Received: 12 Apr 2022 | Revised: 23 Aug 2022 | Accepted: 25 Aug 2022

Electronic Journal of Plant Breeding

Research Article

Combining ability studies through Line × Tester design in sweet sorghum (*Sorghum bicolor* L.) for bioethanol production

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Abstract
In developing of sweet sorghum hybrids, non-additive genetic effects are important in phenotypic expression of the traits of interest. The present experiment was undertaken to assess the combining ability in sweet sorghum genotypes using 16 hybrids developed by crossing four CMS lines with four elite testers in a Line × Tester mating design. The hybrids were evaluated at three locations in Andhra Pradesh in a Randomized Block Design with two replications during *Rabi*, 2018. Among the 13 quantitative characters studied the non-additive genetic variance was higher than the additive variance for all the traits except for days to 50 % flowering, days to maturity, plant height and ethanol yield. Among parents, ICSA 14029, ICSA 14030 (females) ICSV 15006 and GGUB-28 (males) were found to be good general combiners for ethanol yield and its related traits with consistent performance across locations. ICSA 14029 × ICSV-15006 and ICSA 14033 × SEVS-08 were identified as superior crosses for the specific combining ability for high ethanol yield.

Keywords: Sweet sorghum, Brix %, Bioethanol, *gca, sca* components

INTRODUCTION

One of the major millets Sorghum [*Sorghum bicolor* (L.) Moench] is a domesticated species belonging to the family Poaceae and based on their economic use they are classified as grain sorghum, forage sorghum and sweet sorghum which is native to semiarid tropics and subtropics. It serves as a multipurpose crop for food, fodder and fuel. In countries like Brazil, it is grown as the main crop for ethanol production (Doggett, 1988). The main advantage of sweet sorghum is three crops can be taken in a year with low water consumption (Vinutha *et al*., 2014). In India, the current ethanol production raw material is through sugarcane molasses and as a result the distilleries work only during the peak period *i.e.,* harvesting stage. Due to the low supply and huge demand the government policies of blending ethanol to 20 % have become a tough task. The sweet sorghum can be the best alternative for ethanol production to meet up the demand of the country, by providing year around operations to molasses-based ethanol distilleries and provide an assured income to the farmers. The continuous efforts of scientific community have resulted in good sweet sorghum varieties for use in ethanol production, but till date, only reliable hybrid CSH-22SS has been released for commercial cultivation in India. This necessitates the identification of new hybrids with good combining ability to suit under different agro ecological situations for ethanol production. The information on combining ability among sweet sorghum germplasm is needed for maximizing the effectiveness of new hybrid development. (Umakanth *et al.,* 2012).

Combining ability are generally classified into general combining ability (GCA) and specific combining ability (SCA) which have prime roles in inbred line evaluation and population development in breeding experiments. According to Sprague and Tatum (1942) GCA effects is due to the genes which are largely additive in their effects as well as additive × additive interactions. Specific combining ability is an indication of loci with dominance variance (non-additive effects) other epistatic interactions like additive × dominance and dominance × dominance interactions. Among the mating designs Line × Tester is more preferred because under one generation of evaluation overall genetic picture can be understood. The objective of the present study is to find new hybrids and parents with the good combining ability for desirable traits of interest.

MATERIALS AND METHODS

For assessing the general and special combining ability, the parental material consisted of four lines (male sterile) ICSA 14029, ICSA 14030, ICSA 14033 and ICSA 14035 and four testers SEVS-08, GGUB 28, ICSV 15006 and IS 29308 which were crossed in a L × T fashion to generate 16 crosses. In order to achieve synchronization three staggered plantings were adopted. The resultant 16 F_1 hybrids, their corresponding eight parents and hybrid check were evaluated at three different locations within Andhra Pradesh i.e., Agricultural College, Bapatla; RARS, LAM, Guntur and ARS, Garikapadu, *Krishna Dist during Rabi, 2018.* The populations were evaluated in a Randomized Block Design with three replications at all three locations. Each entry was raised in two rows of 4 m length with 45 × 15 cm spacing. Recommended agronomic practices were followed throughout the crop season to maintain a good crop stand. The quantitative traits studied were days to 50% flowering, days to maturity, plant height, the number of nodes per plant, stem girth, panicle weight, 1000-grain weight, fresh stalk weight, juice yield, brix %, total soluble sugars, ethanol yield and grain yield.

