



Research Article

FAISALABAD SORGHUM; MULTICUT, HIGH YIELDING AND NUTRITIOUS LINE TO OVERCOME FODDER SCARCITY IN PAKISTAN

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Abstract

One of the breeding objectives of the time is the development/screening of resilient germplasm, which could satisfy hunger demand. The animal feed and food are the prime concern of time and the existing Pakistan germplasm needs to be updated for green fodder yield and quality traits. Hence, present study aimed to develop agronomic itinerary of the available breeding line and screen out best suited candidate lines. 'Faisalabad Sorghum' first-ever locally developed public sector multicut variety with higher proficiency than first public sorghum sudangrass hybrid (Pak Sudex). This novel variety is a result of hybridization between Australian No. 7 and Sudan Grass followed by back cross breeding method. Faisalabad Sorghum out yielded (Av 133.30t/ha) in station (2016 and 2017), zonal (2018) and national uniform fodder yield trials (2019 and 2020) when compared with check variety. The palatability of Faisalabad Sorghum was more than 80%, with reduced enteric CH₄ and CO₂ emissions by 14% in cattles. The adaptive features of this line are high yielder, resistant to specific pests (borer, shoot fly) and diseases (leaf spot and long smut) that were evaluated, highly nutritious with lower production cost. This line has remarkable energy to minimize the supply and demand gap among summer fodder varieties. The by-products of it could be utilized as an alternative energy sources. Owing to such extraordinary attributes the new line AK-113 proposed as Faisalabad Sorghum was released for general adaptation among farmers during 2021.

Keywords: Multicut sorghum, elite sorghum line, Nutritious summer fodder, Biotic stress resistance, energy-rich crop.

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1. INTRODUCTION

Livestock is the main strength of agriculture because it provides a range of agricultural value-added services. For maintaining herds health, good quality, as well as sufficient fodder production is required. It is predicted that the requirement for fodder production will increase by 631m.ton in 2050. Therefore, the green fodder yield needs to be raised by 1.69% every year to feed the entire dairy population. Green fodder directly involves

in providing the basic nutrients and is responsible for good health of animals (Kumar *et al.*, IGFRI VISION 2050). Sorghum is the supreme multipurpose crop among cereals in terms of grain as well as fodder for rain-fed, irrigated, tropical, arid, and saline areas (Brouk and Bean, 2011). Sorghum enjoys 5th position among cereals and is grown globally at 45.38m.hac area with a production of 6.7m. tons; mostly cultivated in Africa and Asia (Pandey, Madhu, and Bhat, 2019). Sorghum belongs to the Poaceae family and is pivotal summer



(Kharif) crop with multipurpose usage (Mahmud, Ahmad, and Ayub 2003). In semi-arid regions where water limitation is main bottleneck, sorghum is the pivotal fodder here for feeding dairy animals (Chakravarthi *et al.*, 2017).

Numerous sorghum genotypes possess different concentrations of chemical compounds such as proteins, fiber and water-soluble carbohydrates. The quality and quantity of sorghum are mainly determined by factors for instance genetic makeup, reproductive stages, and climatic conditions (Pedersen *et al.*, 1982). Being Kharif fodder, the sorghum forage has 8% protein, 45% NFE (nitrogen free extract), 2.5% fat as well as >50% total palatable nutrient (Mahmud, Ahmad, and Ayub 2003). However, the grain sorghum possesses dry matter (89-90%), ash (1.5-1.7%), Protein (8.9-15%), fiber (2.1-2.3%), and NFE (71.7-72.3%) (Ensminger and Olentine, 1978).

Livestock is the main pillar of the agricultural economy of entire world but owing to the shortage of forage leads towards minimization of farmers' concerns. The only solution to this cause is the breeding of high yielding as well as highly nutritious fodder varieties which will provide sufficient fodder throughout the year (Mishra *et al.*, 2017; Hasnain *et al.*, 2021). Because of its C4 mode of nature, the carbon capture and storage capacity of sorghum is far better than other forage crops which could contribute significantly as an alternative cost-effective energy source.

