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GENETIC TOOLS AND PROXY PLANT CHARACTERISTICS TO EXPEDITE GRAIN YIELD IMPROVEMENT IN SORGHUM HYBRIDS FROM DIVERSE MALE STERILITY CYTOPLASM'S

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ABSTRACT

Correlation, path analytics, and genetic variability were investigated in this work, for yield and yield component traits in 280 sorghum F1 hybrids during *Rabi* 2021 and *Summer* 2022 seasons. Considerable amount of genetic variation was observed for all the traits studied, confirming the scope for further selection for superior and desirable hybrids. The broad sense heritability was high for all traits evaluated, and the correlation between genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) was almost perfect (*r*=0.99, *p*<0.001) and their means comparable (sample estimates mean difference = 1.3), implying the low level of the environmental effect, and a high level additive genetic factors effects on the measured traits. There was a high positive correlation between days to 50 % flowering (DFF) and days to maturity (MAT), MAT and number of leaves per plant (NLP), plant height (PH) and hundred seed weight (HSW), panicle width (PW) and panicle weight per hectare (PWt), PWt and yield (YLD). The correlation coefficients between YLD and the rest of the traits evaluated were low except for PL, PW, and PWt that showed medium, medium, and high positive correlation, respectively. PL, PW, and PWt should therefore be considered in the indirect selection process to identify superior sorghum genotypes with higher grain yield potential. Since the three traits were favorably correlated with grain yield, selection can simultaneously improve all traits by tandem selection, or indirect selection between YLD and PL, PW and PWt. PWt was therefore the sole trait able to determine YLD along with the major component traits in this work. These findings are expected to expedite and enhance genetic gains in sorghum breeding.

KEYWORDS: Grain sorghum hybrids, genetic variability, path analyses, additive gene action, grain yield proxy traits

Sorghum [Sorghum bicolor (L.) Moench] also known as the "King of Millets" is a multifunctional crop that is cultivated for food, feed, fuel, and fodder; it is a staple crop for hundreds of millions of semi-arid people in Asia and Africa (Mwamahonje et al. 2021). Sorghum is a C4 plant with increased photosynthetic and resource use efficiency (RUE), and a strong abiotic and biotic stress tolerance. Its capacity to survive in dry and hot environments, coupled with genetic variability in photoperiod response, explains its extensive and sustainable cultivation under rained conditions of India and other world's drylands, and across the earth's latitudes (Habyarimana et al., 2021). Sorghum is resurging under higher latitudes for feed, biofuel, the manufacture of specialty health-promoting foods rich in antioxidants, and for use as a substitute for climate change susceptible crops (e.g., maize), traditional grains diet, and for meeting gluten-free needs

(Habyarimana et al., 2019). Cytogenetically, sorghum is a diploid crop species with 2n = 2x = 20, where 2n, n, 2x, and 20, respectively, the somatic chromosome number, the gametic chromosome number, two complete sets (2x) of chromosomes, and the total chromosome number of a sorghum cell. Sorghum's small genome (730 Mb) makes it an excellent test bed for the functional genomics studies of C4 grasses and other crop species (Habyarimana et al., 2020). The S. bicolor genome sequence was first released in 2009 (Paterson et al., 2009) and the current version is 3.1.1 released in 2018 reports more than 34,000 annotated genes several of which can be harnessed in genetic introgressions to improve yields and quality plant characteristics (McCormick et al., 2018). Sorghum is the fifth-most significant cereal crop in the world, with Africa and India accounting for the largest share (> 70%) of the world's sorghum area; the top producing nations

are the United States, India, Mexico, Nigeria, Sudan, and Ethiopia (source: https://www.fao.org/faostat/en/ #data/QCL). During the period between 1960 and 2020, sorghum productivity in India increased by more than 175% wherever hybrids were grown, highlighting the importance of heterosis for grain yield in this crop. In sorghum, the superiority of the F1, or hybrid vigour, depends on the environment and the genotypes used (Mindaye, et al., 2016). Sorghum hybrids have been grown by farmers in developed countries since the late 1950s driven by the discovery of the cytoplasmic male sterility system, allowing cost-effective hybrid production, and are increasingly being adopted in the developing world (Mindaye et al., 2016,). For instance, the technology for sorghum hybrid cultivar development is well advanced in Asia, while in Africa open-pollinated varieties are the prevalent products.

The level of genetic variability in a population is crucial to the success of plant breeding efforts, including the development of sorghum hybrid cultivars. The list of key genetic parameters that are used to measure genetic variability includes the coefficient of variation (CV), broad-sense heritability (H), correlation coefficient, and path analytics, which are some of the key tools breeders use for successful crop improvement (Tiwari et al., 2019, Immanuel et al., 2011, and Xu et al., 2017). The correlation coefficients inform the breeder about the magnitude and direction of the relationships that existed between traits; a favorably correlated trait can be used as a surrogate for a corresponding complex trait if it is relatively cheap, has high heritability, and it is easily phenotyped, so it can expedite breeding and enhance genetic gain. The path analytic method extends the correlation analysis as it dissects and better explains the correlation coefficients (Khandelwal et al., 2015; Khadakabhavi et al., 2017). Path analysis is used by the breeding community as a decisionsupport tool to quantify the direct and indirect contributions of proxy traits to the targeted effect or outcome variable and to determine the path model that best fits the pattern of correlations found in the datasets.

The above breeding tools were extensively studied in sorghum (for a review refer to the following references-Habyarimana *et al.*, 2018, Momo *et al.*, 2023, and Patil *et al.*, 2023). For instance, some researchers have used these methods to identify the essential characteristics for increasing yield, such as days to 50% flowering, panicle width, panicle weight, leaf number, and hundred seed weight, as the main determinants of yield (Verma et al., 2019, Momo et al., 2023, and Patil et al., 2023), thereby allowing for direct selection of these traits. Most of the available scientific literature concentrated on OPVs and hybrids derived mainly from the Milo cytoplasm i.e., A, (Reddy et al., 2010, Praveen et al., 2015 and Prasad et al., 2017). The present work was therefore undertaken to close this gap by deriving the breeding parameters and tools from a diverse pool of sorghum F, hybrids produced from crosses involving A₁, A₂, A₃, and A₄ cytoplasm (for a review refer to the following references -Stephens and Holland 1954, Quinby 1970, Reddy et al., 2008 and Jebril et al., 2021). The importance of cytoplasmic and nuclear genetic diversity of male-sterile (A) and restorer (R) lines in sorghum cannot be overemphasized. Particularly, the diversity in A cytoplasms can avoid the disease outbreak that happened in the 1970s for southern corn leaf blight of corn hybrids possessing a uniform Texas (T) cytoplasm (Tatum, 1971).

In view of the aforementioned, this study was conducted to identify the genetic variability and heritability, as well as the characteristics associated with yield enhancements in complex pool sorghum hybrids derived from diverse seed parent cytoplasms and restorer backgrounds.

MATERIAL AND METHODS

We carried out this investigation using 280 genotypes, of which 241 were F1 hybrids, 35 parental lines and 4 standard checks, under 4 environments (2 seasons × 2 irrigation regimes) *i.e.*, during post rainy 2021 and summer 2022 under irrigation and post flowering drought stress. Seed parents included A₁, A₂, A_{a} , and A_{a} cytoplasms. The experimental design was alpha lattice design with 2 replications. The elementary plot consisted of two rows 2 m long with spacing of 60 cm and 15 cm between and within rows, respectively. All the recommended agronomic practices were followed to raise a good crop. For each genotype, plant height (PH), number of leaves per plant (NLP), panicle length (PL), and panicle width (PW), were determined on randomly selected five healthy plants. On a full plot basis, days to 50% flowering (DFF), days to maturity (MAT), hundred seed weight (HSW), panicle weight per hectare (PWt), and yield per hectare (YLD) were recorded. Mean data of each character was subjected to analysis. Burton (1952) and Shafique, *et al.*, 2016 approach was used to quantify the genotypic (GCV) and phenotypic (PCV) coefficients of variation. The genetic advance (GA) was determined using Johnson *et al.*, (1955) method, while broad sense heritability (H) was estimated as suggested by Lush (1940). Correlation between different traits was assessed by the Pearson correlation coefficient Wright (1921) and path analysis as described by Wright (1934). The R software was used for statistical analyses.

RESULTS AND DISCUSSION

Genetic variation among test plant materials is the precondition for efficient breeding and selecting desirable superior candidates. On the other hand, assessing the correlation and the path analytic models between the proxy traits and the outcome dependent variable e.g., the economic trait such as grain yield (Shedge *et al.*, 2019), is critical for the successful deployment of the best selection criteria. Since grain yield is a polygenic and complex trait that is influenced by a huge range of other factors, direct selection based solely on an association pattern between two variables may occasionally mislead the breeder; therefore, direct and indirect effects should be differentiated for effective selection (Awol and Alise fikre, 2018), and it is here that path analytics comes in (Swetha *et al.*, 2019). In addition to evaluating the potential for the simultaneous enhancement of two or more traits, path analytics will help in understanding the desirable and unwanted interaction of plant characteristics (Shanmugam and Kalaimagal, 2019). The heritability of the plant characteristics helps gauge the relative importance of the genetic factors relative to the noise from the environment in order to avoid disturbing breeding surprises when a bred-for trait gets lost at the farmer's level.

Analysis of variance for experimental designs

The analysis of variance for the nine quantitative traits using the four environments and entire data set revealed highly significant differences between genotypes for all the traits studied. A significant genotype × environment interaction was also observed. It can be inferred therefore that genetic variability among the genotypes studied was supported by statistical inference and the performance of the genotypes varied with varying environmental conditions (Table 1). Under such circumstances, techniques such as biplot models e.g.,AMMI biplot = the additive main effects and multiplicative interaction and GGE biplot = genotype+ genotype × environment analyses will be useful to determine the environmental and the genotypic behaviors allowing targeted breeding recommendations.

			Меа	an sum of squa	ares		
S.No.	Characters	Replications	Genotypes	Environment	Genotype: Env	Rep:Block	Residuals
		(D.f. = 1)	(D.f. =279)	(D.f. =3)	D.f=837	D.f=68	D.f=1051
1	DFF	26.29	88.08***	29330.65***	24.28***	5.14	3.99
2	MAT	174.15	107.72***	35159.6***	28.39***	6.57	4.86
3	NLP	7.46	5.31***	2355.04***	1.86***	2.05	0.89
4	PH (cm)	277.68	9237.27***	542736.05***	557.7***	235.5	148.03
5	PL (cm)	0.062	34.48***	1178.35***	3.61***	1.37	1.77
6	PW (cm)	4.92	1.03***	90.62***	0.25***	0.24	0.19
7	HSW (g)	1.51	0.76***	84.65***	0.12***	0.07	0.06
8	PWt (kg/ha)	0.02	3.31***	52.6***	0.97***	0.42	0.38
9	YLD (kg/ha)	0.002	2.11***	52.13***	0.76***	0.2	0.25

Table 1. ANOVA for yield and its attributing parameters in sorghum

*** Significant at 0.001 per cent level,

DFF: Days to 50% flowering, MAT: Days to maturity, PH: Plant Height, NLP: Number of leaves per plant, PL: Panicle length, PW: Panicle width, PWt: Panicle weight per hectare, HSW: 100 seed weight, YLD: Yield per hectare.

Genetic parameters: variability, heritability, and genetic advance

The estimates of genetic parameters which include heritability in broad sense, phenotypic (PCV) and genotypic (GCV) coefficients of variation, and genetic advance are presented in Table 2. The PCV was statistically higher than GCV for all the characters studied with t = 3.2879, p<0.05, 95 percent confidence interval = 0.3915608 - 2.2306614, indicating the existence environmental effects on the observed phenotypic data. However, the correlation between GCV and PCV was almost perfect (r=0.99, p<0.001), and their means comparable (sample estimates mean difference = 1.3) implying the low level of the environmental noise, and a high level of genetic factors effects on the measured traits. This was also confirmed by the high values of the broad sense heritability. The latter ranged from 66% to 94% for grain yield and plant height, respectively. The PCV and GCV ranged from 3.6% (NLP) to 20.9% (YLD) and from 2.9 (NLP, MAT)

(2013), Karpe *et al.*, (2023), Dev *et al.*, (2019), and Toor (2020). For PW and PL, PCV and GCV were low, while GA was moderate (10-20%) and heritability high, which is in agreement with Arunkumar *et al.*, (2022) and Vinodhini *et al.*, (2022). For PH, PWt, HSW and YLD, PCV and GCV were low, while heritability and GA values were high as reported by Hamidou *et al.*, (2018), Nikiema (2023), Gebregergs and Mekbib (2020) and Kalpande *et al.*, (2019). From the high broad sense heritability and the comparable GCV and PCV it can be inferred the existence of low level of the environmental noise, and a high level additive genetic factors effects on the measured traits.

Correlation analysis

The magnitude and direction of the correlation between plant characteristics was interpreted according to Gomez and Gomez (1984) as follows: 0-0.1, 0.1-0.5, 0.5-0.8, and 0.8-1, 1, interpreted as, zero, low, medium, high, and perfect, respectively. There was a high or perfect positive correlation between DFF and

S.No.	Trait	PCV <i>(%)</i>	GCV (%)	Heritability in broad sense (H) (%)	Genetic Advance as % mean
1	DFF	4.2	3.6	73.0	6.4
2	MAT	3.8	2.9	74.0	5.1
3	NLP	16.5	16.0	94.1	31.9
4	PH (cm)	3.6	2.9	68.7	5.1
5	PL (cm)	9.1	8.6	89.6	16.9
6	PW (cm)	7.9	6.9	76.8	12.4
7	HSW (g)	17.3	14.7	72.0	25.7
8	PWt (kg/ha)	12.1	11.1	83.6	20.9
9	YLD (kg/ha)	20.9	16.9	65.8	28.4

Table 2. Heritability, coefficient of variation and genetic advance for yield and its component traits in sorghum hybrids.

DFF: Days to 50% flowering, MAT: Days to maturity, PH:Plant Height, NLP: Number of leaves per plant, PL: Panicle length, PW: Panicle width, PWt: Panicle weight per hectare, HSW: 100 seed weight, YLD: Yield per hectare.

to 16.9% (YLD), respectively. Genetic advance was low (0-10%) for DFF, MAT and NLP, moderate (10-20%) for PL and PW, and high (e"20%) for PH, HSW, PWt and YLD (kg ha⁻¹). The results obtained in this study are in agreement with previous research works. Low GCV, PCV and GA, and high heritability were reported for the DFF, MAT and NLP by Arunkumar *et al.*, (2013), Khandelwal *et al.*, (2015), Nirosh *et al.*, MAT, MAT and NLP, PH and HSW, PW and PWt, and PWt and YLD (Figure 1). Medium positive values of Pearson correlation were observed between DFF and NLP, PH and PW, PL and YLD, PW and HSW, PW and YLD, PWt and HSW. The correlation between PH and NLP, PH and PWt, PH and YLD, NLP and PW, NLP and PWt, NLP and HSW, NLP and YLD, and PL and PW, and YLD and HSW was low and positive, while low and negative correlation was observed between DFF and PWt, DFF and HSW, DFF and YLD, MAT and PWt, MAT and HSW, and MAT and YLD. Similar findings were presented by Khandelwal *et al.*, (2015), Vinodhini *et al.*, (2022), Arunkumar *et al.*, (2020), and Khadakabhavi *et al.*, (2017).

Path coefficient analysis

Identification of breeding parameters and tools to maximize grain yield in sorghum hybrids was the major objective in this work. As in other crops, the efficiency of sorghum breeding program depends on the direction and the magnitude of the correlation among the economic (grain yield) outcome and the different component traits and the breeder's ability to account for the relative direct and indirect importance of each component trait. In this work, the correlation coefficients between grain yield and the rest of the traits evaluated were low except for PL, PW, and PWt that showed medium, medium, and high positive correlation with grain yield, respectively (Figure 1). The latter three plant characteristics should therefore be considered in the selection process to isolate superior sorghum genotypes with higher grain yield potential. Plant breeders routinely select on multiple traits, but progress can be complicated by genetic correlations. As per our findings, since the three traits were favorably correlated with grain yield, selection can simultaneously improve all traits by tandem selection, or indirect selection and index selection. The importance of panicle length on grain yield was also reported by Senbetay et al. (2020), whereas the importance of panicle length and panicle weight was reported by Khandelwal et al. (2015). The direct and indirect effects of PWt were instrumental to the observed correlation between grain yield and PL, PW and PWt. Panicle weight was therefore the sole trait able to determine the grain yield along with the major component traits in this work. This finding is particularly important in situations of shuttle breeding, or when two planting seasons are closely in tandem (e.g., kharif vs. rabi season in Asia) and there is not enough time and resources to thresh entire plots and/or weigh grains from vast breeding pipelines. Here, panicles can be harvested from entire plots, weighed for selection purposes, but only part of them threshed to get seeds to plant selected lines in the following season. Threshing the remnant panicles and/or

weighing the seed can be postponed to a more convenient time or forgone altogether to save resources, depending upon the breeder's instructions.

The direct effects are described as partial regression coefficients, which quantify the influence of one variable on another while accounting for influencing variables. For instance, a change in one standard deviation of PWt in Table 3 showed nearly as much as tenfold the impact on grain yield than a one standard deviation change in the PH. On the other hand, in a path model, the magnitude of indirect effects is determined by the product of the path coefficients along the pathway between the two causally-related variables (Habyarimana *et al.*, 2018 and Khadaka-bhavi *et al.*, 2017).

We observed discrepancies in the direction and/or the magnitude between the direct effects of DFF and MAT, and the correlation between these traits and grain yield. The discrepancy in terms of magnitude between the DFF direct effect and the correlation with grain yield was mainly dependent on the increased MAT indirect effect, while the discrepancy in terms of both magnitude and direction of the MAT direct effect vs. correlation with grain yield was dependent on DFF to a great extent, and on NLP and PWt to a lesser extent. The discrepancy between the HSW direct effect and the correlation with grain yield was mainly influenced by the indirect effect exerted by DFF, MAT and PWt.

CONCLUSION

Grain yield is a complex quantitative trait governed by several genetic factors mostly with minor effects, and showing important interaction with the environment. In several crops, proxy traits were identified for indirect selection for grain yield. In this work, the correlation coefficients between grain yield and the rest of the traits evaluated were low except for PL, PW, and PWt that showed medium, medium, and high positive correlation with grain yield, respectively; these traits should therefore be considered in the selection process to isolate superior sorghum genotypes with higher grain yield potential. Since the three traits were favorably correlated with grain yield, selection can simultaneously improve all traits by tandem selection, or indirect selection and index selection. On the other hand, we were able to identify panicle weight as the

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S. No.	Trait	DFF	MAT	PH	NLP	PL	PW	PWt	HSW	YLD
1	^a DFF	-3.65	<u>3.35</u>	0.005	<u>0.30</u>	-0.001	0.0	- <u>0.14</u>	0.02	-0.11
2	MAT	- <u>3.65</u>	3.35	0.010	<u>0.30</u>	-0.003	-0.01	<u>-0.13</u>	0.02	-0.11
3	NLP	<u>-0.18</u>	<u>0.30</u>	0.11	<u>0.16</u>	0.003	<u>-0.34</u>	<u>0.54</u>	<u>-0.18</u>	0.41 * *
4	PH (cm)	<u>-2.88</u>	<u>2.68</u>	0.04	0.38	0.009	<u>-0.23</u>	<u>0.36</u>	-0.06	0.3**
5	PL (cm)	0.03	-0.06	0.002	0.02	0.16	-0.20	<u>0.60</u>	-0.01	0.54 * *
6	PW (cm)	0	0.06	0.07	<u>0.17</u>	0.06	-0.51	<u>1.0</u>	<u>-0.13</u>	0.75**
7	HSW (g)	<u>0.43</u>	<u>-0.36</u>	0.05	<u>0.11</u>	0.08	<u>-0.44</u>	<u>1.20</u>	<u>-0.11</u>	0.97 * *
8	PWt (kg/ha)	<u>0.51</u>	<u>-0.33</u>	0.09	<u>0.11</u>	0.008	<u>-0.33</u>	<u>0.62</u>	-0.21	0.48**

	Table 3. Phenotypic (P) path coef	ficients of yield and its	s component traits	in sorghum
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^aThe pathways are read horizontally, with each row representing a distinct path from one characteristic (exogenous variable) in the first column to the resulting plot yield (PY) via intermediate variables. Direct and indirect effects are represented by diagonal and off-diagonal values in a row respectively. To make the table easier to read, we high-lighted the indirect effects (Gomez and Gomez, 1976) that were larger than or equal to 0.10. The right-most column represents the Pearson's correlation coefficient between respective exogenous variable and the outcome variable, and each coefficient was decomposed into the corresponding direct and indirect effects (Habyarimana et al., 2018). Traits in the first column from top to bottom: DFF:Days to 50% flowering, MAT:Days to maturity, PH:Plant Height, NLP: Number of leaves per plant, PL: Panicle length, PW: Panicle width, PWt: Panicle weight per hectare, HSW: 100 seed weight, YLD: Yield per hectare. Phenotypic Residual effect = 0.040, * Significant at the 5 per cent and **1 percent probability levels, respectively.



Fig.1: Heatmap visualizing the correlation of yield and its attributing traits in sorghum

The pathways are read horizontally, with each row representing a traits association with corresponding trait falling in that particular column. Traits in the Y-axis from bottom to top: DFF: Days to 50% flowering, MAT: Days to maturity, PH: Plant Height, NLP: Number of leaves per plant, PL: Panicle length, PW: Panicle width, PWt: Panicle weight per hectare, HSW: 100 seed weight, YLD: Yield per hectare.

sole trait to predict the grain yield along with the major component traits with high to very high accuracy, and can therefore be used as a standalone plant characteristic proxy for grain yield in sorghum.

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IN-SITU MOISTURE CONSERVATION AND NUTRIENT MANAGEMENT STRATEGIES ON GROWTH AND YIELD OF RAINFED COTTON IN SEMI ARID REGIONS

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ABSTRACT

A Field study entitled "Influence of resource conservation technologies on cotton productivity, profitability and environment in rainfed *Alfisols* of Semi-Arid Tropics (SAT)" was conducted at CRIDA, Hyderabad, during rainy season, 2021-222 and 2022-23. Experiment was laid out in a split-plot design with five *in-situ* moisture conservation *viz.*, M_1 : Flat bed sowing with CRIDA 6 row planter, M_2 : Flat bed with dhaincha as live mulch, M_3 : Conservation furrow at sowing with CRIDA ridge planter, M_4 : Raised bed and furrow with CRIDA raised bed planter and M_5 : Conservation furrow at 30 - 40 DAS after interculture operations as main plots and nutrient management practices as sub plots *viz.*, S_1 : control (No fertilizer), S_2 : 100% recommended dose of NPK (120:60:60) and S_3 : 125% recommended dose of NPK (150:75:75) allocated randomly and replicated thrice. The *in-situ* moisture conservation furrow at sowing (M₄) recorded higher lint yield (506 kg ha⁻¹) and seed cotton yield (1427 kg ha⁻¹) and was followed by conservation furrow at sowing (M₃), conservation furrow at 30-45 DAS (M₅), flat bed with dhaincha as live mulch (M₂) and flat bed (M₄). These higher yields were due to higher plant height (100.8 cm), monopodial branches plant⁻¹ (3.1), sympodial branches plant⁻¹ (16.0), dry matter production (3567 kg ha⁻¹). Among the nutrient management practice 125 % recommended dose of NPK (150:75:75) recorded performed higher plant height (108.2 cm), monopodial branches plant⁻¹ (3.2), sympodial branches plant⁻¹ (16.1), dry matter production (3812 kg ha⁻¹), lint yield (495 kg ha⁻¹) and seed cotton yield (1412 kg ha⁻¹) and was followed by 100 % recommended dose of NPK (150:75:75) realized higher lint and seed cotton yield (621 and 1717 kg ha⁻¹).

KEYWORDS: Moisture, nutrient, growth, ridge and furrow, yield, conservation furrow and mulch

Cotton (Gossypium hirsutum L.) is an essential cash crop that is known as the 'King of Fibre' and plays a major role in the farmers' as well as country's economy. It is also known as 'White gold'. The cotton crop, contributes significantly to India's foreign exchange, employment and has significant export potential for raw cotton and valueadded goods. This crop provides a source of income for 60 million people in India through cultivation, processing and textiles. It contributes to 29 % of the national gross domestic product. In India it is grown in an area of 13.28 M ha with a production of 35.24 M bales, and productivity of 491 kg ha⁻¹. In India, during 2020-21 higher area (4.54 M ha) and production (10.1 M bales) were recorded in Maharashtra. But higher productivity was recorded from Punjab (690 kg ha⁻¹). Telangana ranked second in area (2.35 M ha) with a production of 5.7 M bales and a productivity of 418 kg ha⁻¹ (CCI, 2021).

Cotton productivity in rainfed areas is very low compared to the potential productivity due to

drought, erratic rainfall, and moisture stress during the crop growth and degraded soils. Cotton, being a long duration crop, suffers from moisture stress during flowering and boll formation which adversely affects the crop growth and yield. Hence measures are needed to conserve the soil moisture and improve the crop productivity. In this background rainwater conservation *in-situ* / *ex-situ* is a critical factor for higher yield and rain water use efficiency in drylands. In-situ moisture conservation is an important method to conserve soil moisture and improve the crop productivity.

Water and nutrients exhibit strong synergistic interaction for their effect in crop growth and yield. But the rainwater uses without balanced nutrient application is ineffective. Cotton requires adequate supply of moisture and nutrients to optimize the seed cotton yield, quality and net profit (Aladakatti *et al.*, 2011). Nutrients are main factors limiting crop production besides moisture. However, imbalanced fertilizer application due to moisture stress is very

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common in rainfed regions. Nutrient-use efficiency in rainfed cropping systems is often very low due to moisture stress that limits the availability of nutrients. Hence nutrient utilization can be improved by optimum and synergistic interaction with other inputs. Hence nutrient and moisture availability should be addressed simultaneously to enhance both water and nutrient use efficiency.

MATERIAL AND METHODS

Field experiment was conducted at Gunegal Research Farm, ICAR-Central Research Institute for Dryland Agriculture (ICAR-CRIDA), Hyderabad, Telangana State during 2021-2022 and 2022-2023 rainy season. The geographical location of the farm is 17.08°N latitude and 78.66°E longitude and 542 m above mean sea level. Average seasonal (June-December) rainfall during the experimental period was 858.5 mm and 833.5 in 2021-22 and 883.5 in 2022-2023. Average annual maximum and minimum temperature during experimental period was 29.93°C and 20.25°C, respectively. The soil of the experimental site is Alfisol with sandy clay loam texture. The experimental site is low in N (195.4 kg ha⁻¹), medium OC (0.6 %), P (18.3 kg ha⁻¹) & K (200.6 kg ha⁻¹). The crop was sown during 29th June of 2021-2022 and 2022-2023 years, respectively. The experiment was laid out in split-plot design with 5 in-situ moisture conservation as main plots include M₁: Flat bed sowing with CRIDA 6 row planter, M₂: Flat bed with dhaincha as live mulch, the dhaincha crop was sown in between the crop rows at 45 DAS the crop was harvested with brush cutter and spread between the rows as mulch. M₃: Conservation furrow at sowing with CRIDA Ridge planter, M₄: Raised bed and furrow with CRIDA Raised bed planter and M₂: Conservation furrow at 30 - 40 DAS, after first interculture operation the furrows were formed with bullock drawn implement at 45 DAS and sub plot treatments 3 nutrient management practices, as subplot were S₁: control (without fertilizer), S2: 100% Recommended dose of NPK (120:60:60) and S₂: 125% Recommended dose of NPK (150:75:75) replicated thrice. In-situ moisture conservation treatments were assigned to the main plots (120 m²) which were split randomly into three sub plots (15 m²). A buffer strip of 3 m was maintained between the main plots. The observations on crop growth, yield attributes of the cotton were recorded at physiological maturity stage of crop growth. Five plants

were selected randomly and labelled in the net plot and all the biometric observations were recorded during different crop growth stages. The lint yield was estimated from the net plot after excluding the border rows *i.e.*, two rows on either side (gross plot size = 12.72 m^2). Data were analyzed statistically by analysis of variance method for split plot design using proc glm of SAS software version 9.2. Tukey's studentized range test (HSD) was employed to offer corrections to Pvalues while doing multiple comparisons. P value less than 0.05 has been used as the criteria for rejecting the null hypothesis of equality of means for main plot treatments, subplot treatments and interaction between main plot and subplot treatments separately.

RESULTS AND DISCUSSION

Effect of *in-situ* moisture conservation and nutrient management practices on crop growth

Growth parameters progressively increased with the ontogeny of the crop up to harvest irrespective of the treatments. Different moisture conservation treatments had significant effect on crop growth at all crop growth stages.

Plant height is a crucial growth factor. It illustrates the plant's vitality. Plant genetical character, management factors and environmental conditions play a significant role in determining plant height. Irrespective of the treatments, plant height increased with the increase in age of the crop. Among in-situ moisture conservation practices raised bed significantly recorded higher plant height (100.8 cm) which is on par with conservation furrow at sowing (99.0 cm) and lowest was recorded in flat bed method (89.8 cm). Raised bed recorded higher plant height (1.81 %, 5.33 %, 9.22 % and 12.29) over conservation furrow at sowing treatment, conservation furrow at 30-45 DAS, flat bed +live mulch and flat bed method without moisture conservation The increase in plant height might be ascribed to the increased availability of nutrients due to moisture supply and restricted weed growth. These results are in conformity with findings of Kumari et al. (2019). Among nutrient management practices 125 % RDF recorded higher plant height (108.2 cm) followed by 100 % RDF (102.1 cm). The lowest was recorded in control treatment (76.3 cm). 125 % RDF recorded 6 % and 41.83 % more plant height over 100 % RDF and control treatment, respectively. Lower plant height is because

of lower nutrient availability and soil moisture conditions. These findings are in agreement with Kumari *et al.,* (2019), Vani *et al.,* (2020) and Siddu and Aladakatti (2021).

Raised bed with 125 % RDF recorded higher plant height (113.1 cm) which was on par with conservation furrow at sowing with 125 % RDF (111.6 cm) and was followed by conservation furrow at 30-45 DAS with 125 % RDF (109.4 cm), raised bed with 100% RDF (107.3 cm), conservation furrow at sowing with 100 % RDF (105.0 cm), flat bed with dhaincha as live mulch with 125 % RDF (104.3 cm), conservation furrow at 30-45 DAS with 100 % RDF (102.9 cm) and flat bed with 125 % RDF (102.9 cm). Lowest plant height (70.0 cm) was recorded with flat bed without moisture conservation with control.

Number of monopodial branches increased with advancement in crop age. Data pertaining to number of monopodial branches plant¹ are influenced with in-situ moisture conservation and nutrient management practices. Raised bed recorded higher number of monopodial branches plant¹ (3.09) due to better soil moisture availability and absorption of more moisture and nutrients this resulted in vigorous plant growth and higher number of monopodial branches. Conservation furrow at sowing (2.84) was on par with conservation furrow at 30-45 DAS (2.74). Lowest (2.41) was recorded in flat bed without moisture conservation. These results are in conformity with findings of Pendke et al., (2001) and Hulihalli and Patil (2004). Among nutrient management practices 125 % RDF recorded higher monopodial branches plant¹ (3.15) and the lower monopodial branches (2.1) was recorded in control. Similar findings were reported by Vora et al., (2016), Muthukrishnan et al., (2017) and Vani et al., (2020).

Raised bed with 125 % RDF recorded higher number of monopodial branches plant¹ (3.5) which was on par with raised bed with 100% RDF (3.4) and conservation furrow at sowing with 125 % RDF (3.3) and was followed by conservation furrow at 30-45 DAS with 125 % RDF (3.1), conservation furrow at sowing with 100% RDF (3.1), flat bed with dhaincha as live mulch with 125 % RDF (3.0), conservation furrow at 30-45 DAS with 100 % RDF (3.0), flat bed with 125% RDF (2.8), flat bed with dhaincha as live mulch with 100 % RDF (2.7), flat bed with 100 % RDF (2.6) and raised bed with control (2.4) they were significantly on par with each other. Lower number of monopodial branches plant⁻¹ (1.8) was recorded with flat bed without moisture conservation with control.