RESULTS AND DISCUSSION

Combined analyses of variance for 13 characters measured over three locations are presented in **Table 1**. Characters measured over three locations in the present investigation revealed significant differences among environments, lines, testers, crosses, environment × line × tester for all the characters studied except panicle weight suggesting that the testers and the interactions for these traits were influenced by the environment. The paramount

Table 1. Pooled analysis of variance for combining ability (Line × Tester design) for 13 traits in Sweet Sorghum

Source of variation	Degrees of freedom	DF 50%	DM	NNS	PH	FSTK	SG	1000 GW	PW	Brix %	TSS	JY	EY	GY
Replications	1	14.69	9.50	12.25	580.81**	30.68**	0.03	604.46	239.94**	1.56*	1.48*	38532.62	4529.62	1.33*
Environments	2	53.52**	37.02**	3.93**	36752.57**	276.53**	3.37**	419.55**	1101.02**	34.17**	25.72**	29092637.07**	18152.66**	3.90**
Parents (line)	3	90.59**	160.48**	14.66**	24260.18**	108.28**	0.64**	153.95**	212.47**	48.55**	42.60**	76247364.84**	201027.46**	1.41**
Parents (testers)	3	4.55**	56.22**	0.26	9077.26**	15.03**	0.12**	27.42**	159.00	1.11*	1.52**	3899348.03**	13786.70*	3.08**
Crosses	15	458.53**	355.55**	9.97**	10204.63**	565.59**	0.99**	51.39**	176.24	21.48**	16.43**	74958299.40**	462077.25**	1.56**
Line * tester effect	9	62.18**	109.64**	8.5**	1358.24**	362.13**	0.59**	63.99**	154.30	12.26**	9.38**	35475264.82**	204317.24**	0.98**
Env *Line effect	6	126.57	85.61	7.70	6322.70	162.63	1.38	43.14	224.13	11.50	8.79	83668964.39*	385546.04	2.78
Env * tester effect	6	108.35	157.78	8.06	8226.78	718.62*	1.50	69.86	148.15	21.04	16.08	40194526.24**	284742.65	5.09
Env * L * T effect	18	89.01**	127.74**	4.68**	3835.91**	203.55**	0.60**	77.34**	152.27	10.09**	7.72**	28797361.51**	14933.95**	2.65**
Error	69	0.73	2.00	0.55	37.01	1.38	0.00	2.86	8.87	0.37	0.37	933387.97	4292.32	0.27

DF 50%= Days to 50% flowering, DM= Days to maturity, NNS= Number of nodes, PH= Plant height, FSTK= Fresh Stalk yield, SG= Stem girth, 1000 GW=1000 grain weight, PW= Panicle weight, TSS= Total soluble sugars, JY= Juice yield, EY= Ethanol yield, GY= Grain yield

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

https://doi.org/10.37992/2022.1303.125

share of variance is through lines for all the characters studied except for grain yield. The L × T interaction was significant for all traits except panicle weight which signifies the involvement of hybrids for *SCA* variance. Similar results were reported by Indhubala *et al.* (2010) and Umakanth *et al*. (2012), Bahadure *et al.* (2015) and Ahmed *et al.* (2014) in earlier studies on sorghum.

The *SCA* variance values were greater than that of *GCA* for the characters (**Table 2**) number of nodes (1.33 > 0.47), fresh stalk yield (60.09 > 36.21), stem girth (0.09 > 0.06), 1000 grain weight (10.14 > 1.22), panicle weight (24.67 > 8.45), brix % (1.98 > 1.45), total soluble sugars (1.51 >1.11), juice yield (5804759.00> 5564006.00) and grain yield (0.11 > 0.08) specifying the non-additive control of genetic variation in these traits. The below mentioned authors reported similar results for different characters *viz.,* Indhubala *et al.* (2010) and Sanjanareddy *et al.* (2011) for juice yield, Bahadure *et al.* (2015) for fresh stalk yield, juice yield and grain yield, Mohammed *et al*. (2015) for plant height and grain yield and Rani *et al.* (2020) for plant height, brix %, total soluble solids, juice yield and grain yield.