The animal feed and food are the prime concern of time and the existing world's germplasm needs to be updated for green fodder yield and quality traits. In the present scenario, there is a dire need to develop suitable high-yielding (multi-cut or re-sprouting) as well as resistant to adverse climate change sorghum varieties. To meet the fodder and forage demand in this scenario, the Fodder Research Sub-Station

(FRSS), Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan has produced a new advanced line of sorghum "AK-113" which is approved by the Punjab Seed Council following rigorous varietal approval procedures and protocols and tested for crude fiber and palatability studies in the course of the year 2021.

2. MATERIALS AND METHODS

The current study was conducted at FRSS, AARI, Faisalabad, Pakistan during 2008-2020. A new sorghum genotype "Australian No. 7 and Sudan grass" included in the study was used as parental material. The hybridization was done followed by a series of back cross of F1 with Australian No. 7 for consecutive years (2009-2012). In brief, the developmental breeding history is elucidated in Table 1. The development of genetic variety for better performance depends on the insertion of necessary genes into the existing sorghum hybrids (Wooten, 2001). After achieving the desired homozygosity and green yield. The performance of the candidate line was evaluated in station yield trials during 2016 and 2017. Zonal yield trials were also conducted in 2018 to evaluate its performance for fodder yield under various types of arid and semi-arid zones. Additionally, its national yield trials were steered all over the country during 2019 and 2020. The final product of hybridization (Code name: AK-113) was achieved after generation of scrutiny for morphological and yield attributes against commercial check variety Sorghum-2011 and Pak Sudex (Afzal *et al.*, 2012). All the parameters were reconfirmed in 2019 and 2020 by the FSC&RD (Federal seed certification department), Islamabad. In all the yield trials, triplicated RCBD (Randomized Complete Block Design) was implemented with plot size (1.8m×5) (Afzal *et al.*, 2012).

Table 1. Detail of breeding methodology opted during variety development process

Year	Filial Generation/Trial	Operation
2007-08	Australian No.7 × Sudan Grass (Hybridization)	<i>F₀ seeds were harvested.</i>
2008-09	F ₁ × Australian No.7 (BC₁)	<i>5 F₁ plants were back crossed with P₁</i>
2009-10	BC ₁ × Australian No.7 (BC₂)	<i>5 BC₁ plants were back crossed with P₁</i>
2010-11	BC ₂ × Australian No.7 (BC₃)	<i>5 BC₂ plants were back crossed with P₁</i>
2011-12	BC ₃ population	<i>Space planting BC₃ seed and harvested in bulk (1200 seed).</i>
2012-13	S-1	<i>Space planting (1200 plants). 100 superior plants were selected based on green fodder yield related traits.</i>
2013-14	S-2	<i>Space Planting (Individual plant progeny of 100 plants). 20 superior progenies with good regrowth after every cut (3 cutts) were retained.</i>
2015-16	S-3	<i>Row planting (20 progenies in 6 rows (5m) each). 10 superior progenies with good regrowth after every cut (3 cutts) were retained</i>
2016-17	Preliminary Green Fodder Yield Trial	Yield Data Recorded
2017-18	Advance Green Fodder Yield Trial	--
2018-19	Zonal Green Fodder Yield Trial	--
2019-20	National Green Fodder Yield Trial/ 1 st DUS Testing	Data recorded for National Trial and 1 st year DUS studies were conducted by FSC&RD
2020-21	National Green Fodder Yield Trial/ 2 nd DUS Testing/Spot Examination	Data recorded for National Trial; 2 nd year DUS studies were conducted by FSC&RD. Spot examination was done
2021-22	ESC and PSC Approval	Recommended for general cultivation as variety following Final approval by Expert Sub Committee and Punjab Seed Council

2.1. DATA COLLECTION

Data was taken for various green fodder yield-related morphological traits viz., plant height, No. of leaves, leaf area, stem thickness, leaf color and days to 50% heading of AK-113 and check (Pak Sudex). Plant height of five plants with help of meter rod in centimeters was taken at soft dough stage. Stem thickness in inches was measured in average with the help of vernier caliper of about five plants individually from 3 points on stem. Data regarding days to heading was counted from the date of sowing till the heads emerges from panicle. The average data of five plants is afterward represented in tables.