In-situ moisture conservation showed significant effect on sympodial branches plant⁻¹. Raised bed recorded higher number of sympodial branches plant¹ (16.00). This was due to availability of adequate moisture, nutrients and light deciding growth and development reflected in higher number of sympodial branches and lowest (11.91) was recorded in flat bed without moisture conservation. Among nutrient management practices 125 % RDF recorded higher sympodial branches plant¹ (16.13) and was followed by 100 % RDF (14.75) and control treatment (11.12). This difference in number of sympodial branches plant¹ can be attributed to genetic makeup and environmental changes, which reduced the number of nodes resulting in reduction in number of sympodial branches plant⁻¹. Similar findings were reported by Singh et al., (2018) and Sankaranarayanan et al., (2021).

Interaction of sympodial branches plant¹ was significantly influenced by *in-situ* moisture conservation and nutrient management practices. Raised bed with 125% recorded higher number of sympodial branches plant¹ (18.6) and was superior over rest of the treatments. Conservation furrow at sowing with 125 % RDF (17.1) and raised bed with 100 % RDF (16.8) were on par with each other. This was due to sufficient amount of moisture with nutrient availability to the crop. This leads to the higher productivity of crop and the lesser number of sympodial branches plant¹ (9.0) was recorded with flat bed without moisture conservation with control.

Dry matter production (kg ha⁻¹)

The crop yield is function of source and sink relationship. Raised bed recorded higher dry matter production (3567 kg ha⁻¹) compared with other treatments. Lower dry matter production (2834 kg ha⁻¹) was recorded in flat bed without moisture conservation and was on par with flat bed with dhaincha as live mulch (2978 kg ha⁻¹). Raised bed method recorded higher dry matter production of 7.85%, 13.76%, 19.80% and 25.86 % over conservation furrow at sowing, conservation furrow at 30-45 DAS, flat bed +live mulch and flat bed without

moisture conservation, respectively. This might be due to soil moisture conservation techniques that facilitate prolonged availability of moisture as compared to flat bed without moisture conservation. These results are in conformity with Dangore *et al.*, (2001) and Ambika *et al.*, (2019).

Nutrient management practices also significantly influenced the dry matter production. 125 % RDF (S₃) recorded higher dry matter production (3812 kg ha⁻¹) and was on par with 100 % RDF (3697 kg ha⁻¹). This might be attributed to higher and more consistent nutrient availability which would have led to an increase in the number and area of leaves, which in turn would have improved photosynthetic activity and eventually led to the generation of biomass. These results are in conformity with those of Maurya *et al.*, (2009). Lowest dry matter production (1988 kg ha⁻¹) was observed in control.

Raised bed with 125 % RDF recorded highest dry matter production (4230 kg ha⁻¹) and was followed by raised bed with 100 % RDF (4113 kg ha-1), conservation furrow at sowing with 125 % RDF (3976 kg ha⁻¹), conservation furrow at sowing with 100 %RDF (3847 kg ha⁻¹), conservation furrow at 30-45 DAS with 125 % RDF (3751 kg ha⁻¹), conservation furrow at 30-45 DAS with 100 % RDF (3662 kg ha-1), flat bed with dhaincha as live mulch with 125 % RDF (3628 kg ha⁻¹), flat bed with dhaincha as live mulch with 100 % RDF (3503 kg ha-1), flat bed with 125 % RDF (3476 kg ha⁻¹) and flat bed with 100 % RDF (3359 kg ha⁻¹) and inturn were on par with each other. The lowest dry matter production (1668 kg ha⁻¹) was recorded in flat bed without moisture conservation with control which is on par with flat bed with dhaincha as live mulch with control (1802 kg ha⁻¹).

Effect of *in-situ* moisture conservation and nutrient management practices on yield

Lint yield (kg ha⁻¹) and seed cotton yield (kg ha⁻¹) was significantly influenced by *in-situ* moisture conservation and nutrient management practices. The higher yields were due to improved yield components *i.e.*, number of squares, number of bolls plant¹, mean boll weight and seed cotton yield plant¹.

Lint yield (506 kg ha⁻¹) and seed cotton yield (1427 kg ha⁻¹) were significantly higher in raised bed. It might be due to increased availability of nutrients, moisture supply, better root penetration, better drainage, less soil compaction and restricted weed growth compared to conventional methods. *In-situ moisture* conservation in the soil profile reservoir plays an important role in conservation of maximum possible rain water in the soil and there by the availability of more moisture for longer time improved the yield of cotton. These results are in conformity with findings of Pendke *et al.*, (2001), Hulihalli and Patil (2004), Arora and Bhatt (2012). Significantly lower lint yield (282 kg ha⁻¹) and seed cotton yield (506 kg ha⁻¹) was recorded with flat bed without moisture conservation this was probably due to restricted water and air movement due to poor soil physical properties results in lower yield. Similar results were reported by Ambika *et al.*, (2019) and Vinay *et al.*, (2022)

Nutrients at different levels significantly influenced the yield. Among nutrient management practices 125 % RDF recorded higher lint yield (495 kg ha⁻¹) and seed cotton yield (1412 kg ha⁻¹). This was due to the favorable effect of improved soil moisture and supply of nutrients throughout the crop growth period. The lowest lint yield (239 kg ha⁻¹) and seed cotton yield (733 kg ha⁻¹) was recorded in control. No fertilizer application and without moisture leads to nutrient imbalance and decreased the lint and seed cotton yield. Similar results were reported by Reddy *et al.*, (2017) and Ali and Ahmad, (2021).

Raised bed with 125 % RDF recorded higher lint yield (621 kg ha⁻¹) and was superior over rest of the treatments. Raised bed with 100 % RDF (583 kg ha⁻¹) followed by conservation furrow at sowing with 125 % RDF (573 kg ha⁻¹), conservation furrow at sowing with 100 % RDF (543 kg ha⁻¹) they were significantly on par with each other. The lowest lint yield (171 kg ha⁻¹) was recorded in flat bed without moisture conservation with control which is on par with flat bed with dhaincha as live mulch with control (201 kg ha-1). Raised bed with 125 % RDF recorded 7.72 %, 19.80 %, 30.27% and 43.96 % higher lint yield over conservation furrow at sowing with 125 % RDF, conservation furrow at 30-45 DAS with 125 % RDF, flat bed with dhaincha as live mulch with 125 % RDF and flat bed with 125 % RDF, respectively.

Highest seed cotton yield (1717 kg ha⁻¹) was recorded with raised bed with 125 % RDF and was on par with raised bed with 100 % RDF (1645 kg ha⁻¹) followed by conservation furrow at sowing with 125 % RDF (1601 kg ha⁻¹), conservation furrow at

in-sit	<i>u</i> moisture	conservat	ion method	ls and nuti	rient manag	ement practi	ices					
Treatments	Ρľ	ant height ((cm)	Monop	odial branch	nes plant ⁻¹	Sympo	dial branche	es plant¹	Dry matt	er productio	n (kg ha ^{.1})
	2021-22	2022-23	Mean	2021-22	2022-23	Mean	2021-22	2022-23	Mean	2021-22	2022-23	Mean
Main plot		_					-					
М1	91.1	88.5	89.8	2.5	2.3	2.4	12.0	11.8	11.9	2912	2757	2834
M2	93.3	91.3	92.3	2.7	2.5	2.6	13.2	12.9	13.0	3028	2928	2978
M3	99.8	98.3	0.66	2.9	2.8	2.8	15.0	14.8	14.9	3361	3255	3308
M4	101.6	100.0	100.8	3.2	3.0	3.1	16.2	15.8	16.0	3612	3522	3567
M5	96.3	95.1	95.7	2.8	2.7	2.7	14.3	13.9	14.1	3210	3073	3141
SE(m)±	1.09	0.26	0.57	0.06	0.04	0.04	0.13	0.14	0.11	59.35	72.42	46.36
CD (p=0.05)	3.56	0.83	1.87	0.20	0.14	0.14	0.43	0.44	0.35	193.52	236.14	151.22
Sub plot:		_	-		_			-				
S1	77.2	75.4	76.3	2.2	2.0	2.1	11.3	11.0	11.1	2014	1963	1988
S2	102.9	101.3	102.1	3.0	2.9	3.0	14.9	14.6	14.8	3791	3602	3697
S3	109.2	107.2	108.2	3.2	3.1	3.2	16.3	16.0	16.1	3869	3755	3812
SE(m)±	0.69	0.33	0.39	0.05	0.05	0.03	0.12	0.11	0.10	83.32	73.94	65.38
CD (p=0.05)	2.05	0.96	1.14	0.15	0.14	0.10	0.35	0.32	0.30	245.75	218.11	192.87
Interaction												
M×S												
SE(m)±	1.67	0.65	0.91	0.11	0.10	0.08	0.26	0.24	0.22	186.30	153.19	128.05
CD (p=0.05)	4.58	2.15	2.56	0.33	0.32	0.22	0.79	0.71	0.67	549.50	487.71	431.27
S×M												
SE(m)±	1.90	0.44	1.00	0.11	0.07	0.08	0.23	0.24	0.19	102.79	125.43	80.29
CD (p=0.05)	3.83	1.48	2.08	0.26	0.22	0.17	0.59	0.55	0.49	373.11	350.04	292.61
Main plot: <i>In-situ</i> sowing	g with CRIE	onservation: DA Ridge pl	: M ₁ : Flatbed anter, M ₄ : Ra	sowing with aised bed a	h CRIDA 6 ro ind furrow wi	w planter, M ₂ : ith CRIDA Rai	Flatbed +live sed bed pla	e mulch with nter, M ₅ : Con	dhaincha as servation fu	live mulch, N urrow at 30 -	1 ₃ : Conservati 40 DAS after	on furrow at interculture
operat of NPI	tions, Sub p K (150:75:75	olot: Nutrieni 5)	t managemen	it-S ₁ : contro	ol (without fer	rtilizer), S ₂ : 10	0% Recomme	ended dose o	f NPK (120:0	60:60) and S ₃ :	125% Recom	mended dose

Table 1. Plant height, number of monopodial branches, number of sympodial branches and dry matter production of cotton at harvest as influenced by

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	Sub trea	tments		
	202	21		
Main treatments	S1	S2	S3	Mean
M1	71.3	97.9	104.0	91.1
M2	75.3	99.6	105.0	93.3
M3	81.1	105.5	112.8	99.8
M4	82.7	108.2	114.0	101.6
M5	75.6	103.4	110.0	96.3
Mean	77.2	102.9	109.2	96.4
	Main	Sub	MXS	SXM
S.Em±	1.09	0.26	1.67	1.90
CD (p=0.05)	3.56	0.83	4.58	3.83
	202	22		
Main treatments	S1	S2	S3	Mean
M1	68.7	95.6	101.2	88.5
M2	73.1	97.3	103.5	91.3
M3	79.7	104.6	110.4	98.3
M4	81.3	106.4	112.3	100.0
M5	74.2	102.3	108.8	95.1
Mean	75.4	101.3	107.2	94.6
	Main	Sub	MXS	SXM
S.Em±	0.26	0.33	0.91	1.00
CD at 5%	0.83	0.96	2.56	2.08
	Pooled mea	an (2021 & 2022)		
Main treatments	S1	S2	S3	Mean
M1	70.0	96.8	102.6	89.8
M2	74.2	98.4	104.3	92.3
M3	80.4	105.0	111.6	99.0
M4	82.0	107.3	113.1	100.8
M5	74.9	102.9	109.4	95.7
Mean	76.3	102.1	108.2	95.5
	Main	Sub	MXS	SXM
S.Em±	0.57	0.39	0.91	1.00
CD at 5%	1.87	1.14	2.56	2.08

Table 1.1. Interaction between in-situ moisture conservation method and nutrient management practices on plant height of cotton

Subtreatments								
	2021	l						
Main treatments	S1	S2	S3	Mean				
M1	1.8	2.7	2.9	2.5				
M2	2.1	2.8	3.1	2.7				
M3	2.2	3.2	3.3	2.9				
M4	2.5	3.4	3.5	3.2				
M5	2.1	3.1	3.2	2.8				
Mean	2.2	3.0	3.2	2.8				
	Main	Sub	MXS	SXM				
S.Em±	0.06	0.05	0.11	0.11				
CD (p=0.05)	0.20	0.15	0.33	0.26				
	2022	2						
Main treatments	S1	S2	S3	Mean				
M1	1.7	2.5	2.7	2.3				
M2	1.9	2.7	2.9	2.5				
M3	2.1	3.0	3.2	2.8				
M4	2.3	3.3	3.4	3.0				
M5	2.1	2.9	3.1	2.7				
Mean	2.0	2.9	3.1	2.7				
	Main	Sub	MXS	SXM				
S.Em±	0.04	0.05	0.10	0.07				
CD at 5%	0.14	0.14	0.32	0.22				
Pooled mean (2021 & 2022)								
Main treatments	S1	S2	S3	Mean				
M1	1.8	2.6	2.8	2.4				
M2	2.0	2.7	3.0	2.6				
M3	2.2	3.1	3.3	2.8				
M4	2.4	3.4	3.5	3.1				
M5	2.1	3.0	3.1	2.7				
Mean	2.1	3.0	3.2	2.7				
	Main	Sub	MXS	SXM				
S.Em±	0.04	0.03	0.08	0.08				
CD at 5%	0.14	0.10	0.22	0.17				

Table 1.2. Interaction between *in-situ* moisture conservation method and nutrient management practices on monopodial branches plant⁻¹ of cotton

	Sub treat	ments						
	202 ⁻	1						
Main treatments	S1	S2	S3	Mean				
M1	9.1	12.9	14.0	12.0				
M2	10.9	13.7	15.1	13.2				
M3	12.1	15.8	17.1	15.0				
M4	12.7	17.1	18.7	16.2				
M5	11.5	15.0	16.4	14.3				
Mean	11.3	14.9	16.3	14.1				
	Main	Sub	MXS	SXM				
S.Em±	0.13	0.12	0.26	0.23				
CD (p=0.05)	0.43	0.35	0.79	0.59				
	202	2						
Main treatments	S1	S2	S3	Mean				
M1	8.9	12.6	13.9	11.8				
M2	10.4	13.4	14.8	12.9				
М3	11.8	15.7	17.0	14.8				
M4	12.5	16.6	18.5	15.8				
M5	11.3	14.7	15.9	13.9				
Mean	11.0	14.6	16.0	13.8				
	Main	Sub	MXS	SXM				
S.Em±	0.14	0.11	0.24	0.24				
CD at 5%	0.44	0.32	0.71	0.55				
Pooled mean (2021 & 2022)								
Main treatments	S1	S2	S3	Mean				
M1	9.0	12.8	13.9	11.9				
M2	10.6	13.6	14.9	13.0				
M3	12.0	15.7	17.1	14.9				
M4	12.6	16.8	18.6	16.0				
M5	11.4	14.8	16.1	14.1				
Mean	11.1	14.8	16.1	13.9				
	Main	Sub	MXS	SXM				
S.Em±	0.11	0.10	0.22	0.19				
CD at 5%	0.35	0.30	0.67	0.49				

Table 1.3. Interaction between *in-situ* moisture conservation method and nutrient management practices on sympodial branches plant⁻¹ of cotton

	Sub tre	atments					
	20)21					
Main treatments	S1	S2	S 3	Mean			
M1	1731	3484	3522	2912			
M2	1832	3588	3663	3028			
М3	2117	3919	4048	3361			
M4	2346	4184	4307	3612			
M5	2042	3781	3806	3210			
Mean	2014	3791	3869	3225			
	Main	Sub	MXS	SXM			
S.Em±	59.35	83.32	186.30	102.79			
CD (p=0.05)	193.52	245.75	549.50	373.11			
	20)22					
Main treatments	S1	S2	S 3	Mean			
M1	1606	3234	3430	2757			
M2	1772	3419	3593	2928			
М3	2085	3776	3904	3255			
M4	2371	4042	4154	3522			
M5	1979	3542	3697	3073			
Mean	1963	3602	3755	3107			
	Main	Sub	MXS	SXM			
S.Em±	72.42	73.94	153.19	125.43			
CD at 5%	236.14	218.11	487.71	350.04			
Pooled mean (2021 & 2022)							
Main treatments	S1	S2	S3	Mean			
M1	1668	3359	3476	2834			
M2	1802	3503	3628	2978			
M3	2101	3847	3976	3308			
M4	2359	4113	4230	3567			
M5	2011	3662	3751	3141			
Mean	1988	3697	3812	3166			
	Main	Sub	MXS	SXM			
S.Em±	46.36	65.38	128.05	80.29			
CD at 5%	151.22	192.87	431.27	292.61			

Table 1.4. Interaction between *in-situ* moisture conservation method and nutrient management practices on dry matter production (kgha⁻¹) of cotton

Treatments	Li	nt yield (kg ha	1 ⁻¹)	Seed o	J ha⁻¹)	
	2021-22	2022-23	Mean	2021-22	2022-23	Mean
Main plot		l			· · · · ·	
M1	295	271	282	896	822	859
M2	341	322	328	990	945	967
M3	494	441	462	1398	1272	1335
M4	539	480	506	1499	1356	1427
M5	424	385	401	1246	1122	1184
SE(m)±	8.73	8.74	7.09	24.83	20.29	20.11
CD (p=0.05)	28.29	28.93	23.46	82.23	67.19	66.59
Sub plot:						
S1	264	224	239	794	671	733
S2	480	440	453	1349	1289	1319
S3	534	477	495	1473	1351	1412
SE(m)±	6.10	7.92	5.11	16.92	20.71	11.64
CD (p=0.05)	18.12	23.53	15.16	50.25	61.53	34.58
Interaction						
M×S						
SE(m)±	14.15	16.89	11.71	39.63	42.91	29.26
CD (p=0.05)	43.88	51.72	36.24	123.03	130.76	91.63
SXM						
SE(m)±	15.13	15.13	12.27	43.01	35.14	34.83
CD (p=0.05)	42.30	54.22	35.34	117.43	141.12	81.52

Table 2. Lint yield (kg ha⁻¹) and seed cotton yield (kg ha⁻¹) of cotton as influenced by *in-situ* moisture conservation methods and nutrient management practices

Main plot: *In-situ* moisture conservation: M₄: Flatbed sowing with CRIDA 6 row planter, M₂: Flatbed +live mulch with dhaincha as live mulch, M₃: Conservation furrow at sowing with CRIDA Ridge planter, M₄: Raised bed and furrow with CRIDA Raised bed planter, M₅: Conservation furrow at 30 - 40 DAS after interculture operations, Sub plot: Nutrient management-S₁: control (without fertilizer), S₂: 100% Recommended dose of NPK (120:60:60) and S₃: 125% Recommended dose of NPK (150:75:75)

sowing with 100 % RDF (1573 kg ha⁻¹) they were significantly on par with each other. Raised bed with 125 % RDF recorded 6.76 %, 14.97 % 27.83 % and 39.19 % higher seed cotton yield over conservation furrow at sowing with 125 % RDF conservation furrow at 30-45 DAS with 125 % RDF flat bed with dhaincha as live mulch with 125 % RDF and flat bed with 125 % RDF. The lowest seed cotton yield (546 kg ha⁻¹) was recorded in flat bed without moisture conservation with control which is on par with flat bed with dhaincha as live mulch with control (622 kg ha⁻¹). Similar results are reported by Kumari *et al.*, (2019) and Ambika *et al.*, (2019)

CONCLUSION

Experimental results revealed that *in-situ* moisture conservation and nutrient management practices significantly enhances the crop growth and yield under rainfed Alfisols. It is concluded that the raised bed conservation method with 125 % recommended dose of NPK (150:75:75 kg ha⁻¹) and their interaction effect shows improved the growth and yield parameters of rainfed cotton.

	Sub trea	tments								
	202	21								
Main treatments	S1	S2	S3	Mean						
M1	178	351	369	295						
M2	204	375	455	341						
Мз	310	568	623	494						
M4	351	622	673	539						
M5	275	482	548	424						
Mean	264	480	534	419						
	Main	Sub	MXS	SXM						
S.Em±	8.73	6.10	14.15	15.13						
CD (p=0.05)	28.29	18.12	43.88	42.30						
	2022									
Main treatments	S1	S2	S3	Mean						
M1	169	312	332	271						
M2	202	345	420	322						
M3	249	519	555	441						
M4	282	566	593	480						
M5	217	457	482	385						
Mean	224	440	477	380						
	Main	Sub	MXS	SXM						
S.Em±	8.74	7.92	16.89	15.13						
CD at 5%	28.93	23.53	51.72	54.22						
	Pooled mean	(2021 & 2022)								
Main treatments	S1	S2	S3	Mean						
M1	171	325	348	282						
M2	201	352	433	328						
M3	271	543	573	462						
M4	314	583	621	506						
M5	239	464	498	401						
Mean	239	453	495	396						
	Main	Sub	MXS	SXM						
S.Em±	7.09	5.11	11.71	12.27						
CD at 5%	23.46	15.16	36.24	35.34						

Table 2.1. Interaction between *in-situ* moisture conservation method and nutrient management practices on lint yield of cotton

Subtreatments									
2021									
Main treatments	S1	S2	S 3	Mean					
M1	567	1019	1100	896					
M2	629	1080	1261	990					
M3	937	1600	1657	1398					
M4	1007	1666	1823	1499					
M5	831	1383	1525	1246					
Mean	794	1349	1473	1206					
	Main	Sub	MXS	SXM					
S.Em±	24.83	16.92	39.63	43.01					
CD (p=0.05)	82.23	50.25	123.03	117.43					
	202	22							
Main treatments	S1	S2	S 3	Mean					
M1	525	955	987	822					
M2	615	1003	1216	945					
M3	727	1547	1544	1272					
M4	833	1625	1610	1356					
M5	656	1315	1396	1122					
Mean	671	1289	1351	1103					
	Main	Sub	MXS	SXM					
S.Em±	20.29	20.71	42.91	35.14					
CD at 5%	67.19	61.53	130.76	141.12					
	Pooled mean	2021 & 2022)							
Main treatments	S1	S2	S 3	Mean					
M1	546	987	1044	859					
M2	622	1041	1239	967					
M3	832	1573	1601	1335					
M4	920	1645	1717	1427					
M5	744	1349	1460	1184					
Mean	733	1319	1412	1154					
	Main	Sub	MXS	SXM					
S.Em±	20.11	11.64	29.26	34.83					
CD at 5%	66.59	34.58	91.63	81.52					

 Table 2.2. Interaction between *in-situ* moisture conservation method and nutrient management practices on seed cotton yield of cotton

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INFLUENCE OF FERTIGATION LEVELS AND DRIP IRRIGATION LEVELS ON YIELD AND QUALITY OF *RABI* CHILLI UNDER MULCH AND NO MULCHED CONDITIONS

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ABSTRACT

The field investigation was carried out during *rabi* season to evaluate the influence of fertigation levels, mulching and drip irrigation levels on fresh fruit yield and yield quality at WTC fields, PJTSAU, Rajendranagar, Telangana in 2020-21 and 2021-22. The study was consisted of 12 treatment combinations *viz.*. 75 %, 100 % (300-60-120 kg ha⁻¹ N-P₂O₅-K₂O) & 125% RDF, drip irrigation levels (0.8 Epan & 1.0 Epan) imposed under factorial combination of mulch (M₁) and no mulch (M₀) conditions. Among the fertigation levels, 100% RDF (31.15 t ha⁻¹, 195.04 mg 100 g⁻¹, 10.33 % & 0.94 %, respectively) recorded the higher fresh fruit yield, ascorbic acid content, oleoresin content and capsaicin content in pooled data. Mulching treatment has recorded the higher values for fresh fruit yield (37.56 t ha⁻¹), ascorbic acid content (224.43 mg 100 g⁻¹), oleoresin content (11.11 %) and capsaicin content (0.74 %) in pooled data when compared to unmulched treatment.

KEYWORDS: Fertigation levels, irrigation levels, mulch, ascorbic acid, oleoresin Content, capsaicin content and chilli fruit yield

Vegetable and fruit production is one of the emerging businesses in India in off and on season. In Telangana, agriculture contributes a large sector of the State's economy and uses around 55.51 lakh ha with 27 different crops though vegetable production is limited due to scarcity of water and labour along with price fluctuations around the year. Inadequate soil moisture and high soil temperature with low organic carbon content are the major factors for lower yield and quality of vegetable production (Yogaraju *et al.*, 2019).

Chilli (*Capsicum annuum* L.) commonly known as hot pepper belongs to the family Solanaceae and is cultivated as an annual crop worldwide. It is an important spice as well as vegetable crop, where both ripe and unripe fruits are used for culinary, salad, anticancer agent and processing purposes. Its extract is used in pharmaceutical industry for colouring the medicines. It is an excellent source of vitamin A and C. chilli has many beneficial effects and sometimes referred as capsule of vitamin C due to high amounts of vitamin C (Davinder singh, 2016).

India contributes about 36.57% to the total world chilli production and occupied an area of 411.0 thousand hectares in the country with a production of

4363.0 thousand tonnes and productivity of 10.6 t ha⁻¹. In Telangana, it occupies an area of 10.9 thousand hectares with a production of 15.78 thousand tonnes (Indiastat-2021-22). India is the largest producer, consumer and exporter of chilli, which contribute to 25 per cent of total India's production. It responds well to split application of nutrients through fertigation in terms of increased growth and yield. Nitrogen is an essential component of nucleic acid and has been suggested to improve the development of vegetative structures. Potassium is well known for its role in improving the quality. Pungency and colour are two important characters liked by consumers. In foreign export and imports, quality is the important factor to be considered which can be achieved only through optimum nutrient application (Biwalkar et al., 2015).

Mulching is a pertinent method to modify the hydrothermal regime of crop and enhance the efficiency of nutrients resulting in more yield over open field condition (Shailendra *et al.*, 2021). The consumption of water varies with method and frequency of application. To achieve higher yield through drip, proper irrigation scheduling is a viable option since it can minimize the water losses of runoff and deep percolation (Supekar *et al.*, 2021).

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MATERIAL AND METHODS

The present experiment was carried out at Water Technology Center, College Farm, College of Agriculture, Rajendranagar, PJTSAU, Hyderabad. The field experiment was laid out in a 2 x 2 x 3 factorial randomised block design, wherein three fertigation levels *viz.*, F₇₅ (225-45-90 kg N, P₂O₅-K₂O ha⁻¹), F₁₀₀ (300-60-120 kg N, P₂O₅-K₂O ha⁻¹) & F₁₂₅ (375-75-150 kg N, $P_2O_5-K_2O$ ha⁻¹) and two drip irrigation levels ($I_{0.8} \& I_{1.0}$) were imposed under factorial combination of with mulch (M_1) and unmulched (M_0) conditions. Thus, the experiment consisted of total 12 treatment combinations which were replicated thrice. Silver-black mulch (30μ) sheet was laid on the field as per the treatments. Robust seeds of chilli hybrid (Sonali) used as test crop and 40 days seedlings were transplanted in paired row method at spacing 45 / 30 cm x 40 cm. Equal amount of irrigation was given in all treatments up to 10 DAS to get better establishment after that irrigation was scheduled at 2 days interval based on daily evaporation data (0.8 Epan & 1.0 Epan) recorded from USWB class 'A' pan evaporimeter in agrometeorological station, ARI Farm, Rajendranagar, Hyderabad. Total 759.30 & 607.44 mm of water given to I₁₀ and I₀₈ treatments in 64 irrigation cycles,

respectively in 2020-21 (I year) whereas 918.00 & 734.40 mm of water given in 72 irrigation cycles at I₁₀ & I₀₈ levels, respectively during II year. The fertigation was carried out through ventury system on every 4th day from 12th DAT at 38 splits as per the treatments. Urea, Urea Phosphate and MoP fertilizers were used as sources of N, P & K, respectively and all the nutrients were applied through fertigation and scheduled according to stage of the crop. The 30%-30%-40% nitrogen applied at 10-40 DAT, 41-70 DAT and 71-150 DAT, respectively. The 35%-35%-30% phosphorus applied at 10-40 DAT, 41-70 DAT and 71-150 DAT, respectively and 30%-30%-40% K applied at 10-40 DAT, 41-70 DAT and 71-150 DAT, respectively. Chilli fresh fruit yield was recorded at different pickings and sum of all the pickings considered as total fresh fruit yield plot¹ and expressed in t ha¹. Quality parameters were analysed immediately after harvesting of fresh fruits during both the years. Quality parameters were analysed at first, third & last picking and the mean results are discussed. The ascorbic acid content, oleoresin content and capsaicin content was estimated as per the procedure given by Sadasivam and Manickam (2005).

Mean of two years (pooled data) - Total fruit yield (t ha-1)									
Treatments	No mulch		h Mean Mulch		ulch	Mean	Overall Mean		
	I _{0.8}	l _{1.0}		I _{0.8}	I _{1.0}				
F ₇₅	16.50	17.12	16.81	30.79	33.01	31.90	24.36		
F ₁₀₀	17.71	23.08	20.40	42.71	41.09	41.90	31.15		
F ₁₂₅	17.62	23.11	20.37	40.61	37.12	38.87	29.62		
Mean	17.28	21.11	19.19	38.04	37.07	37.56			
	Mean of I 0.8			27.66 Mean of I _{1.0}					
Treatments	SEm(±)	CD (P=0.05)	Interactions		SEm(±)	CD (P=0.05)			
Mulch (M)	3.00	8.80	IXM			4.25	NS		
Irrigation (I)	3.00	NS	FxM			5.20	NS		
Fertigation (F)	1.06	3.11	FxI			5.20	NS		
			FxMxI			7.35	NS		

 Table 1. Effect of fertigation and irrigation levels under mulch and non-mulch conditions on total fruit yield (t ha⁻¹) of chilli in pooled data during *rabi* season.

F₇₅= 225-45-90 kg N-P₂O₅-K₂O ha⁻¹;F₁₀₀= 300-60-120 kg N-P₂O₅-K₂O ha⁻¹;F₁₂₅= 375-75-150 kg N-P₂O₅-K₂O ha⁻¹

RESULTS AND DISCUSSION

Yield

The total fresh fruit yield of chilli was found to be significantly influenced by mulch and fertigation levels (Table 1) during both the years. The pooled data pertaining to fertigation levels revealed that yield with F₇₅ (24.36 t ha⁻¹) was significantly lower than the yield with higher fertigation levels. The F_{100} (31.15 t ha⁻¹) recorded significantly higher yield and was on par with higher fertigation levels *i.e.*, F₁₂₅ (29.62 t ha⁻¹). Drip fertigation with 100% RDF positively influenced the total fresh fruit yield of chilli over 75% RDF which was due to positive effect on yield attributing characters ultimately resulting in higher fruit yield. Right amount of NPK during flowering stage favourably increased the number of flowers plant¹ which obviously led to increased fruit setting. Also the timely availability of nutrients led to increased growth, higher uptake of nutrients, better photo assimilation and better translocation of assimilates from source to sink which in turn increased total fresh fruit yield (Subramani, 2008). Ayare et al., (2012) explained that fertigation concentrated near the root zone and resulted in production of more dry matter by more efficient utilization of applied nutrients and ultimately resulted in higher yield of the crop.

Beneficial effect of mulching was observed in both the years, pooled data revealed that mulching recorded significantly higher yield (1.96 times) over nonmulching treatment (19.19 t ha⁻¹). This might be due to the congenial conditions to the plant provided by mulching like water availability, retention of soil moisture for long time and constant availability of nutrients throughout the crop period which improved the growth and yield. Similar results were observed by Kumara *et al.* (2015). Another reason might be that the pest and disease attack was relatively less under mulch and healthier plants were observed in mulch which resulted in higher yield over no mulch (Iftikhar Ahmad *et al.*, 2011).

The irrigation levels did not show any significant difference during both the years. Application of water in $I_{_{0.8}}$ resulted in on par yield with irrigation level at $I_{_{1.0}}$ resulting in 20 % of water without adverse effect. Among the interactions, the various combinations of fertigation levels, mulch and drip irrigation levels were found to be non-significant.

The irrigation requirement of crop varies in mulch and non-mulching conditions, Optimum soil moisture condition near the root zone due to drip irrigation has reduced the moisture losses and proliferate the dry

Mean of two years (pooled data) - ascorbic acid content (mg 100 g^{-1})										
Treatments	Nomulch		Mean	Mean Mulch		Mean	Overall Mean			
	І _{0.8}	I _{1.0}		I _{0.8}	I _{1.0}					
F ₇₅	152.63	152.62	152.63	201.94	207.83	204.88	178.75			
F ₁₀₀	162.07	178.15	170.11	237.92	241.35	239.64	204.87			
F ₁₂₅	149.86	172.78	161.32	232.19	225.34	228.76	195.04			
Mean	154.85	167.85	161.35	224.01	224.84	224.43				
Mean of I _{0.8}			189.43	Mean of I _{1.0}		196.35				
Treatments	SEm(±)	CD (P=0.05)		Interactions		SEm(±)	CD (P=0.05)			
Mulch (M)	11.38	33.38	IX	IXM			NS			
Irrigation (I)	11.38	NS	FxM			19.71	NS			
Fertigation (F)	4.02	11.80	FxI			19.71	NS			
			F :	FxMxI			NS			

Table 2. Effect of fertigation and irrigation levels under mulch and non-mulch conditions on ascorbic acid content (mg 100 g⁻¹) of chilli in pooled data during *rabi* season.