On the opposite way, the *gca* variance showed higher estimates than the *sca* variance for days to 50% flowering (43.84 > 10.21), days to maturity (30.07 > 17.83), plant height (976.35 > 219.42), ethanol yield (35195.36 > 33381.42) indicating the presence of additive gene action. Umakanth *et al.* (2012) reported similar results for ethanol yield. Further these results are also confirmed by the ratios of σ^2*gca* / σ^2*sca*. Characters having ratio σ^2*gca* / σ^2*sca*> 1 indicate additive gene action and < 1 depict the non –additive gene action. Additive gene action implies the general combining ability, while specific combining ability arises due to non-additive gene action *i.e.* dominance and epistasis. So both selection and hybridization can be utilized for the improvement of these traits.

The general combining ability effects for 13 different characters, four lines and four testers are presented in **Table 3** and ranking of parents as high and low with respect to general combining ability effects for ethanol yield and yield component characters is presented in **Table 4**. This ranking identifies the crosses which have both or one of the parents as a good general combiner for the characters studied.

Table 2. GCA and SCA variance for 13 characters in Sweet Sorghum

Source of variation	DF 50%	DM	PH	NNS	SG	PW	1000 GW	FSTK	JY	BRIX%	TSS	EY	GY
σ^2 Gca	43.84	30.07	976.35	0.47	0.06	8.45	1.22	36.21	5564006.00	1.45	1.11	35195.36	0.08
σ^2 Sca	10.21	17.83	219.42	1.33	0.09	24.67	10.14	60.09	5804759.00	1.98	1.51	33381.42	0.11
gca/sca	4.29	1.68	4.44	0.35	0.66	0.34	0.12	0.60	0.95	0.73	0.73	1.05	0.77

Table 3. GCA effects for 13 characters in Sweet Sorghum

Parents	DF 50%	DM	PH	NNS	SG	PW	1000 GW	FSTK	JY	BRIX%	TSS	EY	GY
ICSA 14029	1.760**	1.604**	0.538	-0.406*	-0.015	-0.415	-0.948*	4.978**	803.549**	0.427**	0.374**	84.772**	0.058
ICSA 14030	-0.281	-0.021	0.444	-0.656**	-0.115**	4.961**	1.379**	-1.310**	423.302**	-0.198	-0.173	1.162	0.245*
ICSA 14033	0.552**	-0.063	-2.580	0.969**	0.048*	-0.033	-0.393	0.749**	24.537	-0.073	-0.064	-13.763	0.343**
ICSA 14035	-2.031**	-1.521**	1.599	0.094	0.081**	-4.513**	-0.039	-4.416**	-1251.387**	-0.156	-0.136	-72.171**	-0.646**
SEVS-08	4.760**	4.479**	16.877**	0.344*	-0.277**	0.572	0.796*	-3.874**	-853.241**	-1.490**	-1.303**	-158.727**	-0.033
GGUB-28	-13.573**	-11.438**	-65.857**	-1.031**	0.094**	0.560	1.224*	-1.676**	1777.623**	-1.365**	-1.193**	18.694	0.020
ICSV-15006	2.177**	4.229**	28.811**	0.510**	0.460**	1.146*	-1.715**	11.079**	3201.077**	1.885**	1.649**	356.587**	0.068
IS-29308	6.635**	2.729**	20.169**	0.177 l	-0.277**	-2.278**	-0.306	-5.529**	-4125.458**	0.969**	0.848**	-216.554**	-0.005
CD @ 5%	0.384	0.669	2.654	0.309 l	0.038	1.027	0.726	0.518	330.621	0.250	0.219	26.095	0.227

DF 50%= Days to 50% flowering, DM= Days to maturity, PH= Plant height, NNS= Number of nodes, SG= Stem girth , PW= Panicle weight, 1000 GW=1000 grain weight, FSTK= Fresh Stalk yield, , JY= Juice yield ,TSS= Total soluble sugars, EY= Ethanol yield, GY= Grain yield

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

Table 4. Status of *gca* effects for 13 characters in Sweet Sorghum

Parents	DF 50%	DM	PH	NNS	SG	PW	1000 GW	FSTK	JY	BRIX%	TSS	EY	GY
ICSA 14029	H	H	H	L	L	L	L	H	H	H	H	H	H
ICSA 14030	L	L	H	L	L	H	H	L	H	L	L	H	H
ICSA 14033	H	L	L	H	H	L	L	H	H	L	L	L	H
ICSA 14035	L	L	H	H	H	L	L	L	L	L	L	L	L
SEVS-08	H	H	H	H	L	H	H	L	L	L	L	L	L
GGUB-28	L	L	L	L	H	H	H	L	H	L	L	H	H
ICSV-15006	H	H	H	H	H	H	L	H	H	H	H	H	H
IS-29308	H	H	H	H	L	L	L	L	L	H	H	L	L