2.2. AGRONOMIC CHARACTERIZATION

Data regarding agronomic parameters like date of sowing (1st February, 15th February, 1st March, 15th March and 1st April), fertilizer level (NPK kg/ha; T1 (0-0-0), T2 (23-60-60), T3 (40-60-60), T4 (40-60-0), T5 (50-60-60) respectively), seed rate (broadcast 60, 80, 100 t/ha) and row spacing (15cm, 30cm) trials was also documented during 2018 as well as 2019 at FRSS, AARI, Faisalabad. The screening for disease (leaf spot, long smut) and insect/pest infestation (stem borer, shoot fly) data was recorded as per protocol at the Plant Pathology Department, University of Agriculture Faisalabad (UAF), Pakistan.

2.3. PROXIMATE ANALYSIS AND PALATABILITY STUDIES

The best-performing line AK-113 was analyzed for crude protein, crude fiber, crude fat, ash and dry matter along with the local check as per recommendations given by Association of Official's Analytical Chemists (AOAC, 1990). The palatability and digestibility analysis in studied material along with check variety was performed at the Animal Husbandry Department of UAF.

2.4. DNA FINGERPRINTING

The promising line AK-113 along with the Standard (YS-16) and local check (Ausaf, Pak Sorghum) was analyzed for similarities and differences from available sorghum germplasm accessions. The differences were analyzed in seed samples by Cultivar Identification diagram (CID). Fifty diverse markers were selected using PCR-SSRs method and the size of DNA fragments/amplicon (bp) were analyzed in YS-16 (Standard), Ausaf (Check variety), Pak Sorghum (Check Variety), AK-113 (Sorghum Candidate Variety) in collaboration at Agricultural Biotechnology Research Institute, AARI, Faisalabad, Pakistan.

1980) to dissect out the genetic variation among the recorded traits. All traits depicted the highly significant variation.

3. RESULTS

3.1. GREEN FODDER YIELD EVALUATION AND FEASIBILITY TRIALS

Being a multicut in nature AK-113 showed 203% higher green fodder yield over check variety Sorghum-2011 and 4% higher green fodder production in comparison with SS hybrid check 'Pak Sudex' in Preliminary green fodder yield trial during 2016. The new strain AK- 113 produced 159 and 1.5% higher green fodder yield in advance fodder yield trials during 2017 than the check Sorghum- 2011 and Pak Sudex Hybrid respectively (Figure 1). In 2018, Adaptation Yield Trials were carried out at multi-locations (4 different locations) of Punjab province, the promising line 'AK- 113 was compared with check variety 'Sorghum-2011' and Pak Sudex. The results illustrated that, on the basis of average of four locations, the line 'AK- 113 surpassed the check variety 'Sorghum-2011' by producing 128 % higher green yield and surpassed Pak Sudex by 3% (Figure 2). The performances as well as proposed features