 F_{75} = 225-45-90 kg N-P₂O₅-K₂O ha⁻¹; F_{100} = 300-60-120 kg N-P₂O₅-K₂O ha⁻¹; F_{125} = 375-75-150 kg N-P₂O₅-K₂O ha⁻¹

matter partitioning and helped in the diversion of photosynthesis to the reproductive parts ultimately resulted in higher yield (Rajanee et al., 2017). Due to this positive effect of drip irrigation, application of less water at 0.8 Epan was also sufficient to meet crop demand. Maida et al., (2020) also observed that 0.8 IW/CPE treatment has recorded the maximum fruit length, higher fruit diameter, highest average weight of fruit. Chilli is sensitive to fluctuation in temperature and moisture. Mulch protected the crop from higher temperatures and modified the hydrothermal properties of soil which resulted in better plant growth and yield (Siti Aishai Hassan et al., 1995). Mulch in conjunction with drip irrigation and fertigation hastens the growth and yield of the crop which may be due to favourable moisture maintained in the root zone, its availability to plants, avoiding leaching of soluble fertilizers and provide weed free environment.

Ascorbic Acid (mg 100 g⁻¹)

The results revealed that the highest ascorbic acid content (Table 2) with higher fertigation levels. In pooled data, the F_{100} has recorded the highest ascorbic acid content (204.87 mg 100 g⁻¹) and was on par to the F_{125} (195.04 mg 100 g⁻¹) and the lower value was recorded in F_{75} (178.75 mg 100 g⁻¹). Higher ascorbic acid content was found with optimum level of NPK @

100 % RDF could be due to higher NPK which influenced the carbohydrate synthesis and formation of ascorbic acid, as ascorbic acid is mainly constituted of carbohydrate compounds. These results were in line with Mounika (2016) and Shilpa (2019). Mehnaz Akram *et al.*, (2017) and Janardhanrao (2020) who reported that the highest ascorbic acid content (118 mg 100 g⁻¹) with higher levels of NPK @ 100 % RDF was due to enhancement of enzyme activity for amino acids synthesis leading to higher ascorbic acid content.

With mulching of the chilli crop, the M_1 has recorded 39.09% higher ascorbic acid content over M_0 (161.35 mg 100 g⁻¹) in pooled data. There was insignificant variation found between $I_{0.8} \& I_{1.0}$ irrigation levels during both the years. The interaction effect among fertigation levels, mulch and drip irrigation levels was found to be non-significant. Relatively higher content was observed in F_{100} + $I_{1.0}$ + M_1 (241.35 mg 100 g⁻¹). There was positive correlation observed between yield and mean ascorbic acid content. The determination coefficient (R^2) (Figure 1) was 0.962 in pooled data which showed a linear increase in ascorbic acid content observed with total fresh yield.

Generally ascorbic acid production mainly controlled by changes in the soil temperature (Davinder Singh 2016). Mulch maintained the temperature range

Table 3. Effect of fertigation and irrigation levels under mulch and non-mulch conditions on oleoresin content (%) of chilli during 2020-21 (I year), 2021-21 (II year) and in pooled data during *rabi* season.

Mean of two years (pooled data) - oleoresin content (%)									
Treatments	Nomulch		Mean	Mulch		Mean	Overall Mean		
	I _{0.8}	I _{1.0}		I _{0.8}	l _{1.0}				
F ₇₅	7.81	8.40	8.10	10.15	10.69	10.42	9.26		
F ₁₀₀	8.62	8.63	8.63	12.30	11.78	12.04	10.33		
F ₁₂₅	7.77	7.91	7.84	11.49	10.24	10.86	9.35		
Mean	8.06	8.32	8.19	11.31	10.90	11.11			
Mean of I 0.8			9.69	Mean	of I _{1.0}	9.61			
Treatments	SEm(±)	CD (P=0.05)	Interactions			SEm(±)	CD (P=0.05)		
Mulch (M)	0.51	1.50	IXM			0.72	NS		
Irrigation (I)	0.51	NS	FxM			0.88	NS		
Fertigation (F)	0.18	0.53	Fxl			0.88	NS		
			FxMxI			1.25	NS		

F₇₅= 225-45-90 kg N-P₂O₅-K₂O ha⁻¹;F₁₀₀= 300-60-120 kg N-P₂O₅-K₂O ha⁻¹;F₁₂₅= 375-75-150 kg N-P₂O₅-K₂O ha⁻¹







Fig.1: Regression of chilli fruit yield (t ha⁻¹) with ascorbic acid content (mg 100 g⁻¹), oleoresin content (%) and capsaicin content (%) of fruit in pooled data.

from 24°C to 32°C at different pickings hence higher ascorbic acid content was recorded. Similar results were observed by Ashrafuzzaman *et al.*, (2011) and Maida *et al.*, (2019).

Oleoresin content (%)

Oleoresin is essential oil that gives sharp pungent aroma and the data related to oleoresin content of fresh chilli is presented in Table 3. With respect to the fertigation levels, a 10.48 % & 11.55 % higher oleoresin content recorded in F_{100} over F_{125} (9.35) and F_{75} (9.26), respectively. The highest oleoresin content was observed with F_{100} treatment, it might be attributed due to greater synthesis and translocation of photosynthates in fruits on account of increased uptake of nutrients. These results were in corroborative to Shilpa (2019), Mounika (2016).

There was significant variation found between mulching and non-mulching treatments. The mulching (M_1) treatment was observed 35.62% higher content when compared to the no mulch treatment (8.19%) in

irrigation levels, their interaction with fertigation levels and mulch has not shown significant influence on oleoresin content. The data on correlation between yield and mean oleoresin content (Fig. 1) revealed that there was significant positive correlation observed for oleoresin (0.896) in I, II year and in pooled data respectively.

Capsaicin content (%)

Market demand of chilli depends on the pungency of the fruit, capsaicin is the major alkaloid responsible for pungency in chilli. It is mainly controlled by genetic characters of the plant but external environment and management practices also plays significant role in synthesis of capsaicin and data related to capsaicin content of fresh chilli fruit is presented in Table 4. The capsaicin content was significantly influenced by fertigation levels and mulch during both the years. With respect to the fertigation levels, The F_{100} and F_{125} (0.94 % & 0.91 %, respectively) recorded significantly higher capsaicin content and these

Mean of two years (pooled data) - capsaicin (%)									
Treatments	No mulch		Mean Mulch		Mean	Overall Mean			
	I _{0.8}	I _{1.0}		I _{0.8}	l _{1.0}				
F ₇₅	0.94	0.89	0.91	0.63	0.69	0.66	0.79		
F ₁₀₀	1.03	1.22	1.13	0.72	0.80	0.76	0.94		
F ₁₂₅	0.98	1.07	1.02	0.76	0.85	0.80	0.91		
Mean	0.98	1.06	1.02	0.71	0.78	0.74			
Mean of I _{0.8}			0.84	Mean	of I _{1.0}	0.92			
Treatments	SEm(±)	CD (P=0.05)	Interactions		SEm(±)	CD (P=0.05)			
Mulch (M)	0.06	0.16	IX	I X M			NS		
Irrigation (I)	0.06	NS	FxM			0.10	NS		
Fertigation (F)	0.02	0.06	FxI			0.10	NS		
			FxMxI			0.14	NS		

 Table 4. Effect of fertigation and irrigation levels under mulch and non-mulch conditions on capsaicin content (%) of chilli during in pooled data during rabi season.

 F_{75} = 225-45-90 kg N-P₂O₅-K₂O ha⁻¹; F_{100} = 300-60-120 kg N-P₂O₅-K₂O ha⁻¹; F_{125} = 375-75-150 kg N-P₂O₅-K₂O ha⁻¹ I_{0.8}-0.8 Epan and I_{1.0}-1.0 Epan

pooled data. Under mulch it could be due to optimum soil moisture which hastens the physiological growth that ultimately increased the production of secondary metabolites (Davinder Singh, 2016). With respect to treatments were on par to each other. The lowest capsaicin content was registered in F_{75} (0.79 %). The Capsaicin content increased with increase in the NPK fertigation levels which might be due to external

application of nitrogen that is essential for the synthesis of capsicinoids as nitrogen controls the activity of phenylalaine ammonialyase enzyme and capsaicin synthase enzyme which were responsible for production as well as longitivity of capsaicin content in the chilli. Similar results were observed by Mounika (2016) and Shilpa (2019).

Data related to mulch indicated that the variation between the mulch and no mulch treatments was significant. In pooled data, the maximum capsaicin content was observed in M_0 (1.02 %) over M_1 (0.74 %). No mulch treatment recorded higher capsaicin content due to water stress condition which boosted the activity of antioxidant enzyme in chilli leaves and fruits and leads to the production of high capsaicin content. Another reason might be due to limited moisture content under no mulch condition along with optimum NPK enhances activity of capsaicin synthase enzyme and ultimately increased the production of capsaicin (Subramani 2008). Data pertaining to the irrigation levels, there was no significant variation found between the $I_{0.8} \& I_{1.0}$ during both the years. The interaction effect among the fertigation levels, mulch and drip irrigation levels was found to be non-significant in pooled data. Capsaicin content and fruit yield are highly negatively correlated. The determination coefficient (R²) (Fig.1) showed as non-significant (P=0.01) in pooled data (0.422).

CONCLUSION

From the present study, it can be concluded that application of 100% RDF (300-60-120 kg ha⁻¹ N- P_2O_5 - K_2O) along with $I_{0.8}$ + mulch resulted in higher yield and better quality of chilli. Further increment in the nutrients and water did not increase the spiciness of the chilli and chilli yield. So drip fertigation with 100% RDF along with $I_{0.8}$ + mulch can be recommended for green chilli growers for achieving higher quality, yield and saving irrigation water.

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EVALUATION OF PHYTOTOXICITY OF BLACK SOLDIER FLY LARVAE (Hermetia illucens L.) MEDIATED COMPOST ON CHINESE CABBAGE (Brassica rapa) USING THE SEED GERMINATION BIOASSAY

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ABSTRACT

A lab experiment was conducted at Department of Entomology, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad during September to December 2021. The phytotoxicity of six different entocomposts obtained from rearing black soldier fly larvae on different feed substrate was tested on Chinese cabbage by seed germination bioassay. For each compost type, the effect of four concentrations of water extracts of compost on seed germination and root growth of Chinese cabbage were investigated. Composts were also analyzed for C/N ratio, pH and electrical conductivity. The C/N ratio, pH and EC ranged from 12 to 43.10, 6.87 to 8.81 and 5.70 to 11.30 dSm⁻¹ respectively. Highest relative seed germination (88.44 %) was recorded in frass from Gainesville diet (12.5 %), highest relative root growth (91.23 %) was recorded in frass from fruit waste (12.5 %), and sheep droppings (12.5 %), and highest germination index (73.41) was recorded in the frass from sheep droppings (12.5 %) aqueous extract. Since all the examined BSFL composts extracts were not mature as reflected by germination index of less than 80 resulting in different levels of phytotoxicity, hence suitable neutralizing additives may be added before application in crop.

KEYWORDS: BSFL, compost, Chinese cabbage, phytotoxicity, maturity, germination index

Composting is a natural process that transforms organic waste into nutrient-rich soil conditioner, promoting sustainability and reducing environmental impact. There are various types of composting, one innovative and increasingly popular method is black soldier fly larvae (BSFL) based composting. Black soldier fly larvae efficiently consume various streams of organic waste, converting it into highquality compost while minimizing greenhouse gas emissions and offensive odors associated with traditional composting (Akumah *et al.*, 2021).

One challenge in BSFL mediated composting, is the determination of compost maturity. The rapid and voracious feeding habits of black soldier fly larvae can lead to incomplete decomposition of organic matter, resulting in a compost that may not be fully stabilized. Additionally, the presence of undigested residues or chitin-rich exoskeletons of the larvae in the final compost can affect its quality. Compost maturity is crucial as immature compost may contain phytotoxic substances, inhibiting plant growth and potentially causing harm to the environment. Moreover, mature compost promotes nutrient stability and availability, fostering a balanced microbial community that contributes to disease suppression, improved soil structure, and enhanced plant resistance (Sayara *et al.*, 2020).

Some of the on-site parameters that help in testing the maturity and stability of a compost are phytotoxicity, temperature, colour, odour and moisture. The laboratory parameters that help in testing the maturity and stability of a compost are germination Index (GI), volatile solids (VS), C/N ratio, organic matter, microbial respiration, and biological tests (Oviedo-Ocana *et al.*, 2015).

Among the maturity parameters, GI is considered as the most sensitive parameter for identifying the phytotoxicity of compost and assessing its suitability for use as soil amendments or growing media. It is an integrated parameter which combines the relative radical elongation and relative germination. According to Zucconi *et al.* (1985) GI values below 50% indicate high phytotoxicity, values between 50% and 80% indicate moderate phytotoxicity; and values above 80% indicate absence of phytotoxicity. When

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the index exceeds 100%, the compost can be considered as phytostimulant or phytonutrient.

Application of any compost product is only possible if the compost is mature enough and is free from potentially phytotoxic components. Studying the impact of mature compost on seed germination or plant growth is very much essential for its application.

MATERIAL AND METHODS

Six different feeding substrates *viz*. Vegetable waste (T_1), fruit waste (T_2), ground maize shank (T_3), sheep droppings (T_4), chicken droppings (T_5) and Gainesville diet (T_6) were used for production of BSFL compost. The nitrogen (%), phosphorus (%) and potassium (%) were ranged from 0.98 to 3.20, 0.62 to 2.74 and 0.27 to 0.51 respectively in all the composts. The C/N ratio, pH and EC of all the composts were analyzed as per the standard procedure (Walkely and Black, 1934; Subbaiah and Asija, 1956; Jackson, 1973).

Fresh aqueous extracts (1st extract) were prepared by shaking 10 g of compost with 100 ml of deionized water (1:10 w/v) on a rotary shaker for 1 h. The solutions were centrifuged at 5000 rpm for 15 min, and then filtered through filter paper. A range of dilutions (25%, 50% and 75%) of the aqueous extracts were prepared using deionized water as diluent (Zucconi *et al.*, 1981).

The phytotoxicity of compost extracts was evaluated by the seed germination technique. The test crop Chinese cabbage seeds were surface sterilized by immersion in 75% alcohol for three minutes followed by transferring in 0.001 HgCl₂ solution for two minutes with periodical agitation and finally thoroughly washed with sterilized distilled water to get rid of toxic chemicals. Ten seeds were set for a germination test on Whatman filter paper wetted with 5 ml aqueous extracts (25%, 50%, 75% and 100% dilutions) and deionized water (as control) in 100-mm-diameter and 25-mm-height Petri dishes and incubated at 25 °C. After 3 days, the number of germinated seeds and the radicle length of each germinated seed were recorded. A seed with a radicle length of at least 2 mm was considered as germinated. The whole experiment including preparation of fresh aqueous extracts and germination test was replicated three times, and the results were analyzed by determining the relative seed

germination (RSG), relative radicle growth (RRG), and germination index (GI) as per the Barral and Paradelo (2011)

RESULTS AND DISCUSSION

Relative seed germination (RSG) of Chinese cabbage

At 12.50 % aqueous extract of BSFL compost Gainesville diet showed highest RSG of 88.44 % followed by sheep droppings, chicken droppings, ground maize shank and fruit waste with an RSG of 80.40 %, 77.39 %, 74.37 % and 39.20 % respectively, whereas vegetable waste showed the lowest RSG of 36.18 %. At 25 % aqueous extract of BSFL compost sheep droppings showed highest RSG of 49.25 % followed by Gainesville diet, ground maize shank, chicken droppings and fruit waste with RSG of 48.24 %, 44.22 %, 40.20 % and 30.15 % respectively, whereas vegetable waste showed the lowest RSG of 19.10 %. At 50 % aqueous extract of BSFL compost chicken droppings showed highest RSG of 24.12 % followed by fruit waste, Gainesville diet, ground maize shank and sheep droppings with RSG of 20.10 %, 18.09 %, 17.09 % and 17.09 % respectively, whereas vegetable waste showed the lowest RSG of 13.07 %. At 100 % aqueous extract of BSFL compost fruit waste showed highest RSG of 19.10 % followed by ground maize shank and Gainesville diet with similar RSG of 15.08 %, vegetable waste and sheep droppings with similar RSG of 12.60 %, whereas chicken droppings showed the lowest RSG of 7.04 % (Table 2).

Relative root growth (RRG) of Chinese cabbage

At 12.50 % aqueous extract of BSFL compost fruit waste and sheep droppings showed highest RRG of 91.23 % followed by ground maize shank, vegetable waste and chicken droppings with RRG of 80.70 %, 77.19 %, 77.19 % respectively, whereas Gainesville diet showed the lowest RRG of 66.67 %. At 25 % aqueous extract of BSFL compost fruit waste showed highest RRG of 80.70 % followed by sheep droppings, with RRG of 77.19 %. Vegetable waste, ground maize shank and Gainesville diet showed similar RRG of 66.67 %, whereas chicken droppings showed the lowest RRG of 59.65 %. At 50 % aqueous extract of BSFL compost fruit waste showed highest RRG of 70.18 % followed by ground maize shank, with RRG
of 66.67 %. Sheep droppings and Gainesville diet showed similar RRG of 59.65 %. Whereas vegetable waste and chicken droppings showed the RRG of 56.14 % and 49.12 %, respectively. At 100 % aqueous extract of BSFL compost sheep droppings and Gainesville diet showed highest similar RRG of 49.12 %. Fruit waste ground maize shank and chicken droppings showed similar RRG of 45.61 %, whereas vegetable waste showed lowest RRG of 38.60 % (Table 2).

Germination index (GI) of Chinese cabbage

When Chinese cabbage seeds were treated with 12.50 % aqueous extract of BSFL compost, sheep droppings, ground maize shank, chicken droppings and Gainesville diet and recorded moderate phytotoxicity with a GI of 73.41, 60.07, 59.79 and 59.01, respective-ly. Vegetable waste and fruit waste showed high phytotoxicity with GI of 27.95 and 35.79. Similarly when 25%, 50% and 100% aqueous extract were all the treatments showed high phytotoxicity, where chicken droppings recorded lowest GI of 3.21 (Fig.1).

The dilution of compost consistently led to an increase in RSG, RRG and GI (Table 2). Moldes *et al.* (2006) reported similar findings where GI increased when the extract was diluted or previously washed compost was used, potentially due to a reduction in salinity in the extract.

In the present investigation, the pH of the compost varied from 6.87 to 8.81 (Table 1). The typical pH range for conventional horticulture applications generally falls between 5.5 and 6.5, whereas for organic farming, it may extend from 5.5 to 8.0 (Liu *et al.*, 2021). Different crop seeds exhibit optimal germination at different pH levels, and variations in germination have been observed among different genotypes at various pH levels (Wijayanto *et al.*, 2021). While pH alone

may not always have a significant impact on germination, it can influence nutrient availability, ultimately affecting various germination properties (Follmer *et al.*, 2021).

A high salinity may also cause phytotoxicity. In fact it has been observed that GI is more affected by this property than by degree of stability, in organic amendments with a wide range of EC (Lasaridi and Stentiford, 1998). In present study compost EC ranged from 5.70 to 11.30 dSm⁻¹ (Table 1) which was much higher than threshold value of 3.5 dSm⁻¹ for organic growing media (Liu *et al.*, 2021). Camptelli and Ceppi (2008) observed a strong negative correlation between GI and EC. Similarly, Aslam *et al.* (2008) discovered a negative correlation between relative germination and the electrical conductivity (EC) of the compost.

The Germination Index (GI) of BSFL compost generated from golden needle mushroom was 65.7 for Chinese cabbage and 52.9 for rapeseed (Cai et al., 2017). In Italy, Setti et al. (2019) used lettuce (Lactuca sativa) seeds and found germination indexes above 70%, indicating no signs of phytotoxicity. In another study, Liu et al. (2019) treated chicken, pig, and cow manure with BSF larvae and observed high levels of phytotoxicity in the chicken manure treatment through a seed germination test. In contrast, the compost from the other manures showed stabilized characteristics with reduced electrical conductivity and a higher germination index. The authors suggested that the lack of maturity in the compost derived from chicken manure might be attributed to the high electrical conductivity and concentration of N-NH $_{4}^{+}$ in the feed substrates.

To reduce the pH and EC in compost, various methods can be employed, including the addition of biochar, pine needles, oak leaves, citrus peels, elemental sulfur or sulfur-containing compounds.

 Table 1. Physiochemical properties of black soldier fly larvae compost obtained from different feeding substrates

Treatments	T₁ (Vegetable waste)	T₁ (Fruit waste)	T₁ (Ground maize shank)	T₁ (Sheep droppings)	T₁ (Chicken droppings)	T₁ (G.V. diet)	CD(0.05)	CV
C: N ratio	26.10	25.90	43.10	27.20	19.40	12.00	2.29	5.03
рН	8.81	8.37	6.89	7.76	8.16	6.87	0.71	5.11
EC (d Sm ⁻¹)	11.30	7.83	5.70	9.90	8.73	8.07	0.70	4.80

	%			BSFL Co	mpost		
	aqueous extract	T₁ (Veg. waste)	T ₂ (Fruit waste)	T₃ (Ground maize shank)	T₄ (Sheep droppings)	T₅ (Chicken droppings)	T₀ (G.V. diet)
RSG	12.50%	36.18	39.20	74.37	80.40	77.39	88.44
25% 19.10 30.15 44.22 49.25 40.20	40.20	48.24					
	50%	13.07	20.10	17.09	17.09	24.12	18.09
	100%	12.06	19.10	15.08	12.06	7.04	15.08
RRG	12.50%	77.19	91.23	80.70	91.23	77.19	66.67
	25%	66.67	80.70	66.67	77.19	59.65	66.67
	50%	56.14	70.18	66.67	59.65	49.12	59.65
	100%	38.60	45.61	45.61	49.12	45.61	49.12

 Table 2. Effect of black soldier fly larvae compost obtained from different feeding substrate on RSG (%), RRG (%) and GI of Chinese cabbage



Fig.1: Germination index of Cabbage seeds treated with different aqueous extracts of BSFL composts

Simultaneously, reducing electrical conductivity (EC) in compost can be achieved through dilution with neutral materials, leaching with water to wash out soluble salts, incorporating low-EC ingredients, promoting aeration during composting to enhance microbial activity, and avoiding the inclusion of salty materials. Biochar addition generally resulted in higher GI especially in BSFL treatments. The highest GI (147) was observed for the feedstock amended with 15 % biochar (Akumah *et al.* 2021). Biochar with its alkaline pH must have neutralized the acidic effect of the organic acids produced culminating in higher GI. At maturity, compost with more than 10 % inclusion rate of biochar had the highest cabbage seed germination indices (> 100 %) (Beesigamukama *et al.*, 2020).

CONCLUSION

The results of the present study indicate that the BSFL compost derived from various waste streams have been observed to be immature at all the dilutions exhibiting phytotoxicity to Chinese cabbage. The reasons could be high pH, EC and C/N ratio which should be addressed before application to the crop. Hence there is scope for exploring additives to enhance the maturity of the compost, in view of the rich nutrient (N, P, K and micronutrient) status of ento-composts.

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MOLECULAR CHARACTERIZATION OF Sclerotium rolfsii Sacc. INCITING STEM ROT OF GROUNDNUT PLANT (Arachis hypogea L.)

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ABSTRACT

In the present study, stem rot disease of groundnut was observed in many crop fields of Telangana, India during survey. The most distinguishable symptom is a white fan shaped abundant fungal mycelium mat that appears on the soil surface, leaf fragments and at stem bases of infected plants. The mycelial mat may be seen on the stem a few millimeters above the soil line. Abundant mustard seed-sized, brown to dark brown, spherical sclerotia, initially white later turned to dark brown on maturation was recorded. The fungal pathogen was isolated on potato dextrose agar (PDA) medium. Genomic DNA was isolated and internal transcribed region of ribosomal DNA was amplified using ITS1 universal primers. Based on cultural, morphological and molecular characteristics, the fungal pathogen was identified as *Sclerotium rolfsii*. The rDNA sequence results also showed 98% similarity with reference sequence OR911599 and JF966208.1 confirming pathogen identity. The pathogenicity test was conducted on healthy plants for symptom expression after 6-8 days of inoculation. *S. rolfsii* is known to infect many economically important crop plants at various stages of its growth and development.

KEYWORDS: Groundnut, stem rot, sclerotia, pathogenicity

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop cultivated worldwide in tropical, subtropical and warm areas. Groundnut kernels contain about 26 per cent protein, 48 per cent edible oil, 20 per cent carbohydrates and 3 per cent fiber and also rich in calcium, thiamine and niacin (Haveri, 2017). Globally, groundnut covers an area of 327 lakh ha with the production of 539 lakh tonnes and productivity of 1648 kg per hectare (FAOSTAT, 2021). Groundnut crop suffers from several diseases incited by many fungal, bacterial and viral pathogens, but stem rot caused by *Sclerotium rolfsii* is one of the most important seed and soilborne diseases causing huge economic loss in India and abroad.

According to Grichar and Boswell (1987) yield losses in severely infected areas can range between 10 and 25% and in certain areas, up to 80% losses due to groundnut stem rot occured in India (Mayee and Datar, 1988). In India, stem rot of groundnut was first recorded by Butler and Bisby (1931).

Recently, taxonomic position of *Sclerotium* rolfsii has been assigned by Kirk *et al.* (2008) to the

Kingdom-Fungi, Phylum-Basidiomycota, Class-Agaricomycetes, Order-Polyporales, Family-Atheliaceae, Genus- *Sclerotium* and Species- *rolfsii*. The most distinguishable symptom is a white fanshaped fungal mycelial mat that appears on the soil surface, leaf fragments and near stem bases of infected plants. The mycelial mat may be seen on the stem a few millimeters above the soil line. Abundant mustard seed-sized, tan to brown, spherical sclerotia grow on infected plant material and on the surface of the soil.

This paper describes the isolation and molecular characterization of stem rot disease based on molecular characterization and pathogenicity test.

MATERIAL AND METHODS

Groundnut plants showing typical stem rot symptoms were collected from field during survey in Telangana state. Isolation of fungus was made on PDA medium following standard tissue isolation technique (Wilson, 1953). Infected portion of the root was cut into small pieces (3-4 mm size) and surface sterilized by dipping in freshly prepared 1% sodium hypochlorite solution for one minute followed by rinsing

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three times with sterilized distilled water to remove the traces of disinfectant and dried on sterilized blotter paper. These bits were transferred aseptically into Petri plates containing PDA and incubated in BOD incubator at $25 \pm 1^{\circ}$ C. After 72 hours, the pure culture of the fungus was obtained by following the hyphal tip culture method (Rangaswami, 1971).

A disc of fungal colony was cut with the help of cork borer and placed in the middle of Petri plate containing plain agar and incubated for 2 days. Petri plate was placed under dissecting microscope and mycelial threads of pathogen were located at higher magnification. The pure culture was obtained by hyphal tip method (about 1 mm) and was transferred to PDA plate. After two weeks period, pure culture was prepared by transferring a single sclerotial body on PDA in Petri plates and the pure cultures were maintained in BOD incubator at $25 \pm 1^{\circ}$ C for further studies (Dhingra and Sinclair, 1985).

Morphological characterization

The fungal culture was observed for 4-5 days and the slides of fungal mycelia in the reproductive stage was prepared using lactophenol and cotton blue and observed under the microscope and the colony characteristics were noted (Raper and Fennell, 1965).

Mass multiplication

Sorghum seeds were thoroughly washed with running water and soaked overnight in 1% sucrose solution. Next day, the soaked seeds were transferred to 250 ml conical flasks and autoclaved twice at 15 *psi* pressure (121.6°C) for 20 minutes. After autoclaving, flasks were allowed to cool down. The bits of pure culture mycelium were placed to the conical flask under aseptic condition and were incubated at 25±1°C for 15 days. These seeds were shaken periodically to get uniform growth of fungus.

Pathogenicity test

Susceptible groundnut variety K6 was used for testing the pathogenicity of *S. rolfsii* following soil inoculation technique. The pots were filled with sterilized soil and then each pot was covered with a polyethene cover after being artificially inoculated with 40 g of inoculum that had been multiplied on sorghum grains and carefully mixed. Sterilized soil without inoculation served as control. After 3 days, five groundnut seeds were sown randomly in each pot and three replications were maintained. The pots were maintained under controlled conditions in glass house with adequate soil moisture and watered regularly as per the requirement.

Molecular characterization

DNA extraction, PCR amplification and sequencing

Mycelial disc (5 mm diameter) of an actively growing 5 days old culture was cut off and transferred onto the fresh PDB (Potato Dextrose Broth) and the culture was incubated in shaker at 125 rpm for 3 days. Subsequently, the growing mycelium was scraped with the help of sterilized forceps from the surface and used for genomic DNA extraction by following Cetyltrimethyl Ammonium bromide (CTAB) method.

Protocol for fungal genomic DNA isolation

Mycelial mat was harvested by filtration and stored at -70°C for further use. Mycelial mat was lyophilized and ground in a sterile pre-chilled mortar and pestle to a fine powder. The fine powder was then transferred into a 2 ml Eppendorf tube and 1 ml of CTAB extraction buffer preheated at 65°C was added, mixed thoroughly and incubated in a water bath at 45°C for 45 minutes. After incubation, contents of the tube were cooled to room temperature and an equal volume of chloroform: isoamyl alcohol (24:1) was added, mixed by inversion and centrifuged at 12000 rpm for 15 minutes at room temperature. The upper aqueous phase was transferred to a new tube and an equal volume of chloroform: isoamyl alcohol (24:1) was again added and mixed by inversion and centrifuged at 12000 rpm for 15 minutes at room temperature. The upper aqueous phase was recovered in a fresh tube and DNA was precipitated by adding approximately an equal volume of isopropanol and incubating at room temperature for 30 minutes. After incubation, content in eppendorf tube was centrifuged at 12000 rpm for 20 minutes to pellet DNA. The supernatant was discarded and the DNA pellet was washed with 70 per cent chilled ethanol and centrifuged at 12000 rpm for 5 minutes at 4°C. DNA pellet was dried under vacuum, dissolved in 20 μ l of TE buffer and stored at -20°C for further use.

Quality check and quantification of genomic DNA

A small quantity of extracted genomic DNA was loaded on 0.8 per cent agarose gel stained with ethidium bromide ($0.5 \mu g/ml$) and it was run on gel electrophoresis unit and visualized in gel documentation

system. PCR product was quantified in nanodrop, concentration and purity of DNA were estimated by measuring absorbances at A_{260} and A_{280} nm. Concentration was expressed in ng/ μ l. The remaining DNA was stored at -20°C for further use.

PCR amplification of genomic DNA

PCR amplification of the isolated fungal DNA was amplified at internal transcribed spacer region by ITS1 and ITS4 primers (Table 1). Initially, $10 \,\mu$ I of small

Table 1. Base sequences of ITS primers used

Pri- mers	Sequence	Base pairs
ITS-1	5'TCCGTAGGTGAACCTGCGG-3'	19
ITS-4	5'TCCTCCGCTTATTGATATGC-3'	20

volume PCR product was used followed by $50 \,\mu$ l volume PCR on gel electrophoresis unit and visualized in gel documentation system. DNA ladders (100 bp) were loaded on first well as a size standard. The gel was checked to know whether the PCR amplicon is of the correct size.

Sequencing of PCR products

PCR products of ITS regions of fungal isolate were obtained through amplification and were outsourced for purification and sequencing to Eurofins Genomics, Bengaluru, India.