DF 50%= Days to 50% flowering, DM= Days to maturity, PH= Plant height, NNS= Number of nodes, SG= Stem girth , PW= Panicle weight, 1000 GW=1000 grain weight, FSTK= Fresh Stalk yield, , JY=Juice yield ,TSS= Total soluble sugars, EY= Ethanol yield, GY= Grain yield
H= High; L= Low

Among the lines, ICSA-14029 was found to be a promising general combiner for characters *viz*., fresh stalk yield, brix %, total soluble sugars, juice yield, and ethanol yield While line ICSA-14030 was a good general combiner for panicle weight, juice yield and grain yield and line ICSA-14033 for days to 50 % flowering, the number of nodes per plant, fresh stalk yield, stem girth and grain yield, while line ICSA-14035 for was poor combiner for all the characters.

Among the testers ICSV 15006 showed positive significant *gca* for all the traits *viz*., the number of nodes, plant height, fresh stalk yield, stem girth, panicle weight, brix %, total soluble sugars, juice yield and ethanol yield followed by tester GGUB 28 possessing positive *gca* for juice yield and ethanol yield. Tester IS 29308 had positive *gca* for brix % and total soluble sugars. Tester SEVS-08 showed positive *gca* for characters days to 50% flowering, days to maturity, the number of nodes per plant, plant height and 1000-grain weight. Bahadure *et al.* (2015) and Ingle *et al.* (2018) observed similar results for *gca* effects for plant height, panicle weight and grain yield.

The second important criterion for the evaluation of hybrids is the specific combining ability effects which could be related with hybrid vigour. The *sca* effects signify the role of non-additive gene action in trait expression. *sca* effects pertaining to 16 hybrids are presented in **Table 5**. Among the hybrids, ICSA 14029 × ICSV-15006 and ICSA 14033 × SEVS-08 have excelled with high *sca* effects in respect of brix%, total soluble sugars, juice yield, and ethanol yield while other crosses ICSA 14029 × GGUB 28 and ICSA 14030 × IS 29308 showed high *sca* effects for brix %, total soluble sugars, juice yield and ethanol yield, 1000-grain weight and panicle weight. ICSA 14035 × SEVS-08 recorded significant *sca* effects for juice and ethanol yield. High significant *sca* effects for grain yield in ICSA 14030 × SEVS - 08 and non-significant positive *sca* effects for hybrids ICSA 14029 × GGUB 28, ICSA 14029 × IS 29308, ICSA 14030 × IS 29308, ICSA 14033 × IS-29308, ICSA 14035 × SEVS-08, ICSA 14035 × GGUB 28, ICSA 14035 × ICSV-15006 and ICSA 14035 × IS-29308 was observed.

Most of the hybrids were found to be promising for the characters studied. Out of the 16 hybrids, seven hybrids for days to 50% flowering, six hybrids for days to maturity, four hybrids for plant height and the number of nodes per plant, five hybrids for stem girth, panicle weight, four hybrids for 1000 grain weight, seven hybrids for fresh stalk yield, eight hybrids for juice yield, five hybrids for brix %, total soluble sugars, six hybrids for juice yield and only single hybrid for grain yield showed significant *sca* effects in a desirable direction. Similar results were reported by Umakanth *et al.* (2012), Bahadure *et al.* (2015) for high *sca* effects for all the crosses studied, Soujanya *et al*. (2017) observed similar results for sugar yield. Jadhav and Deshmukh, (2017) and Ingle *et al.* (2018) manifested similar results for grain yield. Rani *et al*. (2020) for ethanol yield. The above presented results indicated that the crosses with high *sca* effects for desirable characters are possible from the parental combinations of (H x H); (L x H); (L x L) and (H × L). The cross between two high *gca* revealed additive and additive × additive genetic components. The cross between high × low *gca* resulted in an exclusive hybrid combination which might be due to both additive and non-additive genetic components. The superiority of the crosses having low × low *gca* parent may be due to the dominance × dominance type of non-allelic gene interaction resulting in over-dominance which is non-fixable.