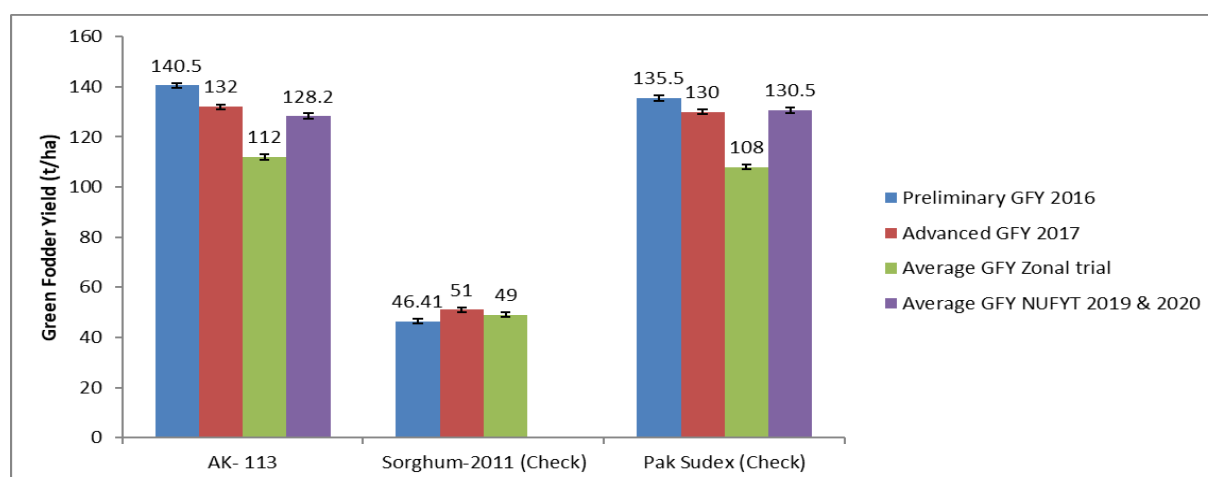


Figure 1: Performance of AK- 113 in Preliminary Green Fodder Yield Trial -2016, Advance GFY trial-2017 at Fodder Research Sub-Station, Ayub Agricultural Research Institute Faisalabad (3 cutts) and under Adaptation/Zonal GFY trial at 3 locations. Note: Error bars denotes Standard Error.

2.5. STATISTICAL ANALYSIS:

Data of all the recorded traits was subjected to analysis of variance (Steel and Torrie,

of the candidate line was recheck in NUFYT (National Uniform Fodder Yield Trials) So, The Coordinator Fodder team, NARC Islamabad tested the promising line

‘AK- 113 in National Uniform Fodder Yield Trials at different locations during 2019 & 2020. In National SS Hybrid green fodder yield trials conducted during 2019 and 2020 the purposed variety remained at par with the check hybrid Pak Sudex.

Green fodder yield-related characters of line “AK-113” with check variety ‘Sorghum-2011’ were compared. It was evident that the line ‘AK-113’ in addition to

as well as seed rate. Planting date studies and fertilizer trials were conducted at FRSS, AARI, Faisalabad during 2018 and 2019 to evaluate specific agronomic requirements of the candidate variety AK-113. From sowing date experiments, it was pragmatic that candidate line AK-113 showed the highest green fodder yield on the 15th of March during 2018 as well as 2019 (Table 2).