Table 2. PCR mixtures for 10 μ l and 50 μ l reaction volumes

Compone	ents	Quality read	for one tion
		Total volume (10 µl)	Total volume (50 µl)
EmeraldAmp GT Master Mix (2X pr	PCR remix)	5 µI	25 <i>µ</i> I
Primers (2.5 pmol/ µl)	Forward	1 <i>µ</i> I	5 <i>µ</i> 1
	Reverse	1 <i>µ</i> I	5 <i>µ</i> l
Template DNA(1	00 ng/ μ l)	1 <i>µ</i> l	5 <i>µ</i> l
dH ₂ O		2 <i>μ</i> Ι	10 <i>µ</i> l
Total volume		10 <i>µ</i> l	50μ l

Table 3. Cycling conditions and amplicon size for ITS amplification

Steps	ITS
Initial denaturation	94°C for 5 minutes
35 cycles of	
Final denaturation	94°C for 45 seconds
Primer annealing	55°C for 45 seconds
Extension	72°C for 1 minute
End of cycle	
Final Extension	72°C for 5 minutes
Amplicon size	~ 600 bp

Sequence data analysis

Sequence results obtained from Eurofins Genomics, Bengaluru, India was analysed using BioEdit, MEGA11 and NCBI-BLAST (https:// blast.ncbi.nlm.nih.gov/Blast.cgi#). A consensus sequence was generated from forward and reverse sequence data using BioEdit software. The consensus sequence was used to perform BLAST against the NCBI GenBank database. The first ten sequences were chosen based on their maximum identity score and aligned using the multiple alignment software program ClustalW. MEGA 11 was used to create the distance matrix and the phylogenetic tree. Using distance matrix and the phylogenetic tree thus formed closest sequence and isolate was identified.

RESULTS AND DISCUSSION

Isolation and Identification of pathogen

The groundnut plants showing typical symptoms were collected during *kharif* 2021-2022 survey and brought to the laboratory. Pure culture of the pathogen was obtained by hyphal tip technique. The mycelium of the isolated organism showed fluffly white growth on PDA medium (Fig.1a and Fig.1b). Based on cultural and morphological characteristics the isolated test pathogen was identified as *S. rolfsii* (Punja, 1985).

In pure culture, the fungal mycelium is initially silky white, but it gradually lost its lustre and later assumes dull appearance. The mycelium is seen

MOLECULAR CHARACTERIZATION OF SCLEROTIUM ROLFSII SACC.

radiating with abundant aerial hyphae that exhibits rapid growth habit. Often, the aerial hyphae appeared as dense tufts and seen dispersed all over the culture medium. The mycelium completely disappeared over a period of three months leaving only sclerotia. Sclerotia are at first white, became light brown to dark brown at maturity. The sclerotia are sub spherical with the surface finely wrinkled and sometimes flattened.

The primary symptoms of stem rot in groundnut are observed as browning and wilting of leaves and branches while still being attached to the plant. The fungus predominantely infected the stem by forming a whitish mycelial mat. However, the infection was also observed in all plant parts including leaf, root and stem (Fig.1a and Fig.1b). The results are in accordance with the findings of Pande and Rao (2000), Rakholia and Jadeja, 2011 and Divya Rani *et al.* (2017) who have noticed similar symptoms on infected groundnut plants in their research experiments.

In pathogenicity test, the inoculated groundnut plants exhibited typical symptoms of stem rot and the pathogen was reisolated and Koch postulates were proved. The pathogen was reisolated and Koch postulate was proved. The results are in similarity with the findings of Le *et al.* (2012) and Eslami *et al.* (2015) on groundnut.

Molecular characterization

Results showed that the test fungal isolate had similar morphological characteristics and its pathogenicity was proved. DNA from the test pathogen isolated from stem rot affected groundnut plants was successfully amplified using universal primer pairs ITS1 targeting the ITS region. The total size of ITS1 region studied was 681 bp. The ITS sequences for *Sclerotium* isolate were compared against the sequences in the NCBI nucleotide database using BLAST tool to confirm the identity of test pathogen. The BLAST results indicated that the pathogen isolated from stem rot infected plants as *Sclerotium rolfsii* Sacc. The query cover of isolate identity was 98% homology to that of *S. rolfsii*. This indicated that the test pathogen belongs to *Sclerotium rolfsii*. The nucleotide sequences of ITS1-4 regions had been deposited in the NCBI database as accession number of OR911599 for the test pathogen.

The present findings were at par with rDNA sequence which showed 99-100% similarity with reference sequence AB075298.1 and confirmed the pathogen identity (Tejaswini *et al.*, 2022).

Morphological approaches can be used for species identification, however this method is not appropriate because high degree of accuracy is required at the species level where most isolates of the same genus are nearly identical. *Sclerotium rolfsii* morphologically very similar and in many cases are phenotypically indistinguishable and can only be reliably identified by molecular characterization. Therefore, consistent and reliable pathogen identification is an essential indicator in plant disease epidemiology and development of management strategies. Currently, molecular methods focusing on genotypic characteristics are used to confirm identification method and accelerate detection of species.



Fig.1a: Stem rot symptoms observed on groundnut



Fig.1b: Pure culture of Sclerotium rolfsii

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Collection of sample



Isolation of test pathogen



Pure culture of S. rolfsii



Mass multiplication on sorghum seeds



Fig.2: Pathogenicity test of S. rolfsii



Fig.3: Phylogenetic tree based on ITS sequence of Sclerotium isolate

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SCREENING OF SORGHUM (SORGHUM BICOLOR L.) GENOTYPES FOR BMR TRAIT BY HISTOCHEMICAL STAINING TECHNIQUE

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ABSTRACT

Reddish to pink coloration of brown-midrib (bmr) mutant tissues upon histochemical staining with Wiesners' reagent has been associated with accumulation of cinnamaldehydes and acts as an indirect indicator of quality improvement in forage. Cinnamaldehydes are phenolic substrates associated with lignin biosynthesis pathway. This study was planned with an objective of understanding the relationship between Wiesner staining in stem, leaf midrib and forage quality traits. Twenty forage sorghum genotypes of both brown-midrib (bmr) and wild-type genotypes were used in the study. The genotypes were planted in a randomized complete block design. Histochemical staining on leaf and stem sections and stem forage quality traits were analyzed at flowering stage. The Wiesners reagent's active ingredient, phloroglucinol (1.0% (w: v)), reacts best in an acidic (10.1 M HCl) environment, and was dissolved in four acid-ethanol (v/v) levels of 35/65, 30/70, 25/75, 20/80. Stained cross-sections images of leaf midrib and stem tissues of the genotypes were captured in flatbed scanner and the staining intensity was scored using traditional histochemical and digitized methods. Forage quality analysis was performed on grounded stem tissue samples using Near Infrared Spectroscopy (NIRS). The analysis of variance revealed significant difference among genotypes for the histochemical staining traits, percent in vitro organic matter digestibility (IVOMD), Metabolizable energy (ME mj/kg), and percent neutral detergent fiber (NDF). Orthogonal contrast analysis revealed genotypes carrying bmr loci (bmr6, bmr6bmr12 and bmr12) had significantly lower ADL (acid detergent lignin) and higher IVOMD than wild-type genotypes. Genotypes BN612, 59353, 59354, N605, N596 N600 had the highest IVOMD and lowest ADL, while wild-type genotypes IS26694, SSV-74, IS11861 and ICSV12006 had the lowest IVOMD and highest ADL. These genotypes had highly red-stained and faintly stained tissues, respectively based on traditional and digitized staining assessment. A highly significant positive correlation between the histochemical staining of leaf midrib and stem tissues was detected suggesting that similar substrates responsible for reaction with phloroglucinol were deposited in both organs. Furthermore, a significantly greater staining in bmr6>bmr6bmr12>bmr12>Bmr were detected. These findings shown promise for employing histochemical staining combined with high-through-put phenotyping in selection of bmr inbred lines of higher forage quality value. However, further studies are recommended using large number of bmr inbred lines to confirm the application of the histochemical staining technique for selection in early and later generation advancement of bmr inbred line development.

KEYWORDS: Sorghum, lignin biosynthesis pathway, flavonoid biosynthesis pathway, phloroglucinol, histochemical staining, near infra-red spectroscopy (NIRS), caffeic acid O-methyltransferase, cinnamyl alcohol dehydrogenase

Evolution of lignin in plants was fundamental in the colonization of terrestrial habitat by vascular plants (Rose *et al.*, 2007). Lignin is a product of phenylpropanoid pathway, it is constituted by three monolignol; guaiacyl (G), syringyl (S) and *p*hydroxyphenyl (H) lignins. The monolignols are polymerized in secondary cell walls of specialized cells/ tissues such as xylem tracheid, vessel elements, and sclereid cell wall, and in spaces in between cellulose, hemicellulose and pectin components (Meents *et al.*, 2018). The dense matrix formed confers mechanical strength to plant cells and whole plant contributing to an erect growth. The inherent hydrophobicity property of lignin (Xin & Herburger, 2021), enables xylem and phloem to translocate water and nutrients to photosynthesizing cells and developing grain. Mutation in the lignin and flavonoid biosynthesis pathways has been associated with reddish brown coloration of the leaf midrib and stem (Bout & Vermerris 2003; Adeyanju et al., 2021). This phenotype called "brown midrib" was isolated in sorghum, maize and pearl millet (Cherney *et al.*, 1988; Sattler *et al.*, 2010). The phenotype appears as early as at three-leaf stage and as late as the sixth leaf stage (Jorgenson, 1931). The central advantage of these *bmr* mutants is their improved biochemical cell wall composition, higher glucose yield (Sattler *et al.*, 2014; Rivera-Burgos *et al.*, 2019) and enhanced fodder digestibility (Oliver *et al.*, 2005). Cattle

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fed with *bmr* fodder show an improved feed intake (Muller *et al.*, 1972), body weight gain (Byers *et al.*, 1964), and milk composition (Byers *et al.*, 1964). The trait also improves process yield of high biomass sorghum for biofuel production (Rivera-Burgos *et al.*, 2019). The first set of 19 *bmr* genotypes in sorghum were isolated and reported by Porter *et al.*, (1978) from mutagenized populations of two genotypes (954114 and 954104). Over the years, a total of 37 *bmr* mutants were isolated from mutagenized populations and naturally occurring spontaneous mutants in existing germplasm materials (Porter *et al.*, 1978; Gupta, 1995; Saballos *et al.*, 2008; Xin *et al.*, 2009).

The brown midrib mutants have been grouped into eight bmr loci (Saballos et al., 2008; Sattler et al., 2014) to foster their utilization in crop breeding programmes. A number of alleles have also been reported within each of these loci (Saballos et al., 2008). bmr6 and bmr12 loci and the associated alleles are the most commonly deployed in sorghum improvement. bmr6, an orthologous of bm1 of maize brown midrib has mutations in cinnamyl alcohol dehydrogenase (CAD) gene, that encodes the enzyme involved in the conversion of each monolignol aldehyde to its corresponding alcohol in the last step of monolignol biosynthesis (Saballos et al., 2009), while bmr12, an orthologous of bm3 of maize harbors mutations in caffeic acid O-methyltransferase (COMT) gene that encodes an enzyme involved in the conversion of 5-hydroxyconiferaldehyde to sinapaldehyde in the synthesis of sinapyl alcohol (Bout & Vermerris, 2003). The single nucleotide mutations in these genes leads to accumulation and incorporation of novel phenolic substrates in the cell wall (Bucholtz et al., 1980; Saballos et al., 2008). Indeed, more coniferaldehyde (Saballos et al., 2008), p-coumaric acid and syringyl content (Akin et al., 1986; Fritz et al., 1990), and vanillin and p-hydroxybenzoic acid (Akin et al., 1986; Fritz et al., 1990) was reported in cell wall of brown midrib mutants than in corresponding normal genotypes. Ferulic acid has been reported to contribute to bonding between polysaccharide and polysaccharide, and between polysaccharide and lignin (Hatfield et al., 1999). Other lignin components that have been hypothesized to copolymerize with lignin monolignol include hydroxycinnamyl alcohols (Blaschek et al., 2020), benzaldehydes (vanillin), and cinnamyl

aldehydes (Kim *et al.*, 2000; Pomar *et al.*, 2002; Blaschek *et al.*, 2020). The mutation of these genes results in changes in chemical composition, which can be detected and quantified using gas chromatography/ Mass spectroscopy (GC/MS) (Ralph and Hatfield 1991; Palmer *et al.*, 2008; Sarath *et al.*, 2006), which are expensive and mostly not accessible by most breeding programmes.

Histochemical staining has been used to monitor changes in cell wall biochemistry, and the characteristics of fundamental tissues, to categorize and evaluate the spatial distribution of cell wall constituents in various plant organs and tissues (Yang et al., 2014; Lux et al., 2005; Sarath; Palmer et al., 2008; de Andrade et al., 2017). Safranin-O.Mäule and Wiesner tests detect regions of plant tissues with low and high aldehyde and syringyl units, respectively (Anderson et al., 2015). In sorghum, Wiesner test is being employed in identification of genotypes with a reduced lignin content. Phloroglucinol, the active ingredient of the reagent, under acidified condition, reacts with the aromatic aldehydes, hydroxyl benzaldehydes and cinnamaldehydes, to form reddish-brown and pink coloration, respectively (Pomar et al., 2002; Blaschek et al., 2020). Cinnamaldehydes was reported to contribute more into the total color production (Pomar et al., 2002). This color formation on staining is independent of the type of lignin monolignol synthesized by a species (Ros et al., 2007). In sorghum, the method has been used to differentiate wild-type allele (Bmr) and bmr2 locus from three other allelic groups (Gorthy et al., 2013; Saballos et al., 2008).

Histochemical staining has mostly been qualitatively measured, and subjectively quantified. Akin *et al.*, (1986) using the Wiesner test with visual scale of 1-3 to quantify the intensity of staining in epidermal tissue, xylem, parenchyma and sclerenchyma leaf midrib tissues of *bmr12* and the corresponding wildtype genotype. Iji *et al.*, (2022) used a visual scale of 0-2 to identify sensitive and less sensitive histochemical staining techniques (Solochrome azurine stain, Walton stain, and the modified Haematoxylin stain) in agar blocks for use in detecting aluminum toxicity in fish. Giaveno and Filho (2000) quantified hematoxylin degree of staining on a 0 (highly tolerant) to 5 (highly sensitive) visual scale to identify aluminum tolerant and sensitive maize genotypes. These staining quantifications were found to correlate with chemical composition under investigation (Giaveno and Filho. 2000; Choi et al., 2007). However, the lack of appropriate tools to quantify the staining has constrained the progress towards deploying histochemical staining in breeding. Tools such as ImageJ and Adobe Photoshop software have been used to improve accuracy in quantification of the staining (Choi et al., 2007; Blaschek et al., 2020). Despite the fact that ImageJ offers a less subjective measurement, the quantification of the area for a specific colour still depends on the thresholds set for image analysis as well as the user's subjective evaluation of the image (Perrier et al., 2017). This introduces random biases that leads to poor differentiation among genotypes (Perrier et al., 2017). Nowadays computer algorithms can be constructed to capture relevant patterns in plant tissues (Da Silva et al., 2015) and application can be built for an automated scanning and quantification of staining. bmr and wild-type genotypes leaf midrib and stem tissue staining can vary in the extent and intensity of their staining, and this information could be captured and utilized in breeding. Hence, the present study was conducted to establish the relationship between the staining of leaf midrib and stem tissues with forage quality traits to fast track bmr phenotyping in the process of bmr cultivar development.

MATERIAL AND METHODS

Experimental Material

Twenty (20) sorghum genotypes were used in the study; the genotypes included 6 introgression bmrlines carrying bmr6 and 3 introgression lines carrying bmr12, 3 double mutant (bmr6bmr12) lines, and 8 white colored midrib (Bmr) wild-type genotypes (Table 1). The genotypes were selected from the breeding material at Sorghum Breeding Programme, ICRISAT, Patencheru, India. The wild-type genotypes included four improved sorghum genotypes and four landraces. The genotypes (bmr and wild-type) were chosen to evaluate the relationships between histochemical staining and fodder quality variables. The bmr genotypes used in the study were developed at USDA-ARS. Details for the development of these bmr introgression lines have been described in the works of Pedersen et al., (2006a); Pedersen et al., (2006b); Pedersen et al., (2006c) and Pedersen et al., (2008).

These introgression lines were developed through four cycles of backcross and selfing. The *bmr6* and *bmr12* alleles were later pyramided through four cycles of backcross and selfing to generate four double *bmr* mutant lines under the genetic backgrounds of Atlas, Wheatland, and RT x 430 (Pedersen *et al.*, 2008). The current study made use of ten *bmr* near isogenic lines developed from the USDA-ARS and two new genetic backgrounds developed at ICRISAT (Table 1).

Experimental design

The genotypes were planted in post-rainy (Rabi) season 2021 in randomized complete block design of two replications. The experiment was conducted at ICRISAT, Patancheru, Telangana, India, in sand-clay soil. Genotype ICSV100305 did not germinate in both replication leaving a total of nineteen genotypes evaluated in the study. All the recommended crop management practices were followed to maintain good crop stand. At 50% flowering stage, six random plants per plot were cut from the base of the plants. The second leaf from the top of these plants were clipped off along with the last internode from the topmost part of the stem for use in the study. The leaves and the stems in uppermost part of the plants were chosen as suggested in earlier studies that reported the region to have high amounts of phenolic compounds in bmr genotypes (Palmer et al., 2008; Dowd & Sattler, 2015; Dowd et al., 2016). In laboratory, leaf midrib from three plants were carefully excised using a twoedged razor blade. Five-cm tissue sections at the base of the node were also obtained and were enclosed in plastic bags. These leaf midrib and stem samples were stored in refrigerator at -20° C until time for grinding and forage quality analysis. However, only the stem samples were later analyzed for forage quality.

Histochemical staining

Leaf midrib and stem tissues from the remaining three plants were used in the histochemical staining study. The sorghum midrib samples were dissected longitudinally to expose the inner tissues. Twenty-one (21) sections of leaf midrib tissues from three plants measuring ~ 2.5 mm in length were sectioned using two-edged razor blade. The histochemical staining protocol was adopted from the works of Saballos *et al.*, (2008). Here, stem and leaf

midrib tissues were soaked in deionized water twice for thirty minutes to remove water-soluble compounds. The samples were then immersed in acetone twice for one hour each to remove unbound phenolics compounds. These pieces were later soaked in 1.0% (w:v) phloroglucinol in 10.1 M hydrochloric acid-ethanol level (25/75, v/v) for 5 minutes as described by Saballos et al. (2008). Eight different proportions of acid-ethanol levels; 35/65, 30/70, 25/75, 20/80, 15/85, 10/90, 5/95 and 0:100 were prepared for the validation study. A set of three midrib pieces for each of the genotypes were submerged in the prepared phloroglucinol solution for 5 minutes. In the acidified solution, phloroglucinol reacts with aldehyde end-groups (Clifford, 1974), forming a stable red-brown pigments (Pomar et al., 2002). The three pieces of midrib tissues from the same leaf per genotype obtained for the seven proportions of acid-ethanol were loaded on microscopic glass slides (Fig.1). The same methodology was repeated in the stem sections staining. They were first sliced into ~1 mm thickness from the base of the node of the internode to generate 21 slices.

The stained tissues on the glass slides were scanned on graphic arts model flatbed scanner manufactured by Epson to generate 24-bit colored (std) images at a resolution of 200 (dpi) and scaling of 13% (Figure 1). The scanned images were imported in Adobe Photoshop (EPSON expression Version 1640XL), and trimmed at boundary of the plant tissues so that only the regions of leaf midrib and stem tissues were analyzed in python. The trimmed plant tissues were imported and analyzed in python using in-house script which has been provided in Appendix. A total of 1396 images for leaf tissues and 1256 images for stem tissues were obtained from the twenty genotypes.

Image analysis

The stained images of stem and leaf midrib of the nineteen genotypes were separately analyzed in Python (Spyder) version 3.9 (Van Rossum & Drake, 2009). Python packages such Panda (McKinney, 2010), Numpy (Harris et al., 2020) and cv2 (Bradski, 2000) were used in executing the analysis. Total number of pixels in the images, and the number of pixels in the highly stained regions were generated using cv2 library. The boundaries for the RED, GREEN, BLUE (RGB) colors were captured at 70, 50, 50 for the lower boundary, and 255, 255, 255 in the upper boundary for highly red-stained regions. The regions for medium and lowly red-stained regions were as well captured. These were optimized from online color picker (https://www.kapwing.com) and color codes chart (https://www.rapidtables.com/web/color/ RGB_Color.html). The number of pixels in the highly red-stained portion per sample (Figure 1) were converted into percent number of pixels, by dividing the respective number of pixels by total number of pixels multiplied by 100. Number of pixels in the medium and lowly red-stained regions were as well recorded. However, there were mostly zero pixels in the medium red-stained regions, while, number of pixels in the low red-stained regions where as expected highly negatively correlated with number of pixels in the highly red-stained regions. Furthermore, the images were scored using a traditional scoring scale. A scale of 1-3 was used, where 1, implied no staining, 2 faint red staining and 3 highly red-stained (Akin et al., 1986).

Forage quality analysis

The leaf midrib and stem tissues stored in the refrigerator were dried in a lyophilizer. The dried samples were grounded to a fine powder in a mix grinder and sieved to obtain ~ 0.75 mm particle size. However, only the stem samples were analyzed for forage quality. Samples of two genotypes (ICSV100453 and ICSV100309) were not analyzed due to the small quantity of biomass samples obtained. The forage quality variables were analyzed using NIRS at the ILRI laboratory, based at the ICRISAT campus, Patancheru, India. The spectral data of the dried, grounded (0.75 mm) samples for forage quality were obtained using the FOSS Forage Analyzer 5000 monochromator spectrophotometer, equipped with a rotation module that performs reflectance measurements in the spectral region between 400 and 12500 nm, at 2 nm intervals. The spectral data and the chemometric analysis were carried out using the WinISI II v program 1.5. "Outliers" samples were determined based on the Global Mahalanobis distance (GH) and NH values. Samples that had GH values greater than 3 and NH values greater than 1 had spectral information outside the calibration model range, and these were re-analyzed by wet chemistry method. The following variables were measured by the NIRS: percent dry matter (%dm), percent nitrogen (nit%dm), percent neutral detergent fiber (NDF%dm), percent acid detergent fiber (ADF%dm), percent acid detergent lignin (ADL%dm), metabolizable energy (ME) (mjkg) and percent *in vitro* organic matter digestibility (invomd%). Estimates of percent hemicellulose and percent cellulose content were calculated by subtracting ADF from NDF, and ADL from ADF, respectively. Percent crude protein was obtained by multiplying percent nitrogen content by 6.25.

Data analysis

The raw datasets for the staining quantification for the individual genotypes were visualized in scatter plot in excel. The average percent number of pixel in highly red-stained portions of plant tissues per plot at each acid-ethanol level were obtained in excel. The data for percent number of pixel in the highly red-stained areas was square root transformed because the percent data were within the range 0 – 30% (Gomez & Gomez, 1984). Similarly, average scores of staining per plot were also obtained from the traditional method in excel. However, data from acid-ethanol levels of 35/ 65, 30/70, 25/75 and 20/80 where considered for presentation in this article. The staining at 0:100, 5/95, 10/90, 15/85 was only conducted in bmr genotypes, and the response of the genotypes were similar as in four acid-ethanol levels.

Forage quality variables were examined in excel, and three plot values were excluded from the analysis because of an unexpected forage variable values. These genotypes had forage quality estimates above the expected performance for wild-type genotypes. Analysis of variance for the histochemical staining quantification, traditional staining visual scores and forage quality traits were conducted following Randomized Complete Block Design model, considering genotypes as a fixed factor according to the model $Y_{ij} = i + a_{i,j} + \tilde{a}_{j,j}$, where Y_{ij} is the performance of genotype in ith replication and ith genotype, i is the mean performance of genotype across replication, á, is the effects of ith replication across genotypes, \hat{a}_{j} is the effects of j^{th} genotype across replications, and \tilde{o}_{i} is the measurement error associated with the *i*th replication and *j*th genotype. Orthogonal contrast analysis was performed to determine bmr (bmr6, bmr12, bmr6bmr12, and Bmr) effects on histochemical staining using digitized staining quantification, traditional visual stain scores and forage quality traits to fast track bmr phenotyping. Furthermore, correlation and partial regression analysis were performed using the genotype mean values to determine the relationships among genotypes, histochemical staining quantification and forage quality variables. The analysis of variance and correlation analysis were conducted in R software version 4.2.1 (R Core Team 2016), while the partial regression analysis was conducted in excel function.

RESULTS AND DISCUSSION

Evaluation for forage quality of the genotypes

There was no significant difference among the genotypes for most of the forage quality traits (percent DM, percent ASH, percent Nit., percent NDF, percent ADF, percent hemicellulose, percent cellulose and percent CP) (Table 2). Significant difference among the genotypes were detected for percent ADL (p=0.008), percent IVOMD (p=0.04), and percent ME (mj/kg) (p=0.03) (Table 2). Orthogonal contrast analysis revealed a significantly higher percent Ash, percent CP, percent IVOMD and ME (mj/kg) in bmr loci groups (bmr6, bmr12, bmr6bmr12 and Bmr) than in the wildtype genotypes (Table 3). On the other hand, genotypes carrying bmr loci (bmr6, bmr12 and bmr6bmr12) had a significantly lower percent ADL and percent ADF than in the wild-type genotypes (Table 3). However, no significant difference between genotypes carrying bmr loci (bmr6, bmr12 and bmr6bmr12) and wild-type genotypes for percent NDF, percent hemicellulose and percent cellulose were detected (Table 3).

The genotypes were compared for percent ADL, percent IVOMD and ME that were found to have significant genotypic effects. bmr genotypes BN612 (mean IVOMD 66.7%, mean ME 9.81), 59353 (mean IVOMD 65.76%, mean ME 9.63), 59354 (mean IVOMD 65.13%, mean ME 9.82), N605 (mean IVOMD 64.38, mean ME 9.56), N608 (mean IVOMD 63.19, mean ME 9.08) and N600 (mean IVOMD 62.87, mean ME 9.44) had the highest percent digestibility and metabolizable energy (Table 4). On the other hand, wild-type genotypes IS26694 (mean IVOMD 58.89, mean ME 8.68), IS93025 (mean IVOMD 58.57, mean ME 8.77), IS11861 (mean IVOMD 57.15, mean ME 8.42) and ICSV12006 (mean IVOMD 56.63, mean ME 8.35), and a bmr genotype N610 (mean IVOMD 56.63, mean ME 8.27), N606 (mean IVOMD 59.25, mean ME 8.94), N597 (mean IVOMD 59.29, mean ME 8.51) had the least percent digestibility and

metabolizable energy (Table 4). Wild-type genotypes IS93025 (mean ADL 3.31), IS11861 (mean ADL 2.96), SSV-74 (mean ADL 2.53) and ICSV12006 (mean ADL 2.53), and *bmr* genotypes N606 (mean ADL 2.97) and RN613 (mean ADL 2.57) had the highest percent ADL while *bmr* genotypes N596 (mean ADL 1.77), BN612 (mean ADL 1.36), 59353 (mean ADL 1.08), BN611 (mean ADL 0.96), N597 (mean ADL 0.79) and N608 (mean ADL 0.60) had the least percent ADL (Table 4).

Evaluation for histochemical staining of the genotypes

Digitized image staining quantification and traditional scoring of degree of staining of the images were employed in both the leaf midrib and stem tissue histochemical staining assessment (Table 5). A highly significant genotypic difference for histochemical staining were detected at each of the four acid-ethanol levels (20:80, 25:75, 30:70 and 35:65) using both methods (Table 5). Mean performance of the genotypes were compared for histological staining. A general increase in the intensity of red staining in most of the genotypes with increases in acid levels under both the traditional and digitization method were observed (Table 6 and Table 7). Leaf midrib and stem tissues for genotypes N600 and 59353 carrying bmr12, 59354, N596 and N605 carrying bmr6, and RN613 and BN612 carrying bmr6bmr12 were highly red-stained, while wild-type genotypes ICSV100453, IS11861, IS26694, ICSV12006 and SSV74 had no or were faintly redstained (Table 6). Un expectedly, bmr genotypes N610 and N606 carrying *bmr12* were faintly red-stained as the wild-type genotypes across the four acid-ethanol levels (Table 6). Similar results were also found using the digitized method (Table 7).

Relationships between leaf midrib and stem tissues histochemical staining quantification

There was highly significant positive correlation for histochemical staining between stem and leaf midrib tissues at the four acid-ethanol levels assessed using both traditional and digitized method (Table 8). Similarly, a highly significant difference was detected for histochemical staining among the acid-ethanol levels within leaf midrib and stem tissues (Table 8).

Application of histochemical staining in breeding

The *bmr* loci effects were analyzed to demonstrate the usefulness of the histochemical

staining in breeding. The comparison among bmr loci groups were made using both traditional and digitized methods (Table 9 and Table 10). In the traditional method, leaf midrib tissues of genotypes carrying bmr6 had a significantly higher intensity of red-stains than leaf midrib tissues of genotypes carrying bmr6bmr12, followed by genotypes carrying bmr12 and wild-type genotypes at acid-ethanol level of 20:80 (Table 9). Similar trend was detected at acid-ethanol levels of 25:75, 30:70 and 35:65 (Table 9). A similar trend was detected in the stem tissues of the genotypes (Table 9). However, there was no significant difference in the intensity of staining between stem tissues of genotypes carrying bmr12 and wild-type genotypes at all the acid-ethanol levels, except at acid level of 20:80 (Table 9).

On the other hand, the digitized method, revealed leaf tissues for genotypes carrying *bmr6* had a significantly higher intensity of staining than genotypes carrying the other loci (*bmr12, bmr6bmr12* and *Bmr*) at acid-ethanol levels of 20:80, 25:75 and 30:70 (Table 10). However, there were no significant difference in the intensity of staining among genotypes carrying *bmr* loci (*bmr12, bmr6bmr12* and *Bmr*) (Table 10). Similar trend where observed in the staining of stem tissues (Table 10). The difference in the intensity of histological staining was in the order of *bmr12>bmr6bmr12>bmr12>Bmr* in the stem tissues (Table 10).

There are several histochemical staining techniques that have been developed for use in quick diagnosis and preliminary analysis of samples before applying more involving and expensive techniques (Pradhan Mitra & Loqué, 2014). Priority in choosing a staining method is based on sensitivity of the staining technique to detect the slightest quantity of the substrate responsible in the reaction to lead in staining of the tissues (Iji et al., 2022). False negative detection may arise from using a lower concentration of the active ingredient involved in the staining or following an inappropriate tissue preparation procedure before staining (Lux et al., 2005). Mäule and Wiesner tests are the major staining methods being employed in bmr screening, and these techniques identify regions of low and high aldehyde and syringyl unit contents, respectively (Anderson et al., 2015). It has been empirically determined that phloroglucinol, the active ingredient in the Wiesner' reagent reacts with coniferaldehyde and related aldehyde residues to form colored pigmentation (Clifford, 1974; Davidson et al., 1995; Pomar et al., 2002). As a result, it was recommended that this method can be used for the detection of the hydroxycinnamyl aldehyde end units contained in lignins (Davidson et al., 1995; Pomar et al., 2002). This method has therefore been employed in differentiating *bmr* from wild-type genotypes (Saballos et al., 2008; Gorthy et al., 2013). The bmr genotypes stain reddish-brown in Wiesner' solution when submerged in the solution for five minutes (Saballos et al., 2008; Gorthy et al., 2013). However, the staining has been subjectively assessed limiting their usage in breeding. Computer algorithms capable of capturing staining intensities can be constructed to quantify the staining intensities. The current study uses Wiesner test to determine the relationship between Wiesner staining in stem, leaf midrib and forage quality variables with a goal of contributing to improve the method to fast-track the phenotyping of bmr trait in early and later generations in sorghum breeding pipelines.

The genotypes used in the study had statistically similar performance for forage quality traits; percent DM, percent ASH, percent NDF, percent ADF, percent hemicellulose, percent cellulose and percent CP (Table 2). These were associated with the less diverse materials evaluated in the study. Similar findings have been reported by de Aguilar et al., (2014) who also found no significant genetotypic difference for NDF and ADF between near isogenic *bmr6* and wild-type genotypes under two genetic backgrounds. Another study, found statistically similar performance for percent CP content in *bmr* hybrids and wild-type hybrids from sorghum x sudan-grass hybrids (Sattler et al., 2010). However, significant genotypic difference where detected for percent ADL, percent IVOMD, and ME (mj/kg) (Table 2). These traits are the most important in forage quality improvement. As a result, the genetic materials are ideal for histochemical staining studies. As expected, orthogonal contrast analysis revealed that there was a significantly higher percent IVOMD and ME (mj/kg) in genotypes carrying bmr mutants (bmr6, bmr12, and bmr6bmr12) than in wild-type genotypes (Bmr) (Table 3) which was reported in other studies (Oliver et al., 2005; Dien et al., 2009; Sattler et al., 2010). The increased percent IVOMD and ME

has been associated to the reduction in lignin content (Oliver et al., 2005; Dien et al., 2009; Sattler et al., 2010). Indeed, genotypes carrying bmr mutants (bmr6, bmr12, and bmr6bmr12) were found to have a significantly lower percent ADL and percent ADF than in wild-type genotypes (Bmr) (Table 3). bmr6 and bmr12 mutants carry mutations in the CAD and COMT genes. The Bmr6 and Bmr12 loci, encodes cinnamyl alcohol dehydrogenase (CAD) and caffeic acid Omethyltransferase (COMT) enzymes, respectively. CAD catalyses the conversion of monolignol aldehyde (p-coumaraldehyde and coniferaldehyde) to its corresponding alcohol (Saballos et al., 2009), while a COMT gene converts 5-hydroxy-coniferaldehyde to sinapaldehyde in the synthesis of sinapyl alcohol (Bout & Vermerris, 2003). These enzymes catalyses the last step of monolignol biosynthesis. As a result, the mutations of these genes results in a reduced lignin content.