Hybrids are best judged by per se performance, *sca* effects, characteristics of parents with regards to *gca* effects for ethanol yield and its component characters

Table 5. SCA effects for 13 characters in Sweet Sorghum hybrids

	HYBRIDS	DF 50%	DM	PH	NNS	SG	PW	1000 GW	FSTK	JY	BRIX%	TSS	EY	GY
H-1	ICSA 14029 x SEVS-08	-3.427**	0.104	-21.165**	-0.844**	0.027	-6.724**	-2.326**	-1.958**	798.301*	-0.885**	-0.775**	-13.185	-0.533*
H-2	ICSA 14029 x GGUB 28	0.073	0.188	2.887	0.031	-0.127**	4.228**	1.989**	-1.357*	1066.203**	1.656**	1.449**	174.823**	0.112
H-3	ICSA 14029 x ICSV-15006	-3.677**	-2.188**	13.072**	1.323**	0.015	-6.821**	-1.558	4.314**	1619.287**	1.406**	1.229**	176.772**	-0.433
H-4	ICSA 14029 x IS 29308	5.698**	1.354*	-0.462	-0.177	0.294**	0.484	0.549	1.395**	-2393.670**	-0.844**	-0.737**	-214.13**	0.023
H-5	ICSA 14030 x SEVS -08	0.948*	-50.938	5.932*	0.573	-0.206**	5.633**	-2.420**	-3.339**	-2875.769**	0.073	0.063	-159.18**	0.586*
H-6	ICSA 14030 x GGUB 28	-0.719	-3.188**	-3.551	-0.385	0.306**	-5.725**	-0.159	5.279**	199.37	-1.385**	-1.212**	-56.500*	-0.355
H-7	ICSA 14030 x ICSV 15006	0. 865*	0.313	0.701	1.240**	0.273**	-2.294*	0.859	7.593**	1190.894**	-0.302	-0.264	49.942	-0.305
H-8	ICSA 14030 x IS 29308	-1.094**	3.813**	-3.082	-1.427**	-0.373**	2.387*	1.720*	-9.534**	1485.338**	1.615**	1.412**	165.746**	0.073
H-9	ICSA 14033 x SEVS-08	0.719	-3.563**	-0.041	0.281	-0.269**	-0.484	5.775**	-5.082**	693.36*	1.115**	0.976**	97.539**	-0.080
H-10	ICSA 14033 x GGUB -28	2.448**	7.688**	5.805*	1.156**	0.077*	-2.385*	-5.217**	-2.061**	2541.510**	0.156	0.136	147.09**	-0.083
H-11	ICSA 14033 x ICSV-15006	-0.802*	-1.146	4.394	-1.719**	0.127**	-1.081	0.153	3.139**	-2523.918**	0.906**	0.791**	-116.24**	-0.174
H-12	ICSA 14033 x IS-29308	-0.927*	-2.979**	10.158**	0.281	0.065	3.949**	-0.711	4.004**	-710.956*	-2.177**	-1.904**	-128.38**	0.337
H-13	ICSA 14035 x SEVS-08	3.198**	4.396**	15.275**	-0.010	0.448**	1.575	-1.029	10.379**	1384.105**	-0.302	-0.265	74.833**	0.027
H-14	ICSA 14035 x GGUB 28	-1.802**	-4.688**	-5.141	-0.802*	-0.256**	3.882**	3.387**	-1.861**	-3807.250**	-0.427	-0.373	-265.41**	0.326
H-15	ICSA 14035 x ICSV-15006	2.281**	2.479**	-23.836**	-0.510	-0.206**	1.364	-0.801	-12.652**	803.858*	-0.677**	-0.591**	13.81	0.081
H-16	ICSA 14035 x IS-29308	-2.344**	-1.646*	18.741**	0.990**	-0.194**	2.011	-0.212	1.920**	529.166	0.073	0.064	52.49*	0.398
	CD @5 %	0.769	1.337	5.308	0.618	0.076	2.054	1.451	1.035	661.242	0.500	0.438	52.190	0.455

