility restoration in many crops including sorghum. Interestingly, a PPR gene (Sb02g004810) was found to co-exist very near to the Xtxp304 marker locus, and was similar to the rice Rf1 locus (LOC\_Os10g35240.2). This locus was identified in a different sorghum genetic cross by Jordan et al. 2010. Therefore, this gene (Sb02g004810) could be the candidate gene involved in fertility restoration in the  $F_2$ s two sorghum genetic crosses (296A x RS29 and 296A x DSV1) of the present study.



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