Novel phenolic substrates such as coniferaldehyde (Saballos et al., 2008), p-coumaric acid and syringyl content (Akin et al., 1986; Fritz et al., 1990), vanillin and p-hydroxybenzoic acid (Akin et al., 1986; Fritz et al., 1990) are deposited in cell wall of the bmr mutants. Phloroglucinol, the active ingredient in the Wieners' test reacts with these components of the plant cell wall (Pomar et al., 2002; Blaschek et al., 2020). Our study detected an increase in intensity of the staining on stem and leaf midrib tissues with the increase in the proportion of HCI in acid-ethanol solution using both the traditional and digitized method (Table 6 & 7). The active ingredient shows a favorable reaction under a more acidic condition (Clifford, 1974; Pomar et al., 2002). There was no major change in the ranking of genotype performance for histochemical staining at the four levels detected (Table 8). Earlier studies detected strong reddish-brown coloration of tissues for reaction with phloroglucinol at 20:80 HCI: Ethanol (Clifford, 1974) and 30:70 HCI: Ethanol levels (Saballos et al., 2008; Gorthy et al., 2013). Therefore, a favorable result on staining of plant tissues with acidified phloroglucinol can be attained at levels of HCI: Ethanol in the range 20:80 to 35:65. The staining method shows promise for deployment in selection as noted from the significant genotypic differences at each of the four acid-ethanol levels (Table 5). One obvious utility of this staining that has been demonstrated is the generally greater intensity of the staining detected on bmr genotypes

than wild-type genotypes. Historically, these method has been qualitatively employed to confirm bmr status (Gorthy et al., 2013) and has been used in differentiating bmr2 loci from bmr6, bmr12 and bmr19 allelic groups (Saballos et al., 2008). bmr genotypes used in the study carry the bmr6 and bmr12 alleles. *bmr*6 alleles are dysfunctional for the cinnamyl alcohol dehydrogenase (CAD) enzyme and are unable to convert monolignol aldehyde to its corresponding alcohol in the last step of monolignol biosynthesis (Saballos et al., 2009), while bmr12 alleles are dysfunctional in caffeic acid O-methyltransferase (COMT) enzyme hence the enzyme fails to catalyze conversion of 5hydroxy-coniferaldehyde to sinapaldehyde in the synthesis of sinapyl alcohol (Bout & Vermerris, 2003). The defect in these enzymes leads to a buildup of hydroxycinnamyl aldehyde, which get integrated in plant cell wall (Bucholtz et al., 1980; Saballos et al., 2008). However, these substrates (hydroxycinnamyl aldehyde) are converted into lignin monolignol in the presence of the fully functional enzymes. This explains why stem and leaf midrib tissues from the wild-type genotypes showed no stain or just faint red staining at the four levels of HCI: Ethanol (Table 6 & 7).

The degree of reddish-brown coloration of stem and leaf midrib in bmr genotypes depend on the growth stage and environment condition. An intense reddishbrown coloration is normally detected on the midrib of younger leaves and along the stem in some genotypes. Leaf and stem tissues from these regions of bmr genotypes have been reported to confer an antibiosis resistance to insect pest (Dowd & Sattler, 2015; Dowd et al., 2016) and are toxic to disease pathogens (Palmer et al., 2008; Funnell-Harris et al., 2017). The current study found strong positive, but significant correlation between the staining of the leaf midrib and stem tissues using both traditional and digitized methods (Table 8), suggesting that the substrates needed for the phloroglucinol reaction accumulated similarly in the stem and leaf midrib tissues. The intensity of red stain was more pronounced in the stem than in the leaf midrib tissues. The chemical composition of upper internodes has been reported as complex, having array of soluble phenolic and associated aromatic chemicals compared to the bottom stem internodes (Sarath et al., 2007; Palmer et al., 2008). Our results suggest that the chemical composition of stem tissues could also be more complex than that of leaf midrib tissues. This result is in accordance with previous studies in sorghum. In sorghum, Akin and colleagues (1989) reported stem to have more specialized cells that can be lignified than in leaf tissues. In their study, xylem, sclerenchyma, epidermis, and parenchyma tissues in stem were extensively and intensely stained using Wiener's test, but only xylem and sclerenchyma tissues were stained in leave tissues. In another study, Pillonel et al., (1991) observed that the shoot tissues of 20-day-old bmr6 mutant had more intense pink staining in comparison to the shoot tissues of wild-type sorghum genotypes. Their analysis found guaiacyl aldehyde and syringyl aldehyde were present in high concentrations in bmr6 mutant than in corresponding wild-type. As a result, either stem or leaf midrib tissues can provide a reliable means for histochemical investigation of bmr expression in sorghum.

This is the first attempt in the quantification of Wiesner's staining, and the results has demonstrated the possibility that the staining method can be improved and deployed in breeding. The intensity of the staining bmr loci was found greater in the order bmr6> bmr6bmr12> bmr12> Bmr using both approaches (Table 9 & 10). Mutations in bmr6 mutants affects two stages in the lignin biosynthesis pathway; the conversion of monolignol aldehyde (p-coumaraldehyde and coniferaldehyde) to its corresponding alcohol (Saballos et al., 2009; Palmer et al., 2008), as compared to bmr12 mutants that affects the conversion of 5-hydroxy-coniferaldehyde to sinapaldehyde in the synthesis of sinapyl alcohol (Bout & Vermerris, 2003; Palmer et al., 2008). It is therefore expected that bmr6 would have a greater effect on lignin reduction and alteration in cell wall chemistry than in bmr12 mutants. Earlier studies such as the works of Palmer et al., (2008) and Dowd and Sattler (2015) found higher soluble phenolic substrates and antibiosis resistance associated with mutants of bmr6 over bmr12. These findings show that histochemical staining could provide an alternative approach in the preliminary selection of brown midrib in sorghum. Digitization of the staining quantification would encourage its deployment in breeding programs.

In summary, the genetic materials of our study provided a good assessment for deployment of

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histochemical staining in breeding. *bmr* genotypes had low lignin content over the wild-type genotypes. Both approaches, traditional and digitized histochemical quantification had corroborated with variation in percent lignin content and percent IVOMD. The staining for leaf midrib and stem tissues were found high in *bmr6* mutants, followed by genotypes carrying double mutant (*bmr6bmr12*), then *bmr12* and *Bmr* (wild-type genotype) using both traditional and digitized method. These findings shown promise for employing histochemical staining combined with high-through-put phenotyping in breeding programs. However, further studies are recommended using a large number of *bmr* inbred lines to determine the relationships between histochemical staining quantification for forage quality assessment. The python algorithms for determining the estimates of staining intensity used in the study once improved can be incorporated in a user friendly device and deployed in large scale phenotyping of the *bmr* trait in breeding programs.

S. No.	Genotype	<i>Bm</i> rlocus	Pedigree	References
1	N597	bmr12	Early Hegari-Sart x F220	Pedersen et al. (2006)
2	59353	bmr12	((IS 21893 x N 595) x IS 21893) x IS 21893	ICRISAT, Sorghum Program
3	N600	bmr12	Atlas(NSL 3986) x F220	Pedersen et al. (2006)
4	N606	bmr12	BTx630 x F220	Pedersen et al. (2006)
5	N608	bmr12	BTx630 x F220	Pedersen et al. (2006)
6	N 610	bmr12	RTx430 x F220	Pedersen et al. (2008)
7	BN 612	bmr6bmr12	N 599 x N 600 (Wheatland bg)	Pedersen <i>et al.</i> (2008)
8	BN 611	bmr6bmr12	N 598 x "Atlas <i>bmr12</i> "	Pedersen <i>et al.</i> (2008)
9	RN 613	bmr6bmr12	N609 x N610 (RT x 430 bg)	Pedersen <i>et al.</i> (2008)
10	59354	bmr6	((IS 23789 x IS 8813) x IS 23789) x IS 23789	ICRISAT, Sorghum Program
11	N596	bmr6	Early Hagari-sart x N 121	Pedersen et al. (2006)
12	N605	bmr6	BTx630 x N 121	Pedersen et al. (2006)
13	ICSV100305	Bmr	(IS 903 x SP 4511-2) -1-1-3-2-1-2	ICRISAT, Sorghum Program
14	ICSV 100453	Bmr	(IS 903 x SP 4511-2) -1-1-6-2-1-2	ICRISAT, Sorghum Program
15	ICSV100309	Bmr	(IS 903 x SP 4511-2) -1-1-6-5-1-1	ICRISAT, Sorghum Program
16	ICSV12006	Bmr	(Ch-1 x (DSV 4 x IS 23568) -1-2-1-1)-13-1-1-4	ICRISAT, Sorghum Program
17	IS 93025	Bmr	IS 93025	ICRISAT, Sorghum Program
18	IS 26694	Bmr	IS 26694	ICRISAT, Sorghum Program
19	IS 11861	Bmr	IS 11861	ICRISAT, Sorghum Program
20	SSV-74	Bmr	IS 23558-2-1	ICRISAT, Sorghum Program

Table 1. List of sorghum genotypes used in the study

Where bg = background

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Fig. 1: leaf midrib pieces scanned by flatbed scanner (A & E), trimmed and magnified in Adobe photoshop (B & D) and image analysis in pthyon for highly, medium and low red stained areas (C). A, B & C represents bmr genotype, BN 612 and D & E represent non-bmr genotype, ICSV100305. Where 0:100, 5:95, 10:90, 15:85, 20:80, 25:75, 30:70 are HCI : Ethanol levels.

Table 2. Analys	sis of variance for	11 forage quality	y analysis conducted c	on stem tissues

	Df	DMC	Ash.	Nit	CP	NDF	ADF	ADL	IVOMD	ME	Hemi- cellu- lose	Cellu Iose
Rep	1	0.14	0.33	0.09	3.42	2.56	0.37	0.01	0.04	0.000	4.89	0.28
Gen	16	0.549	1.686	0.119	4.658	10.548	9.736	1.138 * *	14.086 *	0.323 *	2.236	6.503
Resi- duals	8	0.211	1.016	0.059	2.313	7.528	7.478	0.191	4.15	0.083	0.931	6.405

DMC = percent dry matter content, Ash. = percent ash content, Nit = percent nitrogen content, CP = percent crude protein, NDF = percent neutral detergent fibre, ADF = percent acid detergent fibre, ADL = percent acid detergent lignin, IVOMD = percent *in vitro* organic matter digestibility, ME = metabolizable energy (mj/kg), Hemicellulose = percent hemicellulose, Cellulose = percent

				MC	קעמוויץ יו מויס	-	Ash.		-	
	Estimate	Std.	Error	t_value	Pr(> t)	Estimate	Std.	Error	t_value	Pr(> t)
(Intercept)	900.006	0.1983	453.933	<2e-16	* * *	6.494	0.3561	18.237	3.58E-15	* * *
bmr6 vs bmr12	0.5115	0.3709	1.379	0.1812		0.0835	0.6662	0.125	0.9013	
bmr6bmr12 vs bmr12	0.274	0.3434	0.798	0.4331		-0.766	0.6168	-1.242	0.2268	
Bmr vs bmr12	0.7715	0.2974	2.594	0.0162	*	-1.4752	0.5342	-2.762	0.0111	*
			С С	£			NDF			
	Estimate	Std.	Error	t_value	Pr(> t)	Estimate	Std.	Error	t_value	Pr(> t)
(Intercept)	6.675	0.7334	9.101	4.38E-09	* * *	57.358	1.146	50.037	2.00E-16	* * *
bmr6 vs bmr12	-1.7125	1.3721	-1.248	0.2246		-0.578	2.144	-0.27	0.7899	
bmr6bmr12 vs bmr12	-2.717	1.2704	-2.139	0.0433	*	2.28	1.986	1.148	0.2626	
Bmr vs bmr12	-2.6825	1.1002	-2.438	0.0229	*	3.142	1.72	1.827	0.0807 .	
			A	DF			ADL			
	Estimate	Std.	Error	t_value	Pr(> t)	Estimate	Std.	Error	t_value	Pr(> t)
(Intercept)	27.584	1.091	25.289	2.00E-16	* *	1.249	0.2253	5.544	1.22E-05	* * *
bmr6 vs bmr12	0.481	2.041	0.236	0.8157		0.711	0.4214	1.687	0.105111	
bmr6bmr12 vs bmr12	2.36	1.889	1.249	0.2242		0.431	0.3902	1.105	0.28075	
Bmr vs bmr12	3.793	1.636	2.319	0.0297	*	1.481	0.3379	4.383	0.000217	* * *
			2	OMD			VE (mj/kg)			
	Estimate	Std.	Error	t_value	Pr(> t)	Estimate	Std.	Error	t_value	Pr(> t)
(Intercept)	62.473	0.9657	64.69 2	.00E-16	* * *	9.109	0.1459	62.452	2e-16	* * *
bmr6 vs bmr12	0.807	1.8067	0.447	0.65929		0.2535	0.2729	0.929	0.3625	
bmr6bmr12 vs bmr12	-0.639	1.6727	-0.382	0.70595		0.027	0.2526	0.107	0.9158	
Bmr vs bmr12	-4.4242	1.4486	-3.054	0.00563	* *	-0.5152	0.2188	-2.355	0.0274	*
		Ĭ	emicellulos	e				Cellulose		
	Estimate	Std.	Error	t_value	Pr(> t)	Estimate	Std.	Error	t_value	Pr(> t)
(Intercept)	29.773	0.4228	70.414	2.00E-16	* * *	26.333	0.9815	26.83	2.00E-16	* * *
bmr6 vs bmr12	-1.0555	0.791	-1.334	0.195		-0.2305	1.8362	-0.126	0.901	
bmr6bmr12 vs bmr12	-0.077	0.7323	-0.105	0.917		1.931	1.7	1.136	0.268	
Bmr vs bmr12	-0.6518	0.6342	-1.028	0.315		2.317	1.4722	1.574	0.129	
DMC = percent dry matter conte percent acid detergent lignin, IV(nt, Ash. = percen OMD = percent <i>in</i>	t ash content, l <i>vitro</i> organic n	Vit = percent nit natter digestibil	trogen content, CF ity, ME = metaboli:	^o = percent crude zable energy (mj/k	orotein, NDF = perce g), Hemicellulose = p	nt neutral deterge bercent hemicellul	ent fibre, ADF = p lose, Cellulose =	ercent acid deter percent cellulose	gent fibre, ADL = content

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Table 4. Mean performance of 11 forage quality traits for bmr and wild-type genotypes obtained from top most stem internode sections

IVOMD	66.66	65.76	65.13	64.38	63.19	62.87	61.81	61.41	59.85	59.29	59.25	59.09	58.89	58.57	57.15	56.80	56.63	63.90	1.96	31.66	
ME	9.81	9.63	9.82	9.56	9.08	9.44	9.04	8.99	8.95	8.51	8.94	8.75	8.68	8.77	8.42	8.27	8.35	9.42	0.28	9.13	-
СР	4.83	6.12	1.72	2.86	6.40	4.72	7.64	3.87	3.61	60.9	3.49	4.15	4.56	3.23	6.24	7.90	3.00	4.77	N/A	3.28	
Cellulose	26.05	24.67	26.30	27.99	24.94	26.41	25.06	27.90	29.74	31.03	28.87	26.38	28.30	28.48	30.10	29.22	29.59	26.17	N/A	3.35	-
Hemicellulose	28.38	28.74	28.04	27.89	31.55	28.31	29.47	30.53	29.53	30.21	27.93	28.65	30.46	28.13	28.80	31.40	29.18	29.11	N/A	3.20	
ADL	1.36	1.08	2.11	2.20	09.0	1.93	1.77	0.96	2.57	0.79	2.97	2.53	2.34	3.31	2.96	2.15	2.53	1.50	1.03	22.68	-
ADF	27.41	25.75	28.42	30.19	25.54	28.34	26.83	28.86	32.30	31.82	31.84	28.90	30.63	31.79	33.05	31.37	32.12	27.67	N/A	9.22	-
NDF	55.79	54.49	56.45	58.08	57.08	56.66	56.30	59.38	61.82	62.03	59.77	57.56	61.09	59.92	61.85	62.78	61.29	56.78	N/A	4.65	-
NIT	0.77	0.98	0.27	0.46	1.02	0.75	1.22	0.62	0.58	0.98	0.56	0.66	0.73	0.52	1.00	1.26	0.48	0.76	N/A	31.61	-
ASH	7.07	7.46	5.72	5.73	6.11	5.09	7.43	6.05	4.74	7.19	4.15	4.91	5.36	4.73	5.43	6.13	4.82	6.33	N/A	17.25	-
*DMC	90.67	90.01	90.97	91.22	89.90	89.00	89.94	89.60	90.77	90.19	90.75	90.42	91.01	91.23	90.86	90.89	90.23	90.16	N/A	0.51	-
Genotype	BN612	59353	59354	N605	N608	N600	N596	BN611	RN613	N597	NGOG	SSV-74	IS26694	IS93025	IS11861	N610	ICSV12006	Mean	LSD	CV%	

* DMC = percent dry matter content, Ash. = percent ash content, Nit = percent nitrogen content, CP = percent crude protein, NDF = percent neutral detergent fibre, ADF = percent acid detergent solution, IVOMD = percent *in vitro* organic matter digestibility, ME = metabolizable energy (mj/kg), Hemicellulose = percent hemicellulose, Cellulose = percent cellulose content

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d at each of the			ST 35:65
aditional metho		ssue	ST 30:70
essed using tr		Stem ti	ST 25:75
em tissues ass	staining		ST 20:80
midrib and ste	iistochemical s		LF 35:65
iducted on leaf 5/65)	scoring of the h	rib tissue	LF 30:70
st staining con 75, 30/70 and 3	Visual	Leaf mid	LF 25:75
for Wiesner te /els (20/80, 25/			LF 20:80
of variance ethanol lev			ď
Table 5. Analysis c four acid⊣			

	đ	LF_20:80	LF_25:75	$LF_{30:70}$	LF_35:65	ST_20:80	ST_25:75	ST_30:70	ST_35:65
Replications	١	0.54 **	0.21	0.09	0.17	0.04	00.00	0.03	0.02
Genotypes	18	0.86 ***	1.08 ***	1.12 ***	1.13 ***	0.97 ***	0.86 ***	1.00 ***	0.90 ***
Residual error	13	0.06	0.05	0.07	0.14	0.05	0.06	0.03	0.05
			Histochem	ical staining qua	antification usi	ng python			
			Leaf mid	ib tissue			Stem ti	issue	
	Dť	LF_20:80	LF_25:75	LF_30:70	LF_35:65	ST_20:80	ST_25:75	ST_30:70	ST_35:65
Replications	1	0.43	0.38	1.47	1.89	0.28	0.28	0.02	0.23
Genotypes	18	2.51 ***	3.71 ***	4.02 ***	5.09 ***	0.52 ***	0.68 ***	0.75 ***	0.86 ***
Residual error	15	0.29	0.21	0.78	1.01	0.05	0.02	0.06	0.03

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Table 6. Mean histochemical staining on leaf midrib and stem tissues of 19 genotypes assessed using traditional method (score scale of 1 – 3) at acid-ethanol levels of 20:80, 25:75, 30:70 and 35:65

			Visua	al scoring of the his	stochemical sta	ining		
		Leaf midr	ib tissue			Stem t	tissue	
Genotype	20:80	25:75	30:70	35:65	20:80	25:75	30:70	35:65
59353	2.7	2.8	2.9	3.0	2.7	2.8	3.0	3.0
59354	3.0	3.0	3.0	3.0	2.7	2.8	2.9	3.0
BN611	1.6	2.0	2.4	3.0	2.1	2.4	2.6	3.0
BN612	2.0	2.5	2.8	2.8	2.9	2.9	3.0	2.9
ICSV100309	1.5	1.6	1.8	2.0	1.5	2.0	1.9	2.0
ICSV100453	1.0	1.0	1.0	1.0	1.0	1.0	1.0	2.0
ICSV12006	1.0	1.0	1.0	1.0	1.0	1.9	2.0	1.9
IS11861	1.0	1.3	1.2	1.5	1.0	1.0	1.2	1.5
IS26694	1.0	1.0	1.0	1.0	1.1	1.7	2.0	2.0
IS93025	1.7	2.0	2.0	2.3	1.7	2.0	2.0	2.2
N596	2.5	2.9	3.0	3.0	2.6	3.0	3.0	3.0
N597	1.2	1.3	1.6	1.8	1.0	1.0	1.0	1.0
N600	2.3	2.7	2.7	2.8	2.7	2.6	2.9	3.0
N605	2.7	2.9	3.0	3.0	1.8	2.0	3.0	3.0
NGOG	1.0	1.0	1.2	1.3	1.7	1.9	1.9	2.0
N608	1.6	1.7	1.8	1.8	1.3	1.8	1.7	2.0
N610	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
RN613	2.5	2.8	2.8	2.8	2.8	3.0	3.0	3.0
SSV74	1.0	1.0	1.0	1.0	1.0	1.9	2.0	1.9
Mean	1.7	1.9	2.0	2.0	1.8	2.0	2.2	2.3
LSD	0.4	0.4	0.4	0.6	0.3	0.4	0.3	0.3
CV%	13.9	11.9	13.4	17.8	12.1	11.8	7.8	9.4

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			-	Histochemical sta	iining qualificatio	Ę		
		Leaf midr	ib tissue			Stem	tissue	
Genotype	20:80	25:75	30:70	35:65	20:80	25:75	30:70	35:65
59353	2.81	3.69	4.44	4.84	3.6	5.4	6.4	8.1
59354	3.47	3.74	3.44	1.91	5.7	5.7	6.3	7.6
BN611	0.74	1.06	1.05	0.71	12	1.7	3.4	4.2
BN612	1.88	2.21	3.18	2.17	6.4	7.8	8.2	8.3
CSV100309	0.81	1.00	0.87	0.71	1.8	2.5	2.4	2.5
ICSV100453	0.71	0.71	0.71	0.71	1.0	1.0	1.0	1.0
ICSV12006	0.71	0.71	0.71	0.71	1.1	2.6	2.0	1.9
IS11861	0.71	0.71	0.71	0.71	1.0	1.0	1.0	1.0
IS26694	0.71	0.71	0.71	0.71	1.2	1.7	1.7	2.3
IS93025	1.90	2.46	2.63	2.96	2.2	3.1	3.0	2.7
N596	2.26	3.89	3.85	4.17	5.4	8.2	8.1	8.1
N597	0.71	0.91	0.99	0.91	1.0	1.0	1.0	1.0
N600	3.67	3.90	2.92	4.59	6.5	5.7	6.8	7.2
N605	3.39	4.06	4.43	4.94	4.4	6.5	7.9	8.0
N606	0.71	0.71	0.78	0.78	2.0	2.4	2.5	2.1
N608	0.85	0.92	0.94	1.10	1.4	1.7	2.7	3.7
N610	0.71	0.71	0.71	0.71	1.0	1.0	1.0	1.0
RN613	2.97	3.17	3.28	2.98	6.8	7.4	7.2	7.0
SSV74	0.71	0.71	0.71	0.71	1.0	1.4	1.6	1.9
Mean	1.60	1.89	1.95	1.95	2.9	3.5	3.9	4.2
LSD	0.51	0.44	0.85	0.96	1.3	0.83	1.38	0.95
% CV	32.42	23.11	43.68	49.70	30.1	15.3	23.4	14.9

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Table 8. Correlation analysis for histochemical staining among four acid-ethanol levels within leaf midrib and stem tissues using both traditional and digitized method

	*LT_20.80	LT_25.75	LT_30.70	LT_35.65	*ST_20.80	ST_25.75	ST_30.70	ST_35.65
LT_20.80	-	0.97***	0.91***	0.87***	0.88***	0.83***	0.85***	0.86***
LT_25.75	0.98***	1	0.96***	0.93***	0.87***	0.87***	0.89***	0.89***
LT_30.70	0.96***	0.99***	1	0.92***	0.84***	0.89***	0.91***	0.91 ***
LT_35.65	0.91***	0.96***	0.98***	1	0.74***	0.79***	0.82***	0.81 ***
ST_20.80	0.86***	0.89***	0.90***	0.88***	1	0.95***	0.93***	0.90***
ST_25.75	0.79***	0.81***	0.82***	0.78***	0.92***	1	0.98***	0.94***
ST_30.70	0.84***	0.86***	0.86***	0.82***	0.88***	0.95***	1	0.98***
ST_35.65	0.83***	0.85***	0.85***	0.83***	0.87***	0.89***	0.94***	~
:								

The upper diagonal is the leaf midrib and stem digitized histochemical staining quantification and lower diagonal is the leaf midrib and stem traditional histochemical staining scores. *LT _ leaf midrib tissues, *ST_ upper internode stem tissues