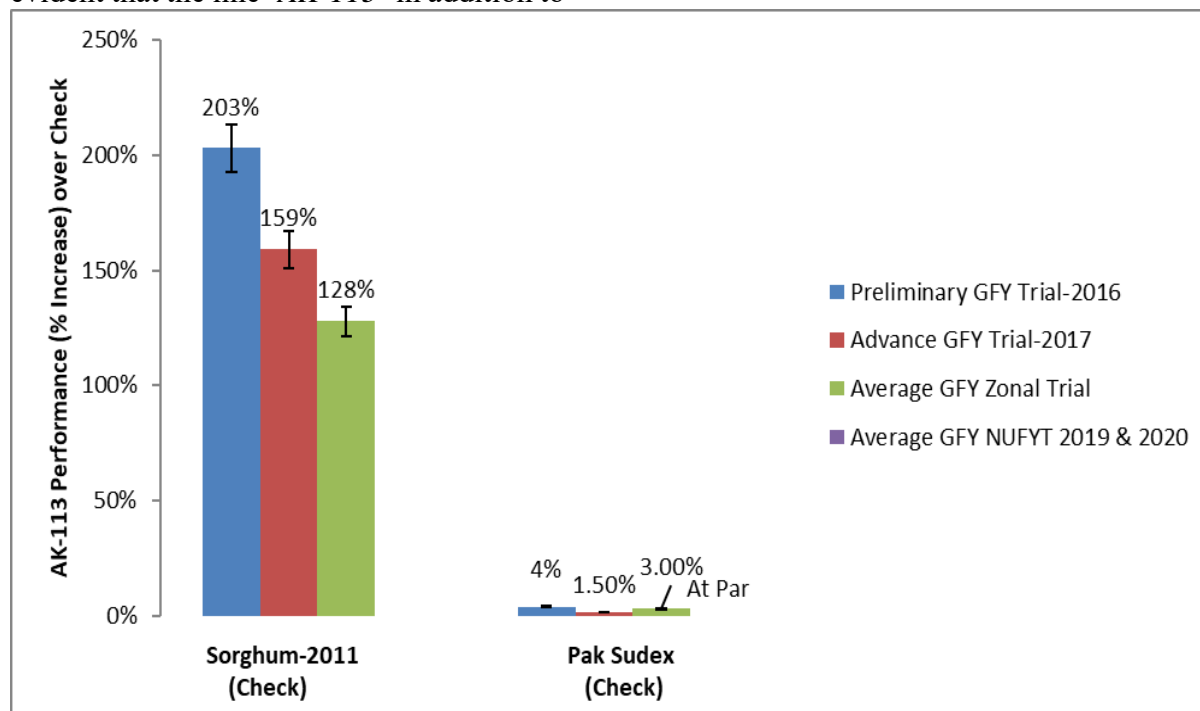


Figure 2: Performance of AK- 113 (Percentage Increase over check) in different trials. Note: Error bars denotes Standard Error

being multicut in nature is superior in plant height, No. of leaves/plant, leaf area and stem thickness to the check variety ‘Sorghum-2011’ (Figure 3).



Figure 3: Comparison of Green Fodder Yield related parameters of line “AK-113” with check variety ‘Sorghum-2011’

3.2. AGRONOMIC PERFORMANCE:

The main indicators for agronomic performance are best sowing date, optimum fertilizer level and the distance among rows

From the experiment of optimum fertilizer level it was depicted that the promising line ‘AK- 113 is more responsive to fertilizer dose of 40-60-60 NPK kg/ha as it produced maximum green fodder yield at the said doses (40-60-60) of NPK during 2018 as well as 2019 (Table 3).

The promising line AK- 113 was sown at Agronomy Forage Production, AARI Faisalabad at different seed rates and row spacing for maximum fodder yield during the year 2018 and 2019. Average green fodder yield of both years (Figure 4) indicated that the promising line AK-113 produced maximum green fodder yield at 40 kg/ha seed rate through broadcast method of sowing during both the studied

Table 2: Planting Date Trial (3 cuttings) [Green Fodder Yield 3 cutts (tons/ha)]

Sowing Date	AK-113		Pak Sudex (Check)	
	2018	2019	2018	2019
1 st February	85.5	96.5	90.5	91.5
15 th February	102.5	116.5	110.5	108.5
1 st March	115.5	121.5	142.5	145.5
15th March	145.5	151.5	132.5	140.2
1 st April	132.0	128.2	140.0	130.8

Table 3: Fertilizer Trial [Green Fodder Yield (tons/ha)] 3cuttings]

Fertilizer level N-P- K (kg/ha)	AK-113		Pak Sudex (Check)	
	2018	2019	2018	2019
T1 (0-0-0)	118.0	116.5	119.5	119.5
T2 (23-60-60)	135.4	134.6	133.5	132.6
T3 (40-60-60)	142.5	143.7	144.0	141.8
T4 (40-60-0)	128.0	127.8	125.0	126.6
T5 (50-60-60)	136.0	135.7	134.0	134.8

Note: 60 kg/ha nitrogen was applied after every cutting

years (2018 and 2019) with green fodder yield 145.5 and 135t/ha respectively.

The major insects of sorghum are stem borer and shoot fly. The average data regarding stem borer and shoot fly infestation on AK- 113 is mentioned as in Table 4. Results revealed that the advance line AK- 113 is resistant to insect/pests in comparison with check hybrid. The line 'AK- 113 was evaluated against the major disease i.e., leaf spot and long smut rot during the year 2019 at FRSS, AARI, Faisalabad. The results revealed that the line 'AK- 113 is moderately resistant to leaf spot and long smut.

3.3. PROXIMATE ANALYSIS OF GREEN FODDER

The promising line 'AK- 113 along with the local check variety 'Pak Sudex' were analyzed for ash, crude fiber, crude protein, and dry matter (Figure 5) at Biochemistry Section, AARI Faisalabad. The results showed that the nutritive value of promising line 'AK- 113 nearly similar to the check variety 'Pak Sudex'. The palatability of advance line AK- 113 is more than 80 %, which is quite similar to the check hybrid (Table 5). In the same way, digestibility of the candidate line AK- 113 was also very good.