DF 50%= Days to 50% flowering, DM= Days to maturity, PH= Plant height, NNS= Number of nodes, SG= Stem girth , PW= Panicle weight, 1000 GW=1000 grain weight, FSTK= Fresh Stalk yield, , JY= Juice yield ,TSS= Total soluble sugars, EY= Ethanol yield, GY= Grain yield

* and ** significant at 5 and 1 % respectively

Table 6. Top ranking hybrids for specific combining ability with their *gca/sca* variance, *gca* status, *sca* effect and *per se* performance

S.No.	Character	gca/sca variance	Crosses	gca status	sca value	Per se performance
1	Days to 50% flowering	4.29 Additive	H-14	L x H	-1.802	59.83
			H-8	L x H	-1.094	82.50
			H-12	H x H	-0.927	83.50
			H-11	H x H	-0.802	79.17
2	Days to maturity	1.68 Additive	H-16	L x H	-1.646	117.83
			H-3	H x H	-2.188	123.00
			H-12	L x H	-2.979	118.50
3	Plant height	4.44 Additive	H-16	H x H	18.741	276.52
			H-13	H x H	15.275	274.00
			H-3	H x H	13.072	289.14
			H-5	H x H	5.932	264.30
4	Number of nodes per plant	0.35 Non-additive	H-16	H x H	0.990	12.67
			H-10	H x L	1.156	12.17
			H-7	L x H	1.240	12.17
			H-3	L x H	1.323	12.17
5	Stem girth	0.66 Non-additive	H-13	H x L	0.448	2.58
			H-6	L x H	0.306	2.62
			H-4	L x L	0.294	2.33
			H-7	L x H	0.273	2.95
6	Panicle weight	0.34 Non-additive	H-5	H x H	5.633	59.74
			H-2	L x H	4.228	52.95
			H-12	L x L	3.949	50.22
			H-14	L x H	3.882	48.51
7	1000 grain weight	0.12 Non-additive	H-9	L x H	5.775	40.52
			H-14	L x H	3.387	38.91
			H-2	L x H	1.989	36.60
			H-8	H x L	1.720	37.13
8	Fresh Stalk yield	0.60 Non-additive	H-13	L x L	10.379	46.26
			H-7	L x H	7.593	61.53
			H-6	L x L	5.279	46.47
			H-3	H x H	4.314	62.15
9	Juice yield	0.95 Non-additive	H-10	H x H	2541.510	17876.53
			H-3	H x H	1619.203	18066.65
			H-8	H x L	1485.338	11316.04
			H-13	L x L	1384.105	12812.33
10	Brix %	0.73 Non-additive	H-2	H x L	1.656	14.00
			H-8	L x H	1.615	15.67
			H-3	H x H	1.406	15.67
			H-9	L x L	1.115	12.83
11	Total soluble sugars	0.73 Non-additive	H-2	H x L	1.449	12.40
			H-8	L x H	1.412	13.86
			H-3	H x H	1.229	13.85
			H-9	L x L	0.976	11.38
12	Ethanol yield	1.05 Additive	H-3	H x H	176.772	1345.27
			H-2	H x H	174.823	1130.31
			H-8	H x L	165.746	802.38
			H-10	L x H	147.09	1004.05
13	Grain yield	0.77 Non-additive	H-5	H x H	0.586	5.34

https://doi.org/10.37992/2022.1303.125

(**Table 6**). Based on these criteria the hybrids ICSA 14029 × ICSV-15006, ICSA 14029 × GGUB 28, ICSA 14030 × IS 29308 and ICSA 14033 × GGUB -28 were found to be suitable for ethanol trait in heterosis breeding.

The present study aimed in selecting good combiners for ethanol yield and other yield contributing characters. Among Lines, ICSA 14029, ICSA 14030 and in testers ICSV 15006, GGUB-28 were found to be the promising general combiners for ethanol yield and its related traits with consistent performance across locations. Among the hybrids ICSA 14029 × ICSV-15006 and ICSA 14033 × SEVS-08 were identified as superior specific combination crosses for high ethanol yield.

ACKNOWLEDGEMENT

The authors are indebted to IIMR, Hyderabad and ICRISAT, Hyderabad for sparing the seed material and package of practices for conducting the trial. The authors are also thankful to RARS, Lam, Guntur; ARS Garikapadu and Agricultural College Farm Bapatla under ANGRAU, Lam Farm, Guntur for providing all the research facilities.

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