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Estimate Std. Conc 20:80 Intercept) 1.62 0.16 9.94 bmr6vs bmr12 1.10 0.29 3.78 bmr6bmr12 vs bmr12 1.10 0.29 3.78 bmr6bmr12 vs bmr12 0.40 0.27 1.45 Bmrvs bmr12 0.41 0.23 -1.78 Bmrvs bmr12 0.41 0.23 -1.78 Bmrvs bmr12 0.17 10.90 1.86 Untercept) 1.86 0.17 10.90 bmr6bmr12 vs bmr12 0.79 0.29 3.72 bmr6vs bmr12 0.79 0.29 2.73 bmr6vs bmr12 0.79 0.29 2.73 bmr6vs bmr12 0.79 0.29 2.73 bmr6vs bmr12 0.69 0.30 2.32 bmr6vs bmr12 0.50 0.24 -2.07 bmr6vs bmr12 0.79 0.23 2.33 bmr6vs bmr12 0.79 0.30 2.33 bmr6vs bmr12 0.69 0.33 <		Leaf histoch	emical stair	ing			
Estimate Std. Error (Intercept) 1.62 0.16 9.94 bmr6vs bmr12 1.10 0.29 3.78 bmr6bmr12 vs bmr12 0.40 0.27 1.45 Bmr vs bmr12 0.40 0.23 1.45 Bmr vs bmr12 0.40 0.23 1.45 Bmr vs bmr12 0.40 0.23 1.45 Bmr vs bmr12 0.14 0.23 1.45 bmr6bmr12 vs bmr12 1.86 0.17 10.90 bmr6bmr12 vs bmr12 0.79 0.29 2.73 bmr6bmr12 vs bmr12 0.79 0.24 2.07 bmr6bmr12 vs bmr12 0.79 0.29 2.73 bmr6bmr12 vs bmr12 0.69 0.17 10.60 bmr6bmr12 vs bmr12 0.28 2.98 2.98 bmr6bmr12 vs bmr12 0.83 0.28 2.98 bmr6bmr12 vs bmr12 0.83 0.28 2.98 bmr6bmr12 vs bmr12 0.83 0.28 2.98 bmr6bmr12 vs bmr12	onc 20:80			0	onc 25:75		
(Intercept) 1.62 0.16 9.94 $bmr6vs bmr12$ 1.10 0.29 3.78 $bmr6bmr12 vs bmr12$ 0.40 0.27 1.45 $Bmrvs bmr12$ 0.41 0.23 -1.78 $Bmrvs bmr12$ -0.41 0.23 -1.78 $Bmrvs bmr12$ 0.041 0.23 -1.78 $Bmrvs bmr12$ 0.186 0.17 10.90 $bmr6vs bmr12$ 1.86 0.17 10.90 $bmr6vs bmr12$ 0.79 0.24 2.73 $bmr6vs bmr12$ 0.79 0.24 2.07 $bmr6vs bmr12$ 0.79 0.24 -2.07 $bmr6vs bmr12$ 0.79 0.24 -2.07 $bmr6vs bmr12$ 0.50 0.24 -2.07 $bmr6vs bmr12$ 0.69 0.30 2.32 $bmr6vs bmr12$ 0.69 0.28 2.98 $bmr6vs bmr12$ 0.69 0.29 2.32 $bmr6bmr12 vs bmr12 0.69 0.23 -2.33 bmr6bmr12 vs bmr$	Error t_value	Pr(> t)	Estimate	Std.	Error	t_value	Pr(> t)
bmr6vs bmr12 1.10 0.29 3.78 bmr6bmr12 vs bmr12 0.40 0.27 1.45 Bmr vs bmr12 -0.41 0.23 -1.78 Bmr vs bmr12 -0.41 0.23 -1.78 Bmr vs bmr12 Estimate Std. Error Intercept) 1.86 0.17 10.90 Intercept) 1.86 0.17 10.90 bmr6vs bmr12 1.14 0.31 3.72 bmr6vs bmr12 0.79 0.29 2.73 bmr6vs bmr12 0.79 0.24 10.90 bmr6vs bmr12 0.79 0.24 2.07 bmr6vs bmr12 0.79 0.24 2.07 bmr6vs bmr12 0.79 0.24 2.07 bmr6vs bmr12 0.69 0.30 2.32 bmr6vs bmr12 0.69 0.23 2.32 bmr6vs bmr12 0.69 0.24 2.07 bmr6vs bmr12 0.69 0.30 2.32 bmr6vs bmr12 0.69 0	9.94 0.00	* * *	1.74	0.17	10.01	0.00	* * *
bmr6bmr12 vs bmr12 0.40 0.27 1.45 Bmr vs bmr12 -0.41 0.23 -1.78 Bmr vs bmr12 Estimate Std. -1.78 Intercept) Estimate Std. -1.78 Intercept) 1.86 0.17 10.90 bmr6bmr12 vs bmr12 1.14 0.31 3.72 bmr6bmr12 vs bmr12 0.79 0.29 2.73 bmr6bmr12 vs bmr12 0.79 0.20 2.73 bmr6bmr12 vs bmr12 0.79 0.24 -2.07 bmr6bmr12 vs bmr12 0.79 0.24 -2.07 bmr6bmr12 vs bmr12 0.69 0.30 -2.32 bmr6vs bmr12 0.69 0.30 2.32 bmr6vs bmr12 vs bmr12 0.69 0.23 -2.33 bmr6vs bmr12 vs bmr12 0.69 0.23 -2.33 bmr6vs bmr12 0.69 0.23 -2.33 bmr6vs bmr12 0.69 -2.33 -2.33 bmr6vs bmr12	3.78 0.00	* * *	1.20	0.31	3.88	0.00	* * *
Bmr vs bmr 12 -0.41 0.23 -1.78 Estimate Std. $Conc 30:70$ Estimate Std. $Error$ Intercept) 1.86 0.17 10.90 bmr 6vs bmr 12 1.14 0.31 3.72 bmr 6vs bmr 12 0.79 0.29 2.73 bmr 6vs bmr 12 0.79 0.24 2.07 bmr 6vs bmr 12 0.750 0.24 -2.07 bmr 6vs bmr 12 0.75 0.17 10.60 bmr 6vs bmr 12 0.69 0.03 2.32 bmr 6vs bmr 12 0.69 0.30 2.32 bmr 6vs bmr 12 0.69 0.30 2.33 bmr 6vs bmr 12 0.69 0.30 2.33 bmr 6vs bmr 12 0.69 0.23 -2.33 bmr 70 0.69 0.30 2.34 0.69 0.79 0.30 2.33 0.70 0.69 0.73 -2.33 0.70 0.69 0.23 -2.33 0.70 0.23 <td>1.45 0.16</td> <td></td> <td>0.70</td> <td>0.29</td> <td>2.39</td> <td>0.02</td> <td>*</td>	1.45 0.16		0.70	0.29	2.39	0.02	*
Intercept) Estimate Std. Conc 30:70 $Intercept$) Estimate Std. Error $Intercept$) 1.86 0.17 10:90 $bmr6bmr12$ vs $bmr12$ 0.79 0.29 2.73 $bmr6bmr12$ vs $bmr12$ 0.79 0.29 2.73 $bmr6bmr12$ vs $bmr12$ 0.79 0.24 -2.07 $bmr6bmr12$ vs $bmr12$ 0.17 10.60 0.24 -2.07 $bmr6bmr12$ vs $bmr12$ 0.17 10.60 0.17 10.60 $bmr6bmr12$ vs $bmr12$ 0.69 0.30 2.32 0.17 10.60 $bmr6vs bmr12$ 0.69 0.30 2.32 0.17 10.60 0.17 10.60 0.160	-1.78 0.09 .		-0.41	0.25	-1.67	0.11	
Estimate Std. Error (Intercept) 1.86 0.17 10.90 bmr6vs bmr12 1.14 0.31 3.72 bmr6vs bmr12 0.79 0.29 2.73 bmr6bmr12 vs bmr12 0.79 0.24 -2.07 bmr6bmr12 vs bmr12 0.50 0.24 -2.07 bmr6vs bmr12 0.50 0.24 -2.07 bmr6bmr12 vs bmr12 0.17 10.60 0.17 bmr6vs bmr12 0.175 0.17 10.60 bmr6vs bmr12 0.83 0.28 2.32 bmr6vs bmr12 0.83 0.23 -2.33 bmr6pmr12 0.83 0.23 -2.33 bmr6pmr12 0.83 0.23	onc 30:70	-		0	onc 35:65	-	
	Error t_value	Pr(> t)	Estimate	Std.	Error	t_value	Pr(> t)
bmr6vs bmr12 1.14 0.31 3.72 bmr6bmr12 vs bmr12 0.79 0.29 2.73 Bmrvs bmr12 -0.50 0.24 -2.07 Bmrvs bmr12 -0.50 0.24 -2.07 Intercept -1.75 0.24 -2.07 Intercept 1.75 0.17 10.60 bmr6vs bmr12 0.69 0.30 2.32 bmr6vs bmr12 0.83 0.28 2.98 bmr6bmr12 vs bmr12 0.83 0.23 -2.33 bmr6bmr12 vs bmr12 0.83 0.23 -2.33 bmr6bmr12 vs bmr12 0.83 0.23 -2.33 bmr6bmr12 vs bmr12 0.83 0.28 -2.33 bmr6bmr12 vs bmr12 0.83 0.28 -2.33 bmr6bmr12 vs bmr12 0.83 0.28 -2.33 bmrv5 bmr12 0.69 -2.33 -2.33 bmrv5 bmr12 -0.55 0.28 -2.33 bmrv5 -0.18 -0.18	10.90 0.00	* * *	1.94	0.19	10.29	0.00	* * *
bmr6bmr12 vs bmr12 0.79 0.29 2.73 Bmr vs bmr12 -0.50 0.24 2.07 Bmr vs bmr12 -0.50 0.24 -2.07 Bmr vs bmr12 Estimate Std. Error Intercept 1.75 0.17 10.60 bmr6 vs bmr12 0.69 0.30 2.32 bmr6 bmr12 vs bmr12 0.63 0.28 2.98 Bmr vs bmr12 0.69 0.23 2.32 bmr6 bmr12 vs bmr12 0.63 0.28 2.38 Bmr vs bmr12 0.655 0.23 2.33 Bmr vs bmr12 0.655 0.23 -2.33 Bmr vs bmr12 0.669 0.69 0.68 -2.33 -2.33 -2.33	3.72 0.00	* * *	1.06	0.34	3.16	0.00	* *
Bmr vs bmr 12 -0.50 0.24 -2.07 Intercept Estimate Std. -2.07 Intercept Estimate Std. -2.03 Intercept 1.75 0.17 10.60 bmr6 vs bmr 12 0.69 0.30 2.32 bmr6 bmr 12 vs bmr 12 0.83 0.28 2.98 Bmr vs bmr 12 0.655 0.23 2.32 Intercept 1.75 0.17 10.60 Intercept 0.83 0.28 2.32 Intercept 0.83 0.28 2.32 Intercept 0.18 0.28 2.38 Intercept 0.18 0.28 2.33 Intercept 0.192 0.23 2.33 Intercept 0.18 0.18 10.88	2.73 0.01	*	0.9	0.32	2.83	0.01	* *
Intercept Estimate Std. Conc 20:80 Intercept 1.75 0.17 10.60 bmr6 vs bmr12 0.69 0.30 2.32 bmr6bmr12 vs bmr12 0.83 0.28 2.98 bmr6bmr12 vs bmr12 0.655 0.23 2.32 bmr6bmr12 vs bmr12 0.655 0.23 2.33 bmr6bmr12 vs bmr12 0.655 0.23 2.33 lmrvs bmr12 0.655 0.23 -2.33 lmtercept 1.92 0.18 10.88 lmtercept 1.92 0.18 10.88	-2.07 0.05	*	-0.44	0.27	-1.64	0.11	
Estimate Std. Conc 20:80 Intercept) Estimate Std. Error (Intercept) 1.75 0.17 10.60 bmr6bmr12 vs bmr12 0.69 0.30 2.32 bmr6bmr12 vs bmr12 0.83 0.28 2.98 Bmrvs bmr12 0.655 0.23 -2.33 Intercept) -0.55 0.23 -2.33 Intercept) 1.92 0.23 -2.33 Intercept) 1.92 0.23 -2.33 Intercept) 1.92 0.18 10.88		Stem histoc	hemical stai	ining	-	-	
Estimate Std. Error (Intercept) 1.75 0.17 10.60 bmr6vs bmr12 0.69 0.30 2.32 bmr6bmr12 vs bmr12 0.83 0.28 2.98 bmr6bmr12 vs bmr12 0.83 0.28 2.32 bmr6bmr12 vs bmr12 0.83 0.28 2.38 bmrcbmr12 vs bmr12 0.83 0.28 2.38 lmrvs bmr12 0.655 0.23 2.33 lmrvs bmr12 0.656 0.636 2.33 lmrvs bmr12 0.656 0.636 2.33 lmrvs bmr12 0.656 0.666 6.666 lmrvs bmr12 0.736 0.766 7.666 lmrvs bmr12 0.766 0.78 7.666	Conc 20:80			0	onc 25:75		
(Intercept) 1.75 0.17 10.60 bmr6bmr12 vs bmr12 0.69 0.30 2.32 bmr6bmr12 vs bmr12 0.83 0.28 2.98 Bmr vs bmr12 0.655 0.23 2.33 Bmr vs bmr12 0.555 0.23 2.33 Intercept) 1.75 0.23 2.33 Intercept) 1.92 0.13 10.88 Intercept) 1.92 0.18 10.88	Error t_value	Pr(> t)	Estimate	Std.	Error	t_value	Pr(> t)
bmr6 vs bmr12 0.69 0.30 2.32 bmr6bmr12 vs bmr12 0.83 0.28 2.38 Bmr vs bmr12 0.55 0.23 -2.33 Immediate -0.55 0.23 -2.33 Immediate -0.55 0.23 -2.33 Immediate -0.55 0.23 -2.33 Immediate 1.92 0.18 10.88 Immediate 1.92 0.18 10.88	10.60 0.00	* * *	1.85	0.17	10.71	0.00	* * *
bmr6bmr12 vs bmr12 0.83 0.28 2.98 Bmr vs bmr12 0.55 0.23 -2.33 Bmr vs bmr12 0.55 0.23 -2.33 Image: State State State State Image: State State State State	2.32 0.03	*	0.83	0.31	2.71	0.01	*
Bmr vs bmr 12 -0.55 0.23 -2.33 Bmr vs bmr 12 -0.55 0.23 -2.33 Estimate Std. Error Intercept 1.92 0.18 10.88	2.98 0.01	* *	0.89	0.29	3.06	0.00	* *
Estimate Std. Error (Intercept) 1.92 0.18 10.88	-2.33 0.03	*	-0.17	0.24	-0.71	0.48	
Estimate Std. Error (Intercept) 1.92 0.18 10.88	Conc 30:70			0	onc 35:65		
(Intercept) 1.92 0.18 10.88	Error t_value	Pr(> t)	Estimate	Std.	Error	t_value	Pr(> t)
	10.88 0.00	* * *	1.99	0.17	11.73	0.00	* * *
DITIO VS DITIFIZ 1.UZ 0.3Z 3.24	3.24 0.00	* *	0.99	0:30	3.26	0.00	* *
<i>bmr6bmr12</i> vs <i>bmr12</i> 0.93 0.30 3.14	3.14 0.00	* *	0.98	0.29	3.42	0.00	* *
<i>Bmrvs bmr12</i> -0.18 0.25 -0.73	-0.73 0.47		-0.07	0.24	-0.30	0.76	

Table 9. Orthogonal contrast analysis for leaf midrib and stem histochemical staining assessed using traditional method at acid-ethanol levels of 20:80.

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Table 10. Orthogonal contrast analysis for leaf midrib and stem histochemical staining assessed using digitized method at acid-ethanol levels of 20:80, 25:75, 30:70 and 35:65

					eaf histoch	emical stain	ning			
		С О	onc 20:80				S	onc 25:75		
	Estimate	Std.	Error	t_value	Pr(> t)	Estimate	Std.	Error	t_value	Pr(> t)
(Intercept)	1.58	0.29	5.51	0.00	* * *	1.81	0.31	5.83	0.00	* * *
bmr6 vs bmr12	1.47	0:50	2.95	0.01	* *	2.09	0.54	3.89	00.0	* * *
bmr6bmr12 vs bmr12	0.29	0.50	0.58	0.57		0.34	0.54	2.64	0.53	
Bmrvs bmr12	-0.65	0.41	-1.61	0.12 .		-0.76	0.44	-1.73	0.09	
		S	onc 30:70			-	S	onc 35:65		
	Estimate	Std.	Error	t_value	Pr(> t)	Estimate	Std.	Error	t_value	Pr(> t)
(Intercept)	1.80	0.36	4.93	0.00	* * *	2.16	0.46	4.69	0.00	* * *
bmr6 vs bmr12	2.11	0.63	3.35	0.00	* *	1.52	0.80	1.91	0.07	
bmr6bmr12 vs bmr12	0.71	0.63	1.12	0.27		-0.20	0.80	-0.26	0.80	
Bmrvs bmr12	-0.74	0.51	-1.43	0.16		-0.07	0.65	-1.65	0.11	
			-	St	em histoch	iemical stai	ning			
		Ö	onc 20:80				S	onc 25:75		
	Estimate	Std.	Error	t_value	Pr(> t)	Estimate	Std.	Error	t_value	Pr(> t)
(Intercept)	2.56	0.52	4.94	0.00	* * *	2.83	0.55	5.20	0.00	* * *
bmr6 vs bmr12	2.74	0.96	2.87	0.01	* *	4.01	0.01	3.99	0.00	* * *
bmr6bmr12 vs bmr12	2.23	0:00	2.48	0.02	*	2.77	0.94	2.93	0.01	* *
Bmr vs bmr12	-1.20	0.73	-1.64	0.11		-0.84	0.77	-1.09	0.28	
		Ö	onc 30:70			-	C	onc 35:65	-	
	Estimate	Std.	Error	t_value	Pr(> t)	Estimate	Std.	Error	t_value	Pr(> t)
(Intercept)	3.38	0.55	6.14	0.00	* * *	3.84	0.58	6.59	0.00	* * *
bmr6 vs bmr12	3.95	0.01	3.89	0.00	* * *	4.02	1.07	3.74	0.00	* * *
bmr6bmr12 vs bmr12	2.88	0.95	3.02	0.01	* *	2.63	1.01	2.60	0.01	*
Bmr vs bmr12	-1.51	0.78	-1.94	0.06		-1.88	0.82	-2.27	0.03	*
Conc_ = concentration	_		_	_		-			_	

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Appendix

The in-house script used	#cv2.destroyAllWindows()
# -*- coding: utf-8 -*-	#Converting image RGB into HSV -Gives more insights from your original image
Spyder Editor	img_hsv = cv2.cvtColor(image,cv2.COLOR_BGR2HSV)
This is a temporary script file.	#cv2.imshow("image",img_hsv)
(87)	#cv2.waitKey(0)
# -*- coding: utf-8 -*-	#cv2.destroyAllWindows()
(877)	def red_intensity_high():
Bruno's Research	lower_bound = np.array([151,87, 95])
@author: xenificity	upper_bound = np.array([166,94, 108])
"""	<pre>msk = cv2.inRange(img_hsv, lower_bound, upper_bound)</pre>
levels\n\n","Please wait!\n\n\n")	#cv2.imshow("image",msk)
#Importing libraries	#cv2.waitKey(0)
import cv2	#cv2.destroyAllWindows()
import numpy as np	height, width = msk.shape[:2]
import pandas as pd	num_pixels = height * width
import os	count_white = cv2.countNonZero(msk)
#directory=os.chdir("C:/Users/Bruno/Desktop/Leaf - Shared")	<pre>percent_high_red_pixels = (count_white/ num_pixels) * 100</pre>
directory=os.chdir("C:/Users/Bruno/Desktop/Leaf - Shared/leaf - plot 7")	<pre>percent_high_red_pixels = round(percent_high_red_pixels,2)</pre>
list_dir=os.listdir(directory)	high red pixels=count white
def identify_stain():	return
result =	num_pixels,high_red_pixels,percent_high_red_pixels
pd.DataFrame(columns=['Image',' I otal_pixels','High_red','	def red_intensity_medium():
mealum_rea;iow_rea;perc_nign_rea;perc_mea_rea;perc_	lower_bound = np.array([102,51, 0])
_low_red])	upper_bound = np.array([255,229, 204])
	msk = cv2.inRange(img_hsv, lower_bound,
#importing image -representing your original image	upper_bound)
$\operatorname{IIII} \operatorname{age} = \operatorname{Cv2.IIII} \operatorname{IIII} \operatorname{Cal}(\operatorname{Str}(I))$	#cv2.imshow("image",msk)
#cv2.imshow("image",image)	#cv2.waitKey(0)
#cv2.waitKey(0)	#cv2.destroyAllWindows()

SCREENING OF SORGHUM (SORGHUM BICOLOR L.) GENOTYPES

height, width = msk.shape[:2]

num_pixels = height * width

count_white = cv2.countNonZero(msk)

percent_medium_red_pixels = (count_white/ num_pixels) * 100

percent_medium_red_pixels =
round(percent_medium_red_pixels,2)

medium_red_pixels = count_white

return

medium_red_pixels,percent_medium_red_pixels

def red_intensity_low():

 $lower_bound = np.array([255,51,51])$

upper_bound = np.array([255,204, 204])

msk = cv2.inRange(img_hsv, lower_bound, upper_bound)

#cv2.imshow("image",msk)

#cv2.waitKey(0)

#cv2.destroyAllWindows()

height, width = msk.shape[:2]

num_pixels = height * width

count_white = cv2.countNonZero(msk)

percent_low_red_pixels = (count_white/ num_pixels) * 100 percent_low_red_pixels =
round(percent_low_red_pixels,2)

low_red_pixels=count_white

return low_red_pixels,percent_low_red_pixels

a,b,c=red_intensity_high()

d,e=red_intensity_medium()

f,g=red_intensity_low()

data = [[str(i),int(a),int(b),int(c),int(d),int(e),int(f),int(g)]]

data_new = pd.DataFrame(data,columns=['Image','Total_pixels',' High_red','m

edium_red','low_red','perc_high_red','perc_med_red','perc

_low_red'])

result = pd.concat([result,data_new])

print(result)

#print(xyz)

#print("Results are: High",result_1,"Medium",result_2,"low",result_3)

return result

#result.to_csv('C:/Users/Bruno/Desktop/Leaf Shared/results.csv')

output=identify_stain()

output.to_csv(".....")

PRODUCTION AND MARKETING CONSTRAINTS IN TURMERIC IN TELANGANA

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ABSTRACT

Turmeric (*Curcuma longa*) is an important spice crop and is popularly known as 'Yellow gold'. Turmeric plays a vital role as its ingredient curcumin is widely utilized for cosmetic, culinary, dye, and medicinal purposes. It also holds significant importance in religious ceremonies. The present study was conducted in Nizamabad, Jagtial and Nirmal districts of Telangana during the year 2021-22 with the objective of identifying the constraints faced by the turmeric farmers in production and marketing of turmeric with a sample size of 240 respondents. The respondents were interviewed personally through a well structured and pre-tested schedule and Garrett's ranking technique was used to rank the problems. The results of the study revealed that, labour shortage and high cost of labour during peak season was identified and ranked as first constraint. This was followed by high cost of FYM, lack of processing facilities, non availability of improved varieties, and inadequate access to credit *etc.*, which were identified as the major constraint followed by price fluctuations, high charges by commission agents, lack of assaying facilities, delay in payment by the marketing agencies and lack of transportation facilities. Provision of MSP, measures to stabilise turmeric prices, and provision of assaying facilities for quality certification would help the farmers to compete in international market and to fetch high prices. Hence, the study suggested for coordinated efforts of various stakeholders to address the constraints in production and marketing of Turmeric.

KEYWORDS: Constraints, production and marketing of turmeric

Turmeric (Curcuma longa L.) is an important spice crop and considered as 'Golden Spice of India'. Due to its high curcumin content, Indian turmeric is considered as the best turmeric in the world market. (Kumar et al., 2020). India is the leading producer, consumer and exporter of turmeric in the world with a global share of 80 per cent in production and 60 per cent in exports (Pushp, 2019). It holds a distinctive place in both the national and international spice markets. India exports 1.53 lakh tonnes of turmeric with a value of Rs.31.47 crores during 2021-22. In India, turmeric is cultivated nearly in 3.3 lakh ha with a production of 12.21 lakh tonnes and with an average productivity of 3668 kg ha⁻¹ (2021-22). Among the major turmeric producing states, Telangana ranks first with an area of about 0.34 lakh ha (10.30%), with production of 2.16 lakh tonnes (17.70%) and with an average productivity of 6600 kg ha⁻¹ (2021-22). Nizamabad, Jagtial and Nirmal are the major turmeric producing districts of North Telangana region of Telangana state. Though there is an increase in area and production of turmeric, in recent times there were large protests by the turmeric farmers of Telangana

state and opined that they were not able to get remunerative prices. Further, they are demanding for inclusion of turmeric under MSP. Turmeric, being a long duration (7-9 months) and labour-intensive crop, needs huge labour in production and post-harvest processing activities like boiling, drying, polishing and processing into powder before it reaches the consumer. Inadequate availability of human labour coupled with increased labour wages and cost of other inputs resulted in high cost of cultivation. On the other hand, farmers felt that due to wide fluctuation in prices and high marketing costs, they were unable to realise high returns. In this context, an attempt was made to know the constraints faced by the farmers in production and marketing of turmeric.

MATERIAL AND METHODS

For the present study, the data was collected from 240 farmers with the aid of a pre-tested schedule. In this method, the turmeric cultivators were asked to rank the constraints faced by them according to the magnitude of the problem. Rank one meant most important and last rank meant least important. Then,

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the rank assigned to each constraint by each individual farmer was converted into per cent position using the Garrett's ranking technique.

Percent position
$$=\frac{100x(R_{ij}-0.50)}{N_j}$$

Where, R_{ij} stands for rank given for the ith constraint (i= 1, 2....n) by the jth individual (j = 1, 2...n) and N_j stands for number of constraints ranked by jth individual. The percentage position of each rank thus obtained was converted into scores by referring to the table given by Henry Garrett (Garret and Woodworth, 1969). Then for each factor the scores of individual respondents were added together and divided by the total number of respondents for whom the scores were added. These mean scores for all the factors were arranged in the order of their ranks and inferences were drawn.

RESULTS AND DISCUSSION

A list of problems that the sample turmeric farmers could have experienced were identified and divided into two categories as production and marketing constraints and were ranked using Garrett's ranking technique and the results are given in Table 1.

Turmeric is labour intensive crop and requires huge labour for carrying out various farming activities such as sowing, weeding, leaf-cutting, digging, and primary processing. Majority of respondents with mean score of 77.55 expressed that shortage of labour is of prime concern particularly during the peak season as majority of labour have migrated to towns in search of better employment opportunities. This resulted in labour scarcity and thereby high cost of labour. This was ranked as first constraint by the farmers in the production of turmeric. The findings are in consistent with the research conducted by Shanmugaraja *et al.*, (2020) and Sahoo *et al.*, (2023).

Farm Yard Manure (FYM) is essential for maintaining soil fertility and promotion of crop growth. Majority of farmers with mean score of 73.58 expressed that they do not have adequate quantities of FYM which made them to purchase from other farmers. This resulted in high cost in procuring of FYM which in turn resulted in increased cost of cultivation of turmeric. Hence, this constraint was identified as second major constraint by the farmers with mean score of 73.58. The findings are in consistent with studies by Ovhar and Dhenge (2014).

Turmeric mostly requires primary processing like curing, boiling and polishing after the harvest of the crop. Sample farmers expressed that they don't have adequate processing facilities such as electrical steam boiling machines and were hiring these machines to carry out processing which resulted in high processing costs. Hence, this problem was ranked as third major constraint by farmers with mean score of 62.10. This limitation hinders the potential for value addition of turmeric, restricting market access and income for farmers. These findings are in line with studies of Sahoo and Sarangi (2018) and Sahoo *et al.*, (2023).

Access to improved varieties with higher yield potential and disease resistance is crucial for realising higher yields. Farmers in Nizamabad and

S.No	Production constraints	Score	Rank
1	Labor shortage and high cost of labour during peak season	77.55	I
2	High cost of FYM	73.58	II
3	Lack of processing facility	62.10	
4	Lack of improved varieties	57.40	IV
5	Unfavourable weather conditions	51.12	V
6	Lack of suitable machinery	47.45	VI
7	Incidence of insect pests and diseases	43.70	VII
8	Lack of technical knowledge	36.02	VIII
9	Inadequate access to credit	28.13	IX
10	Inconsistent quality of rhizomes	20.38	X

Table 1. Constraints in turmeric production

Source: primary data collected from sample farmers

surrounding districts usually grow traditional varieties, which are susceptible to rhizome rot and stated that there are no improved varieties. Farmers felt that lack of improved varieties of turmeric was one of the major problems and ranked as fourth constraint with mean score of 57.40, due to which they were unable to get the expected yields disease infestation *i.e., Dumpa Tegulu* (rhizome rot). Whenever there are heavy rains, the yields of turmeric drops down. Unsuitability of soil also limits the crop options and productivity for improved varieties. The findings are in line with the findings of Sahoo and Sarangi (2018) who reported that majority of turmeric cultivators had serious problem of lack of improved varieties.

Unfavourable weather conditions like heavy rainfall during sowing time and during rhizome formation may affect both the yield and quality of rhizomes. Hence, farmers ranked this factor of unfavourable weather conditions as fifth major constraint with mean score of 51.12.

In turmeric cultivation, most of operations like digging and hand picking of rhizomes, grading, boiling and drying are carried out by manual labour. The absence of suitable machinery results in increased labor requirements and reduced efficiency. Majority of farmers with mean score of 43.70 felt that there is need for suitable machinery to carry out different farm and primary processing operations and ranked lack of suitable machinery as sixth constraint.

Turmeric crop in Telangana is susceptible to pests like rhizome fly and diseases like leaf blotch and rhizome rot that significantly affect yield and quality. Majority of the respondents ranked this problem of incidence of pests and diseases as seventh constraint with mean score of 47.45.

Majority of farmers in the study area are not practising seed treatment of rhizomes due to lack of technical knowledge. The majority of the respondents ranked this problem as eighth constraint with mean score of 36.02. Farmers in the study area did not attend any specific training on production and processing of turmeric and used to follow neighbouring farmers. Only few of the respondents have participated on demonstrations and field days on turmeric organised by the nearby turmeric research station. Farmers hardly visited the nearby research and other resource centres. The findings are in line with the findings of Salunkhe *et al.*, (2017). Production activities of turmeric were highly influenced by the availability of institutional credit. Provision of timely credit, with easy procedures help the farmers in availing the inputs timely and thus, agricultural operations can be carried out at right time. Farmers complained that inadequate access to institutional credit, high rate of interest by money lenders and commission agents was the major problem and ranked it as ninth constraint with mean score of 28.13. Limited access to credit can hinder farmers ability to invest in inputs, machinery, and technology, thereby affecting overall turmeric productivity. This observation supports the conclusions drawn by Viraja *et al.* (2018).

The usage of farm-produced inconsistent quality rhizomes as seed material significantly affects the productivity in turmeric cultivation. Over time, if inconsistent seed material is continuously used, it can lead to a decline in soil health, increased susceptibility to diseases, and have a long-term impact on crop productivity. Thus, the problem of inconsistent quality of rhizomes as a seed material was ranked as the tenth constraint with a mean score of 20.38 by the farmers.

Constraints in turmeric marketing

The respondents were asked to express the problems that are faced by them in the marketing of turmeric and results are presented in Table 2.

From Table-2, it is observed that among the different problems faced by the turmeric farmers, lack of Minimum Support Price (MSP) for turmeric crop was viewed as most serious problem and was given first rank by the respondents with mean score of 78.55. Majority farmers felt that high cost of cultivation of turmeric and unremunerative prices made turmeric cultivation less profitable. Hence, they felt that MSP should be provided for turmeric crop. Similar results were reported by Baral *et al.* (2021).

Price fluctuations of agricultural commodities is common phenomena due to seasonality. Farmers in the study area felt that there were high price fluctuations for turmeric and they were realizing lower prices during post-harvest period due to heavy arrival of the produce in the market. Hence, high price fluctuations in turmeric was ranked as second major constraint with mean score of 70.95. This finding is in line with the study of Sravani *et al.*, (2023)
S.No	Market constraints	Score	Rank
1	Lack of Minimum Support Price (MSP)	78.55	I
2	High price fluctuation of turmeric	70.95	II
3	High charges by commission agents	62.50	III
4	Lack of assaying facilities	53.88	IV
5	Delay in payment	49.83	V
6	Inadequate storage facilities	46.30	VI
7	High marketing costs	35.70	VII
8	Lack of marketing information	33.30	VIII
9	Lack of transportation facilities	19.00	IX

Table 2. Constraints related to turmeric marketing

Source: primary data collected from sample farmers

The problem of high commission charges was ranked as third constraint by the respondents with mean score of 62.50. Usually, the commission agentscum-traders formed in the market would syndicate during the peak arrivals and try to lower the prices offered for farmers, and charge higher prices for the retailers for the same produce. This mutual agreement between the agents caused the farmers to frequently sell their produce at throwaway prices, causing them to incur significant losses. This finding is in line with the results of Sahoo and Sarangi (2018).

Assessing the quality of turmeric is important because the spice's flavour, aroma and health benefits can vary depending on various factors, including its source, processing, and storage conditions. Curcumin is the active compound responsible for many of turmeric's health benefits and lack of quality assessment facility at market yards was ranked as fourth constraint with mean score of 53.88 by the respondents. This finding is in line with the results of Baral *et al.*, (2021)

The delay in payment is a major concern in marketing of turmeric. This problem was ranked as fifth constraint by the respondents with mean score of 49.83. Farmers in the study area felt that there was delay in receiving payments for their agricultural produce from marketing agencies, such as commission agents, and traders. Timely payments not only ensure that farmers receive fair compensation for their hard work but also contribute to the stability and growth of rural economies.

Inadequate storage facilities was ranked as sixth major constraint by the respondents with mean

score of 46.30. Due to the lack of storage units in the villages, most of the small and medium farmers were unable to store their produce. Whereas, large farmers and traders who retained the produce in cold storages were able to get good price for their produce. The findings are in agreement with the findings of Sahoo *et al.*, (2023) who reported that lack of storage facility was main constraint in turmeric marketing.

High marketing costs, including charges related to loading and unloading such as hamali charges was ranked as seventh constraint by the respondents with mean score of 35.70 in marketing of turmeric. High marketing costs can have a profound impact on the profitability of Turmeric farmers.

Access to accurate market information is critical for farmers to make informed decisions about when and where to sell their produce. Efforts to provide farmers with better market information can improve their market participation. Farmers in the study area felt that due to lack of market information, they were unable to realize better price. Thus, lack of market information was ranked as eighth constraint by the respondents with mean score of 33.30. These findings are in accordance with Dhruw *et al.*, (2018).

High cost of transportation was ranked as ninth constraint among all constraints faced by the turmeric farmers with mean score of 19.00. Farmers in the study area felt that due to increase in fuel prices during covid period, they were forced to sell the turmeric in the local market instead of transportation to distant markets like Sangli, Erode *etc.* and could not fetch better prices.

CONCLUSION

The study highlights constraints faced by turmeric farmers in production and marketing of turmeric in Telangana, particularly in Nizamabad, Jagtial and Nirmal districts of North Telangana. The production constraints include labor shortage and high labor costs during peak season, insufficient FYM, absence of processing facilities, lack of improved turmeric varieties, and unfavourable weather conditions. Marketing constraints include the absence of MSP, high price fluctuations, high commission charges, delayed payment, inadequate storage facilities, high marketing costs, limited transportation and lack of marketing information. To address these constraints, policymakers, agricultural authorities, and industry stakeholders should have collaborative efforts in bringing turmeric under MSP ambit and measures to stabilize prices, regulate commission charges, improving storage and transportation facilities, and promoting quality certification. Provision of extension services and training programs would equip farmers with the necessary knowledge and skills. Addressing these constraints is vital for the sustainability and prosperity of turmeric farming in Telangana and could contribute to the state's agricultural growth and the wellbeing of turmeric farmers.

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ADOPTION OF ANGRAU RECOMMENDED REDGRAM PRODUCTION TECHNOLOGY BY THE FARMERS OF GUNTUR DISTRICT OF ANDHRA PRADESH O. SARADA¹ and G. V. SUNEEL KUMAR²

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ABSTRACT

The study analyzed the adoption of recommended redgram production techniques among farmers in Guntur district during 2022-23. Findings revealed that while farmers largely followed advice on soil, spacing, and harvesting, adoption rates were lower for fertilizer and water management, seed treatment, disease and pest management, intercropping, and sowing time. Lack of awareness, confidence, and influence from dealers and neighboring farmers were cited as primary reasons for non-adoption. Positive correlations were found between farmers' education, experience, extension contacts, and training with their adoption levels. Farmers cited constraints such as Maruca and pod fly infestations, low yields, wilt, high cultivation costs, and water scarcity during critical stages.

KEYWORDS: Adoption, redgram, ANGRAU recommended production technology, correlation, constraints

India is the largest producer (25 per cent of global production), consumer (27 per cent of world consumption) and importer (14 per cent) of pulses in the world (Chavan et al., 2017). India accounts for about 80 per cent of the total world redgram production. India ranks first in redgram production globally with 42.8 lakh tonnes cultivated under 48.24 lakh hectares with productivity of 887 kg/hectare in 2020-21 (agricoop.nic.in). It is one of the principal dry land crops in Andhra Pradesh with a very low productivity (601 kg ha⁻¹). Redgram is an important pulse crop of 170 days duration mainly sown in kharif and rabi and harvested in December. It is best suited to areas having low to moderate rainfall. The production is constrained by the use of less productive land, water logging or dry spells during critical stages of crop growth, spotted pod borer, pod fly and wilt problems, and lack of appropriate agronomic management. Andhra Pradesh produced 0.84 lakh tonnes contributing 1.96% to total India's production cultivated in an area of 2.31 lakh hectares with 363 kg/hectare productivity in 2020-21 (Final Estimates). According to 2nd advance estimates during 2021-22, redgram was grown in 2.51 lakh hectares with a production of 1.21 lakh tonnes and productivity was 482 kg/ha. In Guntur district, redgram is cultivated in 20,000 ha. with a production of 15,000 tonnes and with highest (729 kg/ha.) productivity in Andhra Pradesh state. (ANGRAU Redgram Outlook Report-January to December, 2021)

Redgram crop occupy considerable area in Guntur district, keeping the importance of this crop cultivation in the district, a need was felt to analyse and understand the adoption pattern of recommended technology. In this context, an attempt was made by the Extension department of Regional Agricultural Research Station, Lam, Acharya N.G.Ranga Agricultural university during the year 2022-23 to study the extent adoption of ANGRAU recommended Redgram production technology by the farmers of Guntur district, reasons for non-adoption, relationship between profile characteristics of farmers and their adoption and constraints in production.

MATERIAL AND METHODS

The present investigation was conducted during the year 2022-2023 by the extension department of Regional Agricultural Research Station, Lam, Guntur. Multistage sampling design has been adopted for selection of sample. Five mandals with highest redgram areas were selected purposively for the study. From each mandal, two villages with highest redgram areas were purposively selected. From each village, 10 farmers were randomly selected thus making the sample size to 100 redgram cultivating farmers. The data were collected through personnel interview, tabulated and analysed to find out the results and draw the conclusions. The results were analysed with the help of different statistical tools such as frequency, percentage rankings and correlation analysis.