3.4. PALATABILITY STUDY AND ECO-FRIENDLY STATUS

The line AK-113 and Pak-sudex were fed to live stock and the ratio of greenhouse gas emission (in terms of enteric CH₄, CO₂) was detected. The animal category of beef and dairy cattle with sample size of 10 animals/feeding measure were tested (Table 5). The palatability of line AK-113 was proficient with (87%) digestibility than other check material compared. More than 80% of the stock was found palatable for both the sorghum types fed. Cattle on carbohydrate-rich diets with high intake might produce less methane as a percentage of dietary gross energy.

3.5. VARIETY APPROVAL MEETING

The DUS (Distinctness, Uniformity and Stability) testing was performed during 2019 and 2020 by Federal Seed Certification and Registration Department (FSC&RD), Islamabad. Spot examination of given line (AK-113) was conducted at 23.09.2020. However, the final recommendations were made from Expert Sub-Committee (ESC) (12.07.21) and Punjab Seed Council (PSC) (20.09.2021)

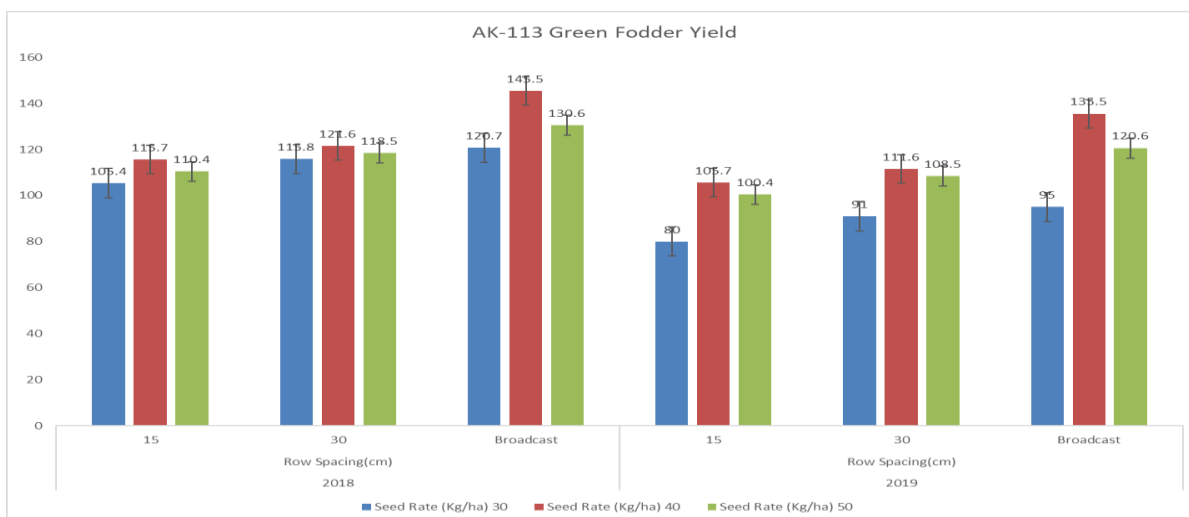


Figure 4. Effect of Different Seed Rates and Row Spacing on AK-113 Green Fodder Yield Green Fodder Yield (Total of 3 cuts). Note: Error bars denotes Standard Error.

Table 4. Insect pest and disease screening

Sr. No.	Name of Variety	Insect** Infestation (%)			Disease* Infestation		
		Year	Borer	Shoot fly	Year	Leaf Spot	Long Smut
1	AK- 113	2018	3.7	7.3	2019	MR	MR
		2019	3.4	7.5	2020	MR	MR
		2020	3.4	7.5	2020	MR	MR
2	Pak Sudex (Check)	2018	5.5	11.3	2019	MR	MR
		2019	4.2	9.5	2020	MR	MR
		2020	4.2	9.5	2020	MR	MR

MR: Moderately resistant (*Plant Pathology Research Institute Faisalabad) (**Entomological Research Institute, Faisalabad);