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Extent of adoption of recommended cultivation practices of Redgram was measured considering the ANGRAU recommended Redgram production technology. Adoption was studied with respect to recommended variety, sowing time, soils, seed rate, spacing, seed treatment, water management, fertilizer management, weed management, pest management, disease management and post-harvest management. Where ever considerable deviation was observed with respect to adoption of recommended practices, respondents were asked for reasons for non-adoption and they were tabulated using frequencies and percentages. Correlation analysis was carried out to understand the relationship between farmers' selective personal, socio-economic and psychological characteristics with their adoption of recommended package of practices. To know the prominent constraints in Redgram cultivation, open ended question was posed to farmers and based on their response constraints were categorized and tabulated using frequency and percentage.

RESULTS AND DISCUSSION

Adoption pattern of recommended Redgram production technology by the farmers

It could be noticed from Table 1 that cent per cent of the Redgram farmers adopted soils, spacing and harvesting as recommended by the ANGRA University. Great majority of the farmers adopted varieties (91.00%), seed rate (80.00%) and weed management (55.00%). But majority of the farmers were not adopting fertilizer management (93.00%), water management (89.00%), seed treatment (88.00%), disease management (77.00%), intercrops (72.00%), sowing time (68.00%) and pest management (62.00%). With respect to soils, spacing and varieties, similar results were reported by Khairnar et al., 2019 in their study on Economic impact of redgram production technology on farm productivity and income in Western Maharashtra. The findings with respect to fertilizer management, irrigation and seed treatment were in conformity with the results of Mohd. Riyaz et al., 2020

Reasons for non-adoption of ANGRAU recommended Redgram package of practices

Sowing time: It is evident from Table 2 that, delayed monsoon was the major reason for deviation in sowing time (66.00%).

Seed treatment : Lack of awareness was the first and foremost reason for non-adoption of seed treatment as expressed by sixty one per cent of farmers, followed by lack of confidence on the practice (32.00%) and non-availability of Trichoderma at the time of sowing (28.00%). Hence to create awareness and to establish confidence, State Department of Agriculture needs to organize demonstrations at village level and ensure availability of Trichoderma at the time of sowings through RBK system.

Fertilizer management: With respect to fertilizer management, non- availability of FYM (74.00%) was the reason for not using FYM as recommended. To overcome this issue, introducing shredders to incorporate redgram plants after harvesting may help to add organic matter to soils to some extent. More than fifty per cent of the farmers were using higher level of fertilizers than recommended due to the assumption, that as their soils are low fertile more fertilizers are required. Other side, forty five per cent of the redgram famers were not at all applying any fertilizer to redgram crop with a confidence that fertilizers applied for tobacco or chilli during the previous year are sufficient for redgram. Some of the farmers due to lack of awareness on exact fertilizer recommendation for redgram crop (23.00%) were not adopting as recommended. In order to address this issue, Integrated Nutrient Management in redgram demonstrations using soil analysis results may convince the farmers to adopt required fertilizers to realize optimum yields.

Water management: Even though redgram is cultivated under rain fed situation, in case of continuous dry spells, delayed sowings and rabi cultivation one or two light irrigations are recommended for redgram. But great majority (83.00%) of the farmers failed to provide irrigations even at critical stages due of nonavailability of irrigation water.

Weed management: Sixty one per cent of the farmers were not using herbicides as the redgram crop cultivation is not remunerative with poor yields, in order to reduce the cost of cultivation they were avoiding herbicide use. Some of the farmers (32.00%) were not adopting weed management as recommended as they were not aware of herbicides recommended for redgram crop.

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Intercrops: Top most reason for avoiding intercrops in redgram was wild boar problem (65.00%), followed by difficulty in inter cultivation (41.00%), yields are effected when intercropped with bajra (38.00%) and difficulty in harvesting (29.00%). With these reasons farmers are preferring redgram as solo crop.

Pest management: Influence of dealers and neighbours was the prime reason for deviation in pest management by the farmers (71.00%). Sixty eight per cent of the farmers were using combination chemicals instead of recommended pesticides. Lack of awareness on pests identification was also one of the major reasons for improper pest management in case of almost fifty per cent of the farmers.

Disease management: Almost seventy per cent of the farmers were using unnecessary fungicides due to lack of awareness on disease management, particularly in case of Sterility Mosaic Disease (SMD) and wilt. Sixty per cent of the farmers were still using locally available varieties as they were not aware of disease resistant or tolerant varieties. Hence introduction of SMD and wilt resistant varieties is the need of the hour to sustain redgram cultivation in pandemic areas.

Based on the understanding of the reasons for the non-adoption of recommended redgram technology, it can be inferred that, majority of farmers lack awareness on crucial practices such as seed treatment, fertilizer management, weed, pest, and disease management. This indicates a significant extension gap in the study area. Consequently, the Department of Agriculture should undertake concerted efforts to bridge this gap through robust awareness programs and field demonstrations. These initiatives aim to enhance knowledge levels among farmers, instill confidence in recommended production techniques, and ultimately boost yield potentials in redgram crops. By improving awareness and demonstrating the effectiveness of recommended practices, farmers can be empowered to adopt these technologies and enhance their agricultural productivity.

Relationship between profile of Redgram Farmers with their Adoption

The findings from Table 3 indicate a significant positive relationship between the education level and farming experience of redgram farmers and their adoption of recommended practices, at a significance level of 5%. Additionally, extension contacts and training undergone showed a highly significant positive relationship with adoption, at 1% significance level.

This result suggests that education, farming experience, extension contacts, and trainings

n=100

Table 1. Adoption pattern of recommended Redgram production technology by the farmers

S.No	Adoption	Adopted		Not A	dopted
		Frequency	Percentage	Frequency	Percentage
1	Varieties	91	91.00	9	9.00
2	Seed rate	80	80.00	20	20.00
3	Sowing time	32	32.00	68	68.00
4	Soils	100	100.00	0	0.00
5	Spacing	100	100.00	0	0.00
6	Seed treatment	12	12.00	88	88.00
7	Fertilizer management	7	7.00	93	93.00
8	Water management	11	11.00	89	89.00
9	Weed management	55	55.00	45	45.00
10	Intercrops	28	28.00	72	72.00
11	Pest management	38	38.00	62	62.00
12	Disease management	23	23.00	77	77.00
13	Harvesting	100	100.00	0	0.00

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			n=	-100
S.No	Technology recommendation		Frequency	Percentage
1	Sowing time	Delayed monsoon	66	66.00
2	Seed treatment	Lack of awareness	61	61.00
		Lack of confidence	32	32.00
		Non availability of Trichoderma at the time of sowing	28	28.00
3	Fertilizer management	With a confidence that fertilizers applied for tobacco or chilli are sufficient for redgram	45	45.00
		Lack of awareness	23	23.00
		With an assumption that as their soils are low fertile more fertilizers are required	51	51.00
		Non availability of FYM	74	74.00
4	Water management	Non availability of irrigation water due to complete rainfed situation	83	83.00
5	Weed management	Lack of awareness on herbicides use	32	32.00
		To reduce cost of cultivation	61	61.00
6.	Intercrops	Wild boar problem	65	65.00
		yields are affected when intercropped with bajra	38	38.00
		Difficulty in inter cultivation	41	41.00
		Difficulty in harvesting	29	29.00
		Bird damage	33	33.00
7	Pest management	Using combination of pesticides	68	68.00
		Lack of awareness on pests identification	49	49.00
		Dealers and neighbor farmers influence	71	71.00
8	Disease management	Lack of awareness on SMD initial symptoms and management	46	46.00
		Lack of confidence on Trichoderma soil application	31	31.00
		Lack of awareness on disease management, using unnecessary fungicides	69	69.00
		Using locally available varieties	60	60.00

Table 2. Reasons for non-adoption of ANGRAU recommended Redgram package of practices

undergone play vital roles in enhancing farmers' knowledge of the latest technological advancements, thereby increasing the likelihood of adoption in their fields. Given the critical importance of extension contacts and training in driving adoption, there is a need to prioritize efforts to encourage farmers to engage more extensively

with extension services and participate in capacitybuilding programs at the village level. By facilitating greater access to extension services and providing comprehensive training opportunities, agricultural authorities can empower farmers with the knowledge and skills necessary to effectively implement

S. No	Independent variable	Correlation co-efficient ('r')
1	Education	0.209 *
2	Land holding	0.152 NS
3	Farming experience	0.251 *
4	Economic motivation	0.142 NS
5	Social participation	0.157 NS
6	Extension contact	0.412 **
7	Risk orientation	0.106 NS
8	Trainings undergone	0.363 **

Table 3. Relationship between profile of Redgram Farmers with their Adoption

* and ** indicate significance of values at P=0.05 and 0.01 respectively NS = Non -significant

Below fifty per cent of redgram farmers felt severe incidence Sterility Mosaic Disease (48.00%), severe flower drop (45.00%), untimely rains (42.00%), no procurement by the Government (40.00%) and low Minimum Support Price (33.00%) were the constraints in redgram cultivation.

The constraints identified underscore the challenges associated with cultivating redgram in poor soils coupled with inadequate management practices, leading to poor yields and increased cultivation costs. Specifically, addressing pest and disease management requires a concerted effort from extension functionaries to bridge the existing extension gap. This can be achieved by organizing a greater number of capacitybuilding training programs and demonstrations, alongside introducing resistant or tolerant varieties.

n=100

S.No	Constraint	Frequency	Percentage	Rank
1	Increased Maruca incidence	81	81.00	I
2	Poor yields due to pests and diseases	77	77.00	П
3	Increased Pod fly incidence	69	69.00	111
4	Severe wilt incidence	65	65.00	IV
5	Increased cost of cultivation	64	64.00	V
6	Non- availability of irrigation water at critical stages	62	62.00	VI
7	Severe Sterility Mosaic Disease incidence	48	48.00	VII
8	Severe flower drop	45	45.00	VIII
9	Untimely rains	42	42.00	IX
10	No procurement by the Government	40	40.00	Х
11	Low Minimum Support Price	33	33.00	XII

Table 4. Constraints experienced by the farmers in Redgram cultivation

recommended redgram production technologies. This, in turn, can lead to improved agricultural productivity and economic outcomes for farmers.

Constraints experienced by the farmers in Redgram cultivation

It is evident from Table 4 that majority of the redgram farmers were experiencing constraints like increased *Maruca* incidence (81.00%), poor yields due to pests and diseases (77.00%), increased pod fly incidence (69.00%), severe wilt incidence (65.00%), increased cost of cultivation (64.00%) and non-availability of irrigation water at critical stages (62.00%).

Moreover, government intervention is crucial in facilitating farmers by ensuring reasonable Minimum Support Prices (MSP) and timely procurement. By providing farmers with adequate support and resources, policymakers can alleviate some of the financial burdens associated with redgram cultivation, thereby promoting sustainable agricultural practices and improving overall crop yields. Vijaya Preethi and Uma Devi (2019), Ashokkumar Bansilal *et al.*, 2020 and Sumitra Singh *et al.*, 2022 in their study reported, increased cost of cultivation, pests and disease incidence were some of the major constraints expressed by the farmers in redgram cultivation.

CONCLUSION

The study indicates a near-complete adoption of basic practices such as soil management, spacing, and harvesting, suggesting that farmers are receptive to fundamental agricultural guidance. While there is substantial uptake of certain practices like selecting varieties and managing seed rates and weeds, there is notably low adoption of crucial practices such as fertilizer and water management, seed treatment, disease and pest management, intercropping, and timely sowing. Lack of awareness, confidence, and influence from dealers and neighbouring farmers emerge as key barriers to adopting recommended technologies. Addressing these barriers could potentially increase adoption rates. The study identifies a positive relationship between education, farming experience, extension contacts, and training with adoption levels. This suggests that targeted education and training programs could enhance adoption rates. Farmers cited various challenges including Maruca and pod fly infestations, low yields, wilt, high cultivation costs, and water scarcity during critical stages. Addressing these constraints through improved pest and disease management and cost-effective agricultural practices could improve overall productivity and profitability. In conclusion, while there are encouraging signs of adoption for certain practices, there is a clear need for targeted interventions to address barriers and constraints hindering the adoption of recommended technologies. This could involve tailored educational programs, extension services, and support systems to empower farmers and enhance agricultural productivity and resilience in Guntur district.

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DIETARY PATTERN OF SMALL AND MARGINAL FARM FAMILIES IN ADILABAD DISTRICT OF TELANGANA STATE

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ABSTRACT

Food plays a critical role in sustaining individuals' health and overall well-being. By keeping the importance of nutrition in daily life the study was formulated. Objectives of the study were to study the profiles of men and women from small and marginal farm families and analyse their dietary pattern. An ex post facto research design was used for the study in Telangana State. Only Adilabad District was purposively selected from Telangana. Among eighteen mandals of Adilabad District, Jainad and Bela mandals were randomly selected. From small and marginal farm families, either a man or a woman was randomly selected, resulting in a sample size of 120 respondents, comprising 60 men and 60 women. The study focused on fifteen independent variables and one dependent variable. The results indicated that most (60.00%) of the people were middle aged, had primary level of education (42.50%), agriculture as an occupation (60.00%) and annual income of 50,000 to 1,50,000 Rs. (74.99%). Exactly half (50.00%) of them were male and female, belonged to nuclear families (70.00%), engaged in cotton cropping (50.00%), had two earners in the family (60.83%) and were living in proximity of <5 kms (50.00%). They had no milch animal (75.00%), debt of >30,000 Rs. (68.33%), medium health orientation (60.00%) and moderate health status (63.33%). Additionally, wives predominantly made decisions about food (50.00%) and wholesale shops were the preferred choice for food purchases (63.33%). Half (50.83%) of the people had insufficient quantity of diet, medium frequency of diet intake (51.66%), sourced their diet diversely (55.83%), spent moderately on their diet (55.00%), stored their food moderately properly (45.00%), engaged in high physical activity (70.83%), and maintained a fair dietary pattern (45.83%). In males, the higher diet quantity difference was found in milk and milk products (190 gr), followed by cereals (150 gr), roots and tubers (75 gr), and other vegetables (75 gr). In females, the higher diet quantity difference was found in milk and milk products (220 gr), followed by cereals (100 gr), roots and tubers (100 gr), and other vegetables (90 gr). Half (54.16%) of the respondents consumed 15%-50% less the recommended quantity of cereals (54.16%) and pulses (52.50%). All (100%) of them consumed cereals pulses, milk and milk products and sugars regularly. Diversity of diet was found in cereals, fruits, other vegetables and green leafy vegetables. Education (0.3452 **), occupation (0.2409 **), number of persons earning (0.2284 **), milch animals (0.3061 **), health orientation (0.3342 **), decision making about food (0.2650 **) and preference of shops (0.2986 **) were found positive and highly significantly associated with dietary pattern. Annual income (0.2184 *), cropping pattern (0.2340 *), and health status (0.1958 *) were found positive and highly significantly associated with dietary pattern. Whereas, gender (-0.1992 *), distance to market (-0.1980 *) and debt (-0.1795 *) were found negative and significantly associated with dietary pattern.

KEYWORDS: Nutritional security, food security, food habit and food consumption

Food plays a critical role in sustaining individuals' health and overall well-being. The right to food is considered as fundamental as the right to life itself, emphasizing its importance in ensuring human dignity and survival. Food security entails ensuring that individuals have consistent access to adequate, nutritious food that caters to their dietary requirements for maintaining a healthy and active lifestyle. This includes considerations of physical, social, and economic factors that influence access to food. Dietary practices vary across regions globally, shaped by local food availability and cultural norms. Over the years, there has been a notable increase in the overall production of food grains such as cereals, millets, and pulses. However, while cereals and millets production seem to meet demand, there's a concerning decline in pulse production, which serves as a crucial protein source for rural populations, highlighting an area of vulnerability (National Institute of Nutrition, 2011). According to the 2022 Global Food Security Index, India ranked 68th among 113 countries, indicating some level of food security with a score of 58.9 (Economist Impact, 2024). However, the 2023 Global Hunger Index paints a different picture, ranking India 111th out of 125 countries, with a score of 28.7, indicating significant challenges regarding hunger and food insecurity within the country (Global Hunger Index, 2024). Hunger levels are affected by structural conditions, shocks and crises,

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levels of inequity and poverty, economic downturns, quality of governance, climate extremes, demographic conditions and conflicts. The crises have exacerbated disparities among regions, nations, and social groups. Areas, countries, and communities with lower resilience are likely to suffer from persistent issues with hunger and nutrition and are less equipped to handle future emergencies. While the global impacts of the COVID-19 pandemic, the Russia-Ukraine conflict, and elevated food costs may be moderating as of 2023, climate conditions continue to deteriorate, and food remains prohibitively expensive for many populations worldwide. Low- and middle-income countries have borne a disproportionate impact. Factors like inequality, fragility of state institutions, inadequate governance, and persistent poverty hinder these countries' recovery from multiple crises. Young people in low- and middleincome countries are particularly at risk from food insecurity and nutritional crises. They are currently grappling with these challenges and will likely bear the brunt of future disasters if immediate and coordinated measures are not implemented (Global Hunger Index, 2024). In September 2022, the cost of food in India rose by 8.6% compared to the previous year, marking the highest increase since November 2020. Vegetables saw a significant rise of 18.05%, spices increased by 16.88%, and cereals recorded an 11.53% increase, marking their highest increases in nine years (Ipe et al., 2022). The National Food Security Act, 2013 represents a significant shift in food security strategy, moving from a welfare-based to a rightsbased approach. NFSA provides coverage to 75% of the rural population and 50% of the urban population through the Antyodaya Anna Yojana and Priority Households (Ipe et al., 2022). As per NFHS data, in India, there is a higher prevalence of stunted children in rural areas (37.3%) compared to urban areas (30.1%). To break the inter-generational cycle of malnutrition, effective interventions targeting both mothers (pre- and post-pregnancy) and children are crucial to address the significant burden of stunting. The problems found in India are stunting, wasting, underweight, micro nutrient deficiency, anaemia, double burden of malnutrition and rising obesity (lpe et al. 2022). Keeping the recent past scenario on hunger status, rising nutritional imbalances and importance of balanced food consumption a special study on "Dietary Pattern of Small and Marginal Farm Families in

Adilabad District of Telangana State" is planned with following hypotheses and objectives. To investigate the research objectives, a null hypothesis formulated, assuming no relationship between the profile of men and women from small and marginal farm families and their dietary pattern.

Objective

To study the profile of men and women from small and marginal farm families

To analyse the dietary pattern of men and women from small and marginal farm families

To find out association between the profile of men and women from small and marginal farm families with their dietary pattern.

MATERIAL AND METHODS

The research study was conducted during the year 2024. An expost facto research design was used. The study was carried out in Telangana State. Adilabad District was purposively selected as the research scholar hails from the district. Among the eighteen mandals of Adilabad District, two mandals viz., Jainad and Bela were selected randomly. From bela mandal, two villages viz., Bhedoda and Khogdur and from Jainad mandal, two villages viz., Pippalgaon and Pendalwada were selected randomly. Thus, total two mandals and four villages were selected for the study. The study targeted individuals from small and marginal farm families, so from each family, either a man or a woman was selected randomly. Thus, from each village 15 men and 15 women respondents were selected randomly. The sample size was 120 respondents, representing a total of 60 men and 60 women. A total of fifteen independent variables and one dependent variable was selected for the study.

Dietary pattern, defined as the extent to which men and women respondents has consistent and equitable access to healthy, safe, affordable foods essential to optimal health and well-being. For studying the dietary pattern, a structure schedule was developed. For studying dietary pattern, six components were identified. Those were quantity of diet, frequency of diet, source of diet, expenditure on food, storage of food and physical activity. The number of items under each component varies. The items under each component were carefully framed with the help of review of literature and discussions with experts in nutrition, diet and other extension personnel. In the quantity of diet, the score three, two and one was assigned for recommended diet, 1-15 per cent less than the recommended and 15-50 per cent less than the recommended for each food item, respectively. In frequency of diet, the scores three, two and one were assigned for regular (5-7 days a week), sometimes (3-4 days a week) and rarely (once a week or while), respectively. In source of diet, scores two and one were assigned for different source/form and same source/form in a week. In expenditure on diet, scores three, two and one were assigned for more than average, average and less than average, respectively. In storage of food, scores two and one were assigned for practiced and not practiced. In physical activity, score one was assigned for each activity carried out. Individual total score was computed by summing the scores of all the items. The maximum and minimum obtained scores were identified. The respondents were categorized into following three groups based on inclusive class interval method. The correlation coefficient was calculated to examine the relationship between the independent and dependent variables. Further, ttest was calculated for testing of significance of the correlation coefficient at 1 per cent and 5 per cent level of significance.

An interview schedule was prepared in line with the objective of the study and it was pre tested in sampling area with non-sampling respondents. The required changes were made and the schedule was corrected as per the requirements. Data was collected by the researcher with personal interview method. The data collected was coded and tabulated for statistical analysis. Statistical tests viz., frequency, percentage, arithmetic mean, correlation coefficient and t-test for testing of significance of the correlation coefficient were employed.

RESULTS AND DISCUSSION

Profile of men and women from small and marginal farm families

A brief description of the profile of male and female respondents of small and marginal farm families is given below. Result revealed that majority (60.00%) of the respondents belonged to middle age group, followed by old age group (20.83%) and young age group (19.16%). The lower number of young respondents could be attributed to their ownership of small and marginal lands and their inclination towards urban employment due to perceiving farming as a tedious job. Transitioning out of farming due to various reasons, such as physical inability to work and delegating farming responsibilities to their offspring or leasing out their land. Two-fifths of respondents had education up to primary school (42.50%), followed by middle school (25.00%), illiterate (13.33%), can read and write (10.00%), intermediate (5.00%) and high school (4.16%). Since, the majority of them had education below middle school level, the reasons could be attributed to the availability of schools in their locality was up to middle school at that time. The majority (60.00%) of the respondents had agriculture as their occupation, followed by Agriculture + livestock (30.00%), Agriculture + Business/service/employee (10.00%). The reason for this distribution could be the availability of resources, which might have compelled them to engage solely in farming. Some respondents engaged in both agriculture and livestock to leverage the benefits of complementary farming. Slightly more than one-third (38.33%) of the respondents belonged to annual income group of 50,000 - 1,00,000 rupees, followed by 1,00,000 - 1,50,000 rupees (36.66%), 1,50,000 – 2,00,000 rupees (11.66%), up to 50,000 rupees (8.33%) and above 2,00,000 rupees (5.00%). The higher number of respondents in lower annual income groups could be attributed to their limited resources, land size, and farming being their sole occupation. Half (50.00%) of the respondents were male and half were female (50.00%). This distribution could be due to the sample selection process conducted by the researcher.

The majority (70.00%) of respondents belonged to nuclear families, followed by a smaller percentage belonging to joint families (30.00%). Half (50.00%) of the respondents solely cultivated cotton, while the remaining respondents cultivated cotton alongside sorghum (25.00%) or soybean alongside Bengal gram (25.00%). The preference for growing cotton alone may be attributed to the predominant reliance on rainfed farming in the area, with those having access to irrigation facilities opting for subsequent crops. A majority (60.83%) of the respondents hailed from families where two individuals were earners, followed by single earners (28.33%) and households with earners more than two individuals (10.83%), possibly

Table 1. Distribution of respondents according to	o profile
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	· · · · · · · · · · · · · · · · · · ·		(n:	=120)
S. No.	Variable	Categories	Frequency(f)	Percentage(%)
1	Age	Young (<30)	23	19.16
		Middle (31-50)	72	60.00
		Old (>50)	25	20.83
2	Education	Illiterate	16	13.33
		Can read and write	12	10.00
		Primary	51	42.50
		Middle school	30	25.00
		High school	5	4.16
		Intermediate	6	5.00
3	Occupation	Agriculture	72	60.00
		Agriculture + livestock	36	30.00
		Agriculture + Business/ service/employee	12	10.00
4	Annual income	< 50,000 Rs	10	8.33
		50,000 - 1,00,000 Rs	46	38.33
		1,00,000 - 1,50,000 Rs	44	36.66
		1,50,000 – 2,00,000 Rs	14	11.66
		>2,00,000 Rs	6	5.00
5	Gender	Male	60	50.00
		Female	60	50.00
6	Family type	Nuclear	84	70.00
		Joint	36	30.00
7	Cropping pattern	Cotton	60	50.00
		Cotton + Sorghum	30	25.00
		Soybean + Bengal gram	30	25.00
8	Number of	1	34	28.33
	persons earning	2	73	60.83
		>2	13	10.83
9	Distance to market	< 5 km	60	50.00
		5 - 15 km	60	50.00
10	Milch animals	0	90	75.00
		1-2	18	15.00
		2 - 4	8	6.66
		4	4	3.33
11	Debt	< 10,000 rupees	14	11.66
		10,000 – 30,000 rupees	24	20.00
		>30,000 rupees	82	68.33

S. No.	Variable	Categories	Frequency(f)	Percentage(%)
12	Health orientation	Low	34	28.33
		Medium	72	60.00
		High	14	11.66
13	Health status	Poor	30	25.00
		Moderate	76	63.33
		Good	14	11.66
14	Decision making	Head of the family	15	12.50
	about food	Husband	25	20.83
		Wife	60	50.00
		Both husband and wife	20	16.66
15	Preference of shops	Retail shop	34	28.33
		Wholesale shop	76	63.33
		Marts	10	8.33

Table 1. contd..

indicating the involvement of both spouses in agricultural activities. Half (50.00%) of the respondents resided within a 5 km radius from the market, followed by with the remaining respondents living within a proximity of 5 - 15 km (50.00%). The reason could be the location of their village from towns and mandals. Majority (75.00%) of the respondents did not own milch animals, while the remaining had 1-2 milch animals (15.00%), 2-4 milch animals (6.66%) and (3.33%).

The majority (68.33%) of respondents had debts above 30,000 rupees, followed by those with debts ranging between 10,000 - 30,000 rupees (20.00%). Only a few (11.66%) had debts below 10,000 rupees. This trend may be attributed to lower annual incomes, leading to additional financial requirements for farming and maintenance purposes. The majority (60.00%) of respondents exhibited a medium level of health orientation, followed by those who showed low health orientation (28.33%) and high health orientation (11.66%). This distribution could be attributed to a lack of awareness regarding the importance of maintaining a healthy lifestyle. The majority (63.33%) of respondents exhibited a moderate health status, followed by those who showed poor health status (25.00%) and good health status (11.66%). In half (50.00%) of respondents' households, decisions regarding food choices were made by the wife (50.00%), followed by the husband (20.83%), both husband and wife (16.66%), and the head of the family

(12.50%). This could be due to the involvement of women in cooking, thus influencing decision-making in this regard. The majority (63.33%) of the respondents preferred shopping at wholesale shops, followed by retail shop (28.33%) and marts (8.33%). The reason could be that the mandals were near to their village as compared to the towns and the availability of multiple items at cheaper prices at wholesale shops as compared to the retail shops. These findings align with previous studies conducted by Mahmoud and Daoud (2023), Wetal *et al.*, (2023), and Hamba *et al.*, (2024).

Dietary pattern of respondents

Dietary pattern of respondents had been studied with the help of six components viz., quantity of diet, frequency of diet, source of diet, expenditure on food, storage of food and physical activity. By summing up the scores of all the components dietary pattern was obtained. The results had indicated that half (50.83%) of the respondents had insufficient quantity of diet, followed by moderate quantity of diet (29.16%) and sufficient quantity of diet (20.00%). The probable reason could be the low awareness regarding the healthy diet. Half (51.66%) of the respondents had followed medium level of frequency of diet, followed by low level of frequency (28.33%) and high level of frequency (20.00%). The reason could be that they were not having their own milch animals, unavailability of fruits in the locality and the lower annual income

			(
S. No.	Components	Categories	Frequency(f)	Percentage(%)		
1	Quantity of diet	Insufficient	61	50.83		
		Moderate	35	29.16		
		Sufficient	24	20.00		
2	Frequency of diet	Low	34	28.33		
		Medium	62	51.66		
		High	24	20.00		
3	Source of diet	Similar	14	11.66		
		Moderately diverse	36	30.00		
		Diverse	67	55.83		
4	Expenditure on food	Low	37	30.83		
		Medium	66	55.00		
		High	17	14.16		
5	Storage of food	Improper	18	15.00		
		Moderately proper	54	45.00		
		Proper	48	40.00		
6	Physical activity	Low	0	0.00		
		Moderate	35	29.16		
		High	85	70.83		
7	Overall dietary pattern	Poor	42	35.00		
		Fair	55	45.83		
		Good	23	19.16		

	Table 2. Distribution	of respondents	according to diet	pattern
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might not have allowed them to consume fruits, milk *etc.* on daily basis. More than half (55.83%) of the farmers had followed diverse type of diet, followed by moderately diverse (30.00%) and similar type of diet (11.66%). The probable reason could be their limited land holding, cropping pattern followed by them, cultural reason and lower annual income levels.

Slightly more than half (55.00%) of the respondents had medium expenditure on food, followed by those who had low expenditure on food (30.83%) and high expenditure on food (14.16%). The reason could be limited land holding, lower level of educational status, lack of knowledge regarding a healthy diet, unavailability of food items in the locality *etc.* Less than half (45.00%) of the respondents had moderately proper storage of food followed by, those who had proper storage of food (40.00%) and improper storage of food (15.00%). The probable reason for such result could

following hygiene and another reason could be the drudgery involved in farming and multiple roles performed by them might not allowed them to draw their attention on proper storage. Majority (70.83%) of the respondents had high physical activity, followed by moderate level of physical activity (29.16%). The reason for such result was all of the respondents were belonged to farm family and actively engaged in farming. Slightly less than half (45.83%) of the respondents had fair level of dietary pattern, followed by those with poor dietary pattern (35.00%) and good dietary pattern (19.16%). The reason could be the lack of awareness, limited sources, unavailability, unaffordability and lack of time to give attention to diet. The results are in line with the findings of Manikanta and Satpathy (2023), Elinur et al., (2024), Sanz-Martín et al., (2024) and Yildirim et al., (2024).

be that they were not aware of negative effects of not

(n=120)

Quantity of food consumed by men and women

The results presented in Table 3 indicate that men were consuming quantities of food below the recommended diet for individuals engaged in heavy activity. Men exhibited deficits of 150 grams in cereals, 60 grams in pulses, 190 grams in milk and milk products, 75 grams in roots and tubers, 40 grams in green leafy vegetables, 75 grams in other vegetables, 40 grams in sugar and 20 grams in fats. Only fruits were consumed in sufficient amounts, with consumption typically occurring once a week. Regarding cereals, In the case of women, they were also consuming a deficit quantity of food compared to the diet recommended for women engaged in heavy activity. Women exhibited deficits of 100 grams in cereals, 50 grams in pulses, 220 grams in milk and milk products, 100 grams in roots and tubers, 50 grams in green leafy vegetables, 90 grams in other vegetables, 30 grams in sugar and 10 grams in fats. Only fruits were consumed in sufficient amounts per day, but the consumption was rare, occurring only once a week. Apart from cereals, women were consuming a greater deficit quantity of food compared to men.

(n=120)

	1	1	1				-
S. No.	Food items	Recommen- ded for a Man (gr)	Mean consumption by a man (gr) (B)	Differ- ence (gr)	Recommen- ded for a woman (gr)	Mean consumption by a woman (gr) (D)	Difference (gr)
		(~)	(8)	(,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	(0)	(8)	(0-0)
1	Cereals	600	450	150	480	380	100
а	Rice	NA	300	NA	NA	250	NA
b	Wheat (or)		150			100	
с	Sorghum						
2	Pulses	120	60	60	90	40	50
3	Milk and milk products	300	110	190	300	80	220
4	Roots and tubers	200	125	75	200	100	100
5	Green leafy Vegetables	100	80	40	100	50	50
6	Other Vegetables	200	125	75	200	110	90
7	Fruits	100	125	-25	100	110	-10
8	Sugar	55	15	40	45	15	30
9	Fats	40	20	20	30	20	10

men consumed 300 grams of rice per day, 150 grams of wheat, and occasionally sorghum as a substitute for wheat. The consistent consumption of wheat (with occasional sorghum) may be attributed to factors such as proximity to Maharashtra, cultural dietary habits, the tradition of consuming Jwari Kanya (a local sorghum-based dish), and the predominant Marathi ethnicity within the sampled villages.

Item wise analysis of quantity of diet, frequency of diet and source of diet

The majority of respondents had consumed recommended quantity of fruits (66.66%), followed by sugar (51.66), other vegetables (38.33%) and green leafy vegetables (33.33%). Half of the respondents had consumed 1%-15% less to the recommended quantity of milk and milk products, followed by fats

(n=120)	ce	Same	19(15.83%)		٩A		83(69.16%)			٩A			70(58.33%)	110(91.66%)	33(27.50%)	22(18.33%)	5(12.50%)	110(91.66%)	97(80.83%)
	Sour	Different	101(84.16%)		Z		37(30.83%)		2		50(41.66)	10(8.33%)	87(72.50%)	98(81.66%)	105(87.50%)	10(8.33%)	23(19.16%)		
urce of diet		Rarely	0(00:00)	0(0:00)	0(00:00)	73(60.83)	0(0:00)	0(00:00)	97(80.83%)	120(100%)	120(100%)	120(100%)	0(0.00%)	115(95.83%)	105(87.50%)	32(26.66%)	80(66.66%)	0(0.00%)	75(62.50%)
t diet and sou	Frequency	Sometimes	0(0:00)	0(0:00)	38(31.66)	47(39.16)	0(0:00)	0(0:00)	23(19.16%)	0(0.00%)	0(0:00%)	0(0.00%)	0(0.00%)	5(4.16%)	15(12.50%)	28(23.33%)	25(19.16%)	0(0.00%)	36(30.00%)
trequency o		Regular	120(100%)	120(100%)	82(68.33)	0(0:00)	120(100%)	120(100%)	0(0.00%)	0(0:00%)	0(0.00%)	0(0:00%)	120(100%)	0(0.00%)	0(0.00%)	60(50.00%)	7(5.83%)	120(100%)	97.50
uantity of diet,		15% -50% recommended	65 (54.16%)	i			63(52.50%)						30(25.00%)	38(31.66)	45(37.50%)	32(26.66%)	15(12.50%)	22(18.33%)	30(25.00%)
according to di	Quantity	1%-15% <recommended< th=""><th>31(25.83%)</th><th></th><th>NA</th><th></th><th colspan="2">38(31.66%)</th><th></th><th>AN</th><th></th><th>-</th><th>65(54.16%)</th><th>62(51.66%)</th><th>35(26.66%)</th><th>42(35.00%)</th><th>25(20.83%)</th><th>36(30.00%)</th><th>65(54.16%)</th></recommended<>	31(25.83%)		NA		38(31.66%)			AN		-	65(54.16%)	62(51.66%)	35(26.66%)	42(35.00%)	25(20.83%)	36(30.00%)	65(54.16%)
of respondents		Recommended	24(20.00%)				19(15.83%)						25(20.83%)	20(16.66)	40(33.33%)	46(38.33%)	80(66.66%)	62(51.66)	25(20.83%)
. Distribution		Food items	Cereals	Rice	Wheat (or)	Sorghum	Pulses	Red gram	Bengal gram	Black gram	Moth bean	Lentil	Milk and milk products	Roots and tubers	Green leafy Vegetables	Other Vegetables	Fruits	Sugar	Fats
lable 4		S.No.	-	а	q	U	2	a	q	υ	q	e	3	4	5	9	7	8	6

Table 4. Distribution of respondents according to quantity of diet, frequency of diet and source of diet

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(54.16%), roots and tubers (51.66%), other vegetables (35.00%), and sugar (30.00%). Similarly, half (54.16%) of the respondents had consumed 15% -50% less to the recommended quantity of cereals, followed by pulses (52.50%) and green leafy vegetables (37.50%).

In case of frequency of diet, all (100%) respondents had consumed cereals, pulses and milk and milk products and sugar on regular basis, followed by half (50.00%) who consumed other vegetables regularly. The majority (95.83%) of respondents consumed roots and tubers rarely, followed by green leafy vegetables (87.50%), fruits (66.66%) and fats (62.50%). All (100%) of the respondents consumed rice and red gram on regularly, with the majority (68.33) also consuming wheat regularly. Additionally, all (100%) respondents consumed black gram, moth bean and lentil rarely, with Bengal gram (80.83%) and sorghum (60.83) following suit. Two-fifths (39.16%) of the respondents consumed sorghum occasionally, followed by wheat (31.66%). These results align with the findings of Sabu (2019).

In case of source of diet, the majority (84.16%) of the respondents consumed different sources of diet in cereals, followed by green leafy vegetables (72.50%), other vegetables (81.66%) and fruits (87.50%). Similarly, most (69.16%) of them consumed similar sources of diet in pulses, milk and milk products (58.33%), roots and tubers (91.66%), sugar (91.66%), fats (80.83%).

Association between the profile of respondents and their overall diet status

In consideration of the significance of dietary patterns, an attempt was made to delineate the association between the profiles of respondents and their overall dietary pattern using correlation coefficients, and the results are provided in Table 5.

The data presented in Table 5 indicated that education (0.3452**), occupation (0.2409**), number of persons earning (0.2284**), health orientation (0.3342**), milch animals (0.3061**), health orientation (0.3342**) decision making about food (0.2650**) and preference of shops (0.2986**) were found to be positively and highly significantly associated with dietary patterns. Conversely, annual income (0.2184*), cropping pattern (0.2340*) and health status (0.1958*) were found positive and significant with dietary pattern.

Family type (-0.1140^{NS}) was found positive but not significantly associated with dietary patterns. Gender (-0.1992*), distance to market (-0.1980*) and debt (-0.1795*) were found to be negatively and significantly associated with dietary patterns. Meanwhile, age (-0.1284^{NS}) was found to be negatively but non-significantly associated with dietary patterns. Therefore, the null hypothesis was rejected, and the empirical hypothesis was accepted. These findings are consistent with the results reported by Jebessa *et al.*, (2024).

From the above results, it can be concluded that, with higher and increase in education, occupation number of persons earning, milch animals, health orientation, decision making about food, preference of shops, annual income, gender, cropping pattern, distance to market and health status led to an improvement in dietary patterns. The reasons for this trend could be attributed to various factors. Firstly, improved education might have led to increased awareness and knowledge regarding health and diet. Secondly, engagement in additional occupations alongside farming could boost annual income, enabling individuals to afford a wider variety of food items. Moreover, an increase in the number of earners within a household could correlate with higher annual income, facilitating greater spending on food. Additionally, an increase in the availability of milch animals may lead to a greater abundance of milk and milk products, positively impacting dietary patterns. Furthermore, heightened health orientation may result in increased interest and importance placed on health, thereby influencing dietary choices. Women's involvement in decision-making about food exhibited a positive impact on dietary patterns. This may be attributed to their heightened concern for the health and nutritional needs of their families. Preference for wholesale shops may enhance accessibility to food items at lower prices, thereby improving dietary patterns. Those cultivating a diverse range of cereals and pulses may have greater access to grains and pulses from their own farms, resulting in a positive relationship with dietary patterns. Individuals with good health statuses may be more conscientious about their diet and overall health. Gender dynamics may also play a role, with female respondents potentially prioritizing the health and nutrition of their families over their own dietary patterns. Increased distances to markets may limit access to a

S. No.	Variables	'r' value
X1	Age	-0.1284 ^{NS}
X2	Education	0.3452 **
ХЗ	Occupation	0.2409 * *
X4	Annual income	0.2184 *
X5	Gender	-0.1992 *
X6	Family type	0.1140 ^{NS}
X7	Cropping pattern	0.2340 *
X8	Number of persons earning	0.2284 **
X9	Distance to market	-0.1980 *
X10	Milch animals	0.3061 **
X11	Debt	-0.1795 *
X12	Health orientation	0.3342 **
X13	Health status	0.1958 *
X14	Decision making about food	0.2650 **
X15	Preference of shops	0.2986 **

Table 5. Association between the profile of respondents and their overall diet pattern (n=120)

NS Non-significant, * Significant at 0.05 level, ** Significant at 0.01 level

variety of food items, including fruits and vegetables, negatively impacting dietary patterns. Moreover, increased debt may lead to a cautious mindset when spending on food items.

CONCLUSION

The results indicated that half of the participants had insufficient quantity of diet, with medium frequency of diet, diverse source of diet and medium expenditure on diet. Their storage of food was moderately proper and they engaged in high physical activity with a fair amount of dietary pattern. In the association between profile and dietary pattern, education, occupation, number of persons earning, health orientation, milch animals, health orientation, decision making about food, preference of shops, annual income, cropping pattern, and health status had positively affected the dietary pattern. On the other hand, gender, distance to the market, and debt negatively affected dietary pattern. Thus, it can be concluded from the results that, there is difference between the consumption of female and male and respondents. Vast differences were found in the food intake and pattern than the recommended. Hence,

to improve the condition, inclusion and distribution of millets and pulses in Public Distribution System should be done. People were still not aware about importance of maintaining healthy diet, those who aware were not taking practicing it seriously. Hence, extension systems should focus on conducting mass awareness programmes on importance of maintaining healthy diet. extension systems should conduct trainings in collaboration with Anganwadi and women associations on preparation of highly nutritional diets, practicing healthy diets and identifying symptoms of malnutrition.

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EFFECT OF DESIZING AND SCOURING ON GEOMETRICAL PROPERTIES OF THE COTTON FABRIC

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ABSTRACT

Textiles have constantly played a crucial role in the evolution of human culture by being at the forefront of both technological and artistic development. The study investigates the impact of desizing and scouring processes on the geometrical properties of grey cotton fabric, crucial for its functionality in various applications. Through a series of preparatory treatments, including desizing to remove stiffening agents and scouring to eliminate impurities, the fabric's appearance and physical attributes were enhanced. Geometrical properties such as fabric count, weight, and thickness were examined before and after treatment. The results indicate a notable increase in fabric count, with a 12.38% rise in warp and a 6.4% increase in weft, post-desizing and scouring. Additionally, fabric weight and thickness showed significant increases of 9.5% and 8.6%, respectively, after treatment. These enhancements suggest improved fabric quality, likely attributed to the removal of impurities and the resulting compactness of the weave. Overall, the study underscores the importance of preparatory processes in optimizing cotton fabric properties for diverse textile applications.

KEYWORDS: Textiles, geometrical properties, cotton fibre, desizing and scouring

Textiles have constantly played a crucial role in the evolution of human culture by being at the forefront of both technological and artistic development. Clothing, considered to be the second skin, is responsible for a healthy and contagious free living (Hooda *et al.*, 2013). Cotton is a natural fibre of great economic importance as a raw material for cloth (Saleemuddin, 2013). Cotton fibre is mainly compiled with cellulose and some noncellulosic constituents surrounding the cellulose core (Chung, 2004). Physical properties of fabric are vital because these properties decide the functionality of the fabric. There were various physical properties such as geometrical, mechanical and comfort properties. The geometrical properties of the cotton fabric comprised of fabric count, weight and thickness etc. (Poonia, 2018).

The pretreatment of cotton fabric, involving desizing and scouring processes, is a critical phase in textile production, bringing about significant improvements in both appearance and geometrical properties. Cotton, a ubiquitous natural fiber in the textile industry, undergoes a series of manufacturing stages before becoming the final fabric. Desizing and scouring are pivotal steps in this journey, preparing the fabric for further processing while enhancing its quality. Desizing, as the initial step, focuses on removing sizing agents applied during weaving. These agents, while essential for yarn strength and weaving efficiency, can leave the fabric feeling stiff and textured. Desizing effectively eliminates these agents, resulting in a softer, more pliable cotton fabric. This transformation enhances the fabric's comfort and drape, making it more appealing for various applications.

Following desizing, the scouring process tackles impurities and natural waxes that may linger on the fabric's surface. Scouring ensures the removal of these unwanted substances, promoting fabric cleanliness and improving its overall aesthetic appeal. The fabric emerges from this stage cleaner, smoother, and ready for further treatment or processing. In addition to these changes in appearance, the geometrical properties of the treated cotton fabric also undergo significant alterations. These properties include fabric count, weight, thickness, and weave pattern. These changes are essential as they directly impact the fabric's functionality and performance characteristics. For instance, a fabric with improved geometrical properties *i.e.*, fabric weight, fabric count and thickness may exhibit better dye absorption, print adhesion, and overall durability, making it a versatile choice for a wide range of textile applications. Thus, the work has been planned to find the "Effect of Desizing and Scouring on Geometrical Properties of the Cotton Fabric".

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MATERIAL AND METHODS

Selection and Procurement of Cotton Fabric

A small survey was undertaken in the local market of Hisar city to acquire cotton fabric and samples were collected from various sources. The collected fabric samples were screened visually and through physical and chemical tests. Physical (microscopic analysis), burning and solubility tests were performed on the collected samples to ensure the purity. A medium weight grey cotton fabric was chosen from the collected cotton fabric samples. Grey cotton fabric was chosen since it has no finishing treatments, making it more suitable for the study as no previous treatment will interfere with the research, resulting in unbiased data.

Determination of the Geometrical Properties of the Cotton Fabric

Geometrical properties of the selected cotton fabric were examined for three parameters: fabric count, weight, and thickness. Prior to determining fabric measurements, the fabric samples were conditioned before desizing and scouring under standard test circumstances, which included a relative humidity of 65 ± 2 per cent and a temperature of 27 ± 20 °C.

Desizing of the Fabric

It is very usual for the fabric to have leftover sizing material on the warp yarn after weaving; this makes the yarn less porous and may obstruct the penetration of the test extract, thus it is critical to remove it. The prewetted cotton fabric was desized in Water Bath rectangular 6 holes for 60 minutes at 50 °C with MLR 1:40 in a solution containing 1 per cent sulphuric acid (H_2SO_4). The fabric was rinsed thoroughly to remove loose starch and other residues, if left (Singh *et al.*, 2017).

Table 1.	Instruments	and Test	Standards	Used
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Scouring of the Fabric

As woven fabrics include natural and added impurities such as wax, oils, dirt and non-cellulosic material (in the case of cotton fabric), scouring (wetting procedure) is used to remove the dirt and extra impurities without affecting the fabric's physical and chemical qualities. It entails washing the textile material in a water bath with chemical (Soda Ash and Sodium Sulphite) or enzymatic agents for a set period of time. In an aqueous solution, the cloth was properly squeezed before being immersed in a mixture comprising 1 per cent soap, 3 per cent soda ash, and 0.5 per cent sodium sulphite at boiling temperature (100°C) with MLR 1:40 for 60 minutes. The fabric was further rinsed thoroughly to remove any residues, if left and dried on a flat surface (Singh *et al.*, 2017).

Determination of Geometrical Properties after Preparatory Processes

Fabric's geometrical properties are crucial because they determine the fabric's functionality. When a fabric is treated, it is critical that the geometrical properties are not significantly altered and if they are, the fabric's quality is improved. The geometrical qualities of the desized and scoured samples were examined and compared in order to see the influence of the fabric treatment. According to the standards, each sample was subjected to the recommended number of tests. The samples were trimmed to the size of the template, leaving the selvedge on both sides and ensuring that neither warps nor weft yarn was repeated. The influence of preparatory processes on the geometrical properties of finished fabric samples were investigated using a variety of standard test methods, as detailed below.

Physical properties	Samplesize	Instruments	Standard Methods
Fabric count (Ends and picks per sq. inch.)	6"x6"	Paramount Pick Glass with (1"x1")	ASTM-D123
Fabric weight (g/m²)	5"x5"	GSM Quadrant Balance	ASTMD-377960
Fabric thickness (mm)	4"x4"	Paramount Thickness Tester	BS 2544:1967

RESULTS AND DISCUSSION

Effect of Preparatory Processes on Geometrical Properties on Cotton Fabric

Grey cotton fabric exhibiting medium weight was selected for the study. Three parameters i.e. fabric weight, count and thickness of the cotton fabric were evaluated. The data in Table 2 indicated that the fabric count of grey cotton fabric was 55×41 picks/sq. inch with 0.23 mm thickness and 120 g/m² weight.

Table 2. Preliminary Data of the Cotton Fabric

 $62.6\pm0.88\times44.4\pm1.20$ ends and picks per inch with 12.38 and 6.4 per cent increase and 4.23 and 3.12 t-values, respectively. The increase in fabric counts in both warp and weft direction was found to be non-significant.

The thickness and weight of the grey fabric was found to be 0.23 ± 0.006 mm and 120 ± 3.39 g/m²which increased to 0.25 ± 0.021 mm and 131.4 ± 1.87 g/m², respectively after desizing and scouring with +

Fabric	Fabric count	Fabric weight	Fabric thickness (mm)
Properties	(ends and picks/sq. inch)	(g/m²)	
Cotton fabric	55×41	120	0.23

Effect of Preparatory Processes on Geometrical Properties of Grey Cotton Fabric

The selected cotton fabric underwent preparatory processes like desizing and scouring which involves the removal of impurities and starch from the fabric and makes it more absorbent for further textile processing. Scouring was done after desizing to ensure that all contaminants and starch were removed. The change in physical attributes of the pretreated grey cotton fabric, such as geometric, mechanical and comfort aspects was investigated further.

Geometrical Properties of Cotton Fabric

Geometrical properties i.e. fabric count, weight and thickness were tested to analyze the effect of preparatory processes on the geometrical properties of grey cotton fabric. The data in Table 3 highlighted that the fabric count of grey fabric was $55.7\pm0.33 \times$ 41.7 ± 0.66 ends and picks per sq. inch. It was found that after desizing and scouring, it increased to 9.5 and + 8.6 per cent increase and 8.25 and 2.87 tvalue respectively. The increase in fabric weight and thickness of desized and scoured fabric was found to be non-significant. Medha (2019) reported nonsignificant increase in geometrical properties of cotton fabric after giving pretreatment to cotton fabric. Sushila et al., (2021) reported non-significant gain in fabric count, weight and thickness of fabric after scouring of cotton fabric. After desizing and scouring, there was removal of starch as well as cellulosic matter and other impurities which resulted in compactness and closeness of weaves. The change in physical properties of the fabric may therefore be attributed to the compactness of the weave. Decrease in air permeability may be owing to increased fabric count and thickness of the fabric.

Thus, it was perceptible from the results presented in Table 3 that after desizing and scouring there was non- significant increase in all geometrical properties of grey cotton fabric.

Physical Parameters	Fabric	Grey fabric Mean±S.E	Desized and scoured fabric Mean ± S.E	Per cent change	t-value
Fabric count	Warp	55.7±0.33	62.6±0.88	+ 12.38	4.23
(ends and picks per square inch)	Weft	41.7±0.66	44.4±1.20	+ 6.4	3.12
Weight per unit area (g/m²)	1	120±3.39	131.4±1.87	+ 9.5	8.25
Thickness (mm)		0.23±0.006	0.25±0.021	+ 8.6	2.87

Table 3. Effect of Preparatory Processes on Geometrical Properties

**Significant at 1% level of significance, *Significant at 5% level of significance

CONCLUSION

The pretreatment of cotton fabric through desizing and scouring processes not only enhances its visual and tactile qualities but also refines its geometrical properties, making it a more adaptable and valuable material in the textile industry. These treatments set the stage for further processing, ensuring that the fabric meets quality standards and performance expectations while opening doors to a variety of creative possibilities in the world of textiles.

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ATTITUDES OF AGRICULTURAL STUDENTS TOWARD CAREERS IN THE AGRICULTURE SECTOR

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The agriculture sector, as a linchpin of global food security and sustainable development, relies heavily on a skilled workforce. Recognizing the pivotal role of undergraduate agriculture students in shaping the future of agriculture, this research seeks to unravel the intricacies of their attitudes towards choosing a career in the agriculture sector. (Onu and Ikehi, 2013). By understanding the underlying factors influencing their career choices, educational institutions and policymakers can tailor strategies to attract and retain talent in this vital industry. (Eck and Torres, 1996). Agricultural progress serves as a crucial pillar for the economic advancement of any nation, with the agricultural sector being a focal point for overall economic growth. Contrary to historical perceptions, agriculture is no longer confined to uneducated farmers. In India, the agriculture and allied sectors stand as the primary source of livelihood, particularly in rural regions. (Zaki et.al., 2018) In the early 1950s, agriculture contributed to half of India's GDP; however, this share dwindled to approximately 25% by 1995 and further diminished to a mere 13.9% by 2011. Evidently, the agricultural sector has witnessed a significant reduction in its labor force, especially among the youth, primarily males. This decline can be attributed to a lack of incentives for choosing agriculture as a career, prompting the youth to migrate from rural to urban areas in pursuit of whitecollar employment opportunities. Regrettably, the youth harbor a pessimistic perception of agriculture as a viable career option, leading to a decline in interest in pursuing agricultural professions. Agriculture as a profession represents the backbone of human civilization, providing sustenance, raw materials, and economic stability. This multifaceted field involves various activities, ranging from cultivating crops and managing livestock to embracing cutting-edge technologies for sustainable practices. Professionals in agriculture contribute

significantly to global food security, economic development, and environmental stewardship. (Osborne et.al., 2000). The problem under consideration pertains to the prevailing negative attitude of undergraduate agriculture students towards pursuing a career in the agriculture sector at G.B. Pant University of Agriculture and Technology, Uttarakhand. Despite the crucial role agriculture plays in India economy, there is a noticeable decline in student interest in pursuing agricultural careers. This issue poses a significant threat to the future of the agriculture sector, as the youth, particularly undergraduate students, are crucial stakeholders for its sustainable development. The primary objective of this paper is to assess and comprehend the attitudes of undergraduate agriculture students towards their careers in the agriculture sector. This involves a detailed exploration of the factors influencing their career decisions, shedding light on their expectations, aspirations, and concerns. The research aims to contribute valuable insights that can inform educational practices, policy formulation, and industry initiatives to enhance the appeal of agriculture as a career choice.

- 1. To assess the socio-personal, communication and psychological characteristics of students.
- 2. To study the attitudes of undergraduate agriculture students towards their career in the agriculture sector.
- Universe of the study: G.B.Pant University of Agriculture and Technology, Uttarakhand was selected as it has maximum number of agriculture undergraduate students.
- Respondents: The study involves undergraduate agriculture students from diverse backgrounds, ensuring a representative sample. Final year students will be selected purposively as they are or might be

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willing to start their professional carrier in various fields. Thus, total 56 respondents were selected.

- Data Collection: Data was gathered through online questionnaire.
- Variables: The research examines various variables, including gender, CGPA, place of residence, parents annual income, mass media exposure, self confidence, achievement motivation, attitudes, influencing factors, and career expectations.

Gender: It refers to the difference in male and female in terms of their role and status in society, values and attitudes. From the Table 1 it can be observed that there were maximum (66 %) of female and male (34.00%). This could be due to the reason that the university allows 60 % enrollment of female candidates and 40% to male candidates. **Place of residence:** It refers to the original place of habitat of the respondent at the time of investigation. The results in the above Table 1 delineates that half (55.35%) of the students reside in urban areas, then 25.00 per cent student live in rural area and some (19.64%) live in semi urban areas. This could be due to the reason that students' parent might have been migrated to urban areas to provide their child a good quality of education and other facilities.

Medium of education: It refers to the language in which students have pursuid education. From the above Table 3, it can be seen that a maximum (84.00%) students have English as a medium of instruction followed by 16.00% Hindi.

Family type: It refers to group of two or more individuals residing together who are related by blood, marriage or adoption. An apprehension of the above Table 1

S.No.	Gender	Frequency	Percentage
I	Male	19	34.00
II	Female	37	66.00
	Place of Residence		
I	Rural	14	25.00
II	Semi Urban	11	19.64
	Urban	31	55.35
	Medium of Education		
I	Hindi	9	16.00
II	English	47	84.00
	Any other	0	0
	Family Type		
I	Nuclear Family	44	78.57
II	Joint Family	12	21.43
Ш	Extended Family	0	0
	Family Size		
I	Small	6	10.71
II	Medium	46	82.14
	Large	4	7.14
	'Parent's Annual Income'		
I	Low	14	25
	Medium	21	37.5
Ш	High	21	37.5

Table 1. Distribution of students according to general information (N=56)

reflects that 78.57 per cent of the students Nuclear family, whereas 21.43 per cent belong to joint and none belong to extended family type.

Family size: It refers to the total number of family member in a single family unit whether nuclear, joint or extended family. The Table 1 represents family size into three categories in accordance with mean value (4.76) and standard deviation (1.59). Greater majority (82.14%) of the students belonged to medium family size, whereas 10.71per cent belonged to small and 7.14 students belonged to large family size.

Parent's Annual Income: The total annual income of the students' family from all the sources was considered as Parent's annual income. From the above table it can be concluded that equal per cent of students *i.e.*, 37.5 per cent belonged to medium and high income

colleges followed by low category of Parent's annual income (25%).

From the above Table it can be comprehended that most (41.07%) of the students fathers were in government service, then 21.42 per cent in business, 14.28 per cent in private services, 14.28 per cent in farming sector and a few (5.35%) were working as labour (skilled/ unskilled). Whereas, majority (76.78%) of the mother of students were housewives and 12.5 per cent were in private services, 5.35 per cent were in government services, 3.57 per cent were in farming sector and few (1.78%) were working as labour.

Sources of information about the job: It refers to the various sources of information, *i.e.*, from where the respondents seek about his job/ employment. The data related to this can be observed in the Fig. 2.

(N=56)

S.No.	Parent's Occupation	Fat	her	Mother		
		Frequency	Percentage	Frequency	Percentage	
1	Government service	23	41.07	3	5.35	
2	Private service	8	14.28	7	12.5	
3	Business	12	21.42	0	0	
4	Labour	3	5.35	1	1.78	
5	Farming	8	14.28	2	3.57	
6	Caste occupation	0	0	0	0	
7	Housewife/Husband	0	0	43	76.78	

Table 2. Distribution of students according to their 'Parent's Occupation'



Fig.1: Distribution of students according to 'Parent's Occupation'



Fig.2: Distribution of students according to 'Sources of Information about the job' (N=56)

mass media exposure								
S.No.	Mass Media Exposure	Frequency	Percentage					
1	Low	11	19.64					
2	Medium	37	66.07					
3	High	8	14.28					

Table 3. Distribution of students according to their mass media exposure

Table 4. Distribution of	f students ac	cording to
achievement m	notivation	(N=56)

S.No.	Family Size	Frequency	Percentage
1	Low	13	19.64
2	Medium	36	66.07
3	High	7	14.28

Mass Media Exposure: It refers to the exposures of the respondents to various mass media such as radio, television, reading newspaper, magazines, etc. The observations for the same can be seen in the Table 3.

From the above Table 3, it can be concluded that more than half (66.07%) of the students had medium mass media exposure, followed by low (19.64 per cent) and 14.28 per cent students had high mass media exposure.

Achievement motivation: It is an internal desire of a person to do something unique and excellent with minimum utilization of resources.

It can be observed from the Table 4 that maximum (66.07%) of the students has medium, 19.64 per cent students had low and 14.28 per cent students had high achievement motivation.

Career Aspiration: The term career aspiration often refers to profession/ occupation. Career aspiration represents an individuals orientation towards a particular professional or occupational goal. The Table 5. shows the data related to career aspiration.

Students' Attitude towards Career in Agriculture: It refers to your feeling and preferences towards career in agriculture sector as a profession. The Table 5 shows the responses related to students' attitude towards career in agriculture.

The above Table 5 shows that maximum (75.00%) of the students had undecided attitude and rest in equal percentage (12.50%) had negative and positive attitude towards career in agriculture. This could be due to the unawareness and lack of information for the jobs available after graduation, so that this type of attitude i.e. a kind of dilemma developed among the undergraduate students. Thus, they are still in undecided attitude situation for career in agriculture sector.

CONCLUSION

The research explores the attitude of undergraduate agriculture students at G.B. Pant University of Agriculture and Technology, Uttarakhand, focusing on their attitude towards career in agriculture.

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S.No.	Career aspiration	Frequency	Percentage
1	To become a Teacher/Professor in an SAU	8	14.28
2	To become a Scientist in ICAR institute	16	28.57
3	To pursue higher studies (MSc./PhD.)	25	44.64
4	To pursue an MBA degree in Agribusiness Management or any other MBA degree	4	7.14
5	To secure administrative position in an Agriculture University	10	17.85
6	To secure administrative position in the State Department of Agriculture	17	30.36
7	To secure job in Central Government	33	58.93
8	To secure job in National Bank	29	51.76
9	To secure job in Private Banks	2	3.57
10	To secure job in Private Agriculture Company	4	7.14
11	To secure job in NGO	5	8.93
12	To start own Agri-business /Agri-clinic	3	5.36
13	To start own Consultancy in Agriculture	0	0
14	To become Farm Manager	1	1.78
15	To become Horticulture Inspector	5	8.93
16	Any other (Please specify)	0	0

Table 5. Distribution of students according to 'career aspiration' (N=56)

Table 6. Distribution of students according to 'Stu-
dents' Attitude towards Career in Agricul-
ture'ture'(N=56)

S.No.	Family Size	Frequency	Percentage
1	Negative	7	12.50
2	Undecided	42	75.00
3	Positive	7	12.50

Among the demographics analyzed, the maximum observation unit is female students, constituting 66.00%. The study also reveals that 55.35% of the students reside in urban areas, potentially due to migration for better education and facilities. In terms of the medium of education, 84.00% study in English, emphasizing the language's prominence in academic pursuits. Family dynamics, such as nuclear family structures (78.57%), and moderate family sizes (82.14%), are prevalent among the surveyed students. The occupation of students' fathers is predominantly in government services (41.07%), while mothers are primarily

housewives (76.78%). The annual income distribution indicates 37.5% in the medium category, pointing to a varied socio-economic background. Mass media exposure among students is mostly medium (66.07%), and achievement motivation is observed to be predominantly at a medium level (66.07%). Regarding attitudes towards a career in agriculture, a significant 75.00% of students remain undecided, indicating a critical need for information dissemination and career awareness initiatives. This research illuminates the multifaceted factors influencing the attitudes of undergraduate agriculture students and underscores the need for interventions to address the prevailing negative perceptions. The detailed examination of demographics and motivational aspects provides a comprehensive understanding of the challenges faced by these students. Initiatives promoting awareness about diverse career opportunities in agriculture are crucial for fostering a positive attitude and steering students towards a sector fundamental to the nation's economic progress.



Fig.3: Distribution of students according to 'Mass Media Exposure' (N=56)



Fig.4: Distribution of students according to 'Achievement Motivation'



Fig.5: Distribution of students according to 'Students' Attitude towards Career in Agriculture'

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- 8. **RESULTS AND DISCUSSION** : Great care should be taken to highlight the important findings with support of the data well distinguished by statistical measures like CD, r, Z test etc. Too descriptive explanation for the whole data is not desirable. The treatments should be briefly expressed instead of abbreviations like T₁, T₂ etc. The discussion should be crisp and relate to the limitations or advantages of the findings in comparison with the work of others.

Tables

The data in tables should not be duplicated in graphs and vice versa. Mean data for main treatment effects should be presented with appropriate SE± and CD values wherever necessary. The 2 or 3 way tables should be furnished only if the results are consistent over years and are distinguished to have consideration of significant practical value. SE± and CD values however, should be furnished in the tables for all interactions and should be explained in the results and discussion. The treatments should be mentioned at least in short forms if they are lengthy, but not abbreviated as T_1 , T_2 and T_3 etc. The weights and measures should be given in the metric system following the latest units eg. kg ha⁻¹, kg ha⁻¹ cm, mg g⁻¹, ds m⁻¹, g m-3, C mol kg⁻¹ etc.

- + Figure/bar diagram/artwork with caption. Tables must be numbered in the run of the text. Text should include references to all tables
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Journals and Bulletins

- Abdul Salam, M and Mazrooe, S.A. 2007. Water requirement of maize (*Zea mays* L.) as influenced by planting dates in Kuwait. Journal of Agrometeorology. 9 (1): 34-41.
- Hu, J., Yue, B and Vick, B.A. 2007. Integration of trap makers onto a sunflower SSR marker linkage map constructed from 92 recombinant inbred lines. Helia. 30 (46): 25-36.

Books

- AOAC. 1990. Official methods of analysis. Association of official analytical chemists. 15th Ed. Washington DC. USA. pp. 256.
- Federer, W.T. 1993. Statistical design and analysis for intercropping experiments. Volume I: two crops. Springer Verlag, Cornell University, Ithaca, New York, USA. pp. 298-305.

Thesis

Rajendra Prasad, K. 2017. Genetic analysis of yield and yield component in hybrid Rice (*Oryza sativa*. L). Ph.D Thesis submitted to Professor Jayashankar Telangana State Agriculutural University, Hyderabad.

Seminars / Symposia / Workshops

Naveen Kumar, P.G and Shaik Mohammad 2007. Farming Systems approach – A way towards organic farming. Paper presented at the National symposium on integrated farming systems and its role towards livelihood improvement. Jaipur, 26 – 28 October 2007. pp.43-46

Proceedings of Seminars / Symposia

Bind, M and Howden, M. 2004. Challenges and opportunities for cropping systems in a changing climate. Proceedings of International crop science congress. Brisbane –Australia. 26 September – 1 October 2004. pp. 52-54

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