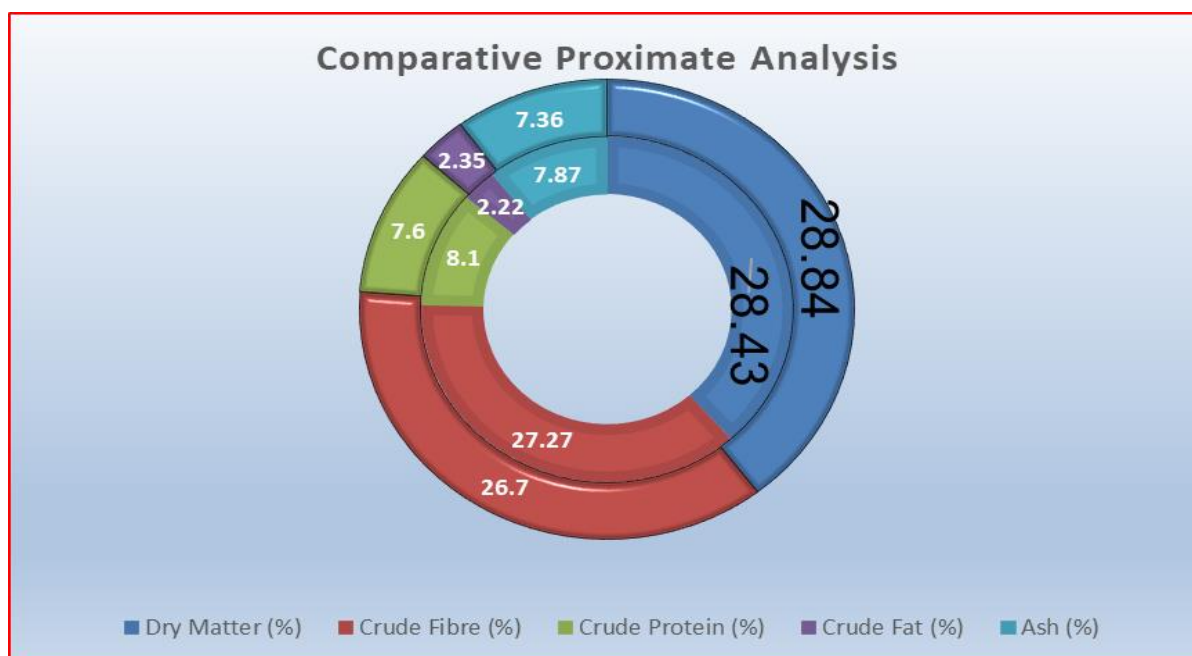


Figure 5: Proximate Analysis of AK-113(Inner Circle) & Pak-Sudex (Outer Circle)

Table 5. Impact of green fodder (AK-113, Pak Sudex) supplementation on direct and indirect GHG emissions and palatability studies in beef and dairy cattles

Animal Category	No.	Fodder feed/ Feeding Measures	Quantity of Fodder Offered (Kg)	Quantity of Fodder Consumed	Palatability (%)	Measured Gas	GHG's observational changes
Beef and dairy cattle	10	AK-113 (Faisalabad Sorghum)	60	53	87	Enteric CH ₄ , CO ₂	When compared to Pak-Sudex, AK-113 forage consumption reduces enteric CH ₄ and CO ₂ emissions by 14%.
Beef and dairy cattle	10	Pak Sudex (Check)	60	46	82	Enteric CH ₄ , CO ₂	Lower CH ₄ and greater CO ₂ concentrations were associated with Pak Sudex diet.

3.6. CULTIVAR IDENTIFICATION DIAGRAM (CID)

Cultivar Identification Diagram (CID) presenting the association among standard variety (YS-16) check varieties (Pak Sorghum and Ausaf) and candidate variety (AK-113) was generated by unweighted paired group method with arithmetic means (UPGMA). X-axis represents genetic similarity coefficient between genotypes which ranges from 0.54 to 0.86 (Figure 6). CID results indicated that candidate line AK-113 was 40% different from YS-16, 23% from Pak Sorghum and 16% from Ausaf.

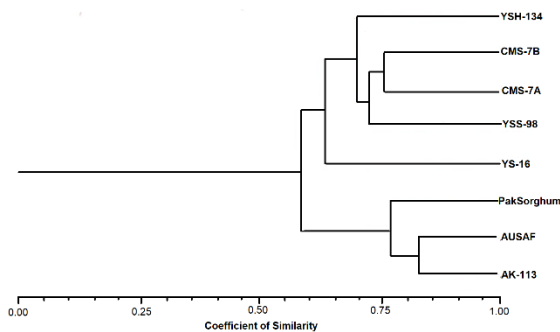


Figure 6: DNA fingerprinting and cultivar identification phylogeny

4. DISCUSSION:

Sorghum is a nutrient-packed grain that you can use in many ways. It's rich in vitamins and minerals like B vitamins, magnesium, potassium, phosphorus, iron, and zinc. It's also an excellent source of fiber, antioxidants, and protein. What's more, it's

easy to replace rice or quinoa with whole sorghum in most recipes. The best alternative to maize on nutritional scale is Sorghum grain. The yield of Sorghum is more with fewer inputs, thus making it useful for animal and poultry feed in future. Development of such varieties is pivotal which are adaptive to a vast range of environmental conditions. Thus, performance of Sorghum advance line AK-113 was computed with the check in preliminary as well as in advance fodder yield trials at FRSS, AARI, Faisalabad during 2016 and 2017. AK-113 outperformed throughout the assessment period. Highly significant results for various green yield contributing traits were observed (Khan *et al.*, 2013) during station yield trials of sorghum lines. The agronomic behaviour of this strain amazingly highly correlates with the findings of Reddy *et al.*, 2014 (Reddy *et al.*, 2014). The growth and high productivity of sorghum varieties depend greatly on the uptake of nutrients. Application of balanced amount of macro and micro nutrients to plants enhanced the uptake of fertilizers (such as NPK) as well as nutrient use efficiency in sorghum genotypes (Weldegebriel *et al.*, 2018). The combination of NPK (40-60-60 kg/ha) might be responsible for the maximum shoot height and number of green leaves, which in turn results higher green fodder yield potential (Ramadhani *et al.*, 2019).

Sorghum green fodder yield mostly relies on seed rate as well as row spacing. Seed rate had indirect relation with row spacing; higher seed rate along with narrow spacing ultimately produce high fodder yield and vice versa (Malik *et al.*, 2007).

The major insects, pests and diseases of sorghum are stem borer, shoot fly, leaf spot and long smut. The performance of AK-113 was better than other tested materials. The genetic architecture of breeding material significantly determines the resistance and intensity of insect/pest and disease attack (Shyam *et al.*, 2015). Maximum fodder yield in case of AK-113 can be obtained when it is sown on 15th March. The seed rate of 40 kg/ha, through broadcast method and fertilizer rate of 40-60-60 NPK kg/ha (apply 60kg Nitrogen after each cutting) is recommended to obtain economically maximum green fodder yield.

It is pertinent from the study that we should adopt alternate strategies to fulfill this gap. The palatability of Faisalabad Sorghum was more than 85%, with reduced enteric CH₄ and CO₂ emissions by 14% in cattle. Enteric fermentation leads to the production of methane emissions from ruminant animals raised for their meat and milk account for as much as 30% of global anthropogenic methane emissions (Dini *et al.*, 2017). The fodder AK-113 developed after years of breeding is beneficial in many aspects, even the eco-friendly status of it is high in terms of less contribution to GHG's production. DNA fingerprinting results can help researchers understand how environmental factors influence genetic diversity and adaptation in a population. Overall, evaluating DNA fingerprinting results involves analyzing banding patterns and using statistical methods (Cluster analysis or principal component analysis (PCA) to identify similarities and differences between samples. While evaluating the similarity and differences for AK-113 using CID, the coefficient of similarity indicated a difference of 40%, 23% and 16% than the check varieties (YS-16, Pak Sorghum and Ausaf respectively).

This study will lay basis for DNA based identification of relationship regarding sorghum germplasm. Faisalabad Sorghum is first locally developed open-pollinated multicut advance line of sorghum, with numerous other characteristics as high green fodder yield, resistance to insect/pest and diseases, tall growth and a greater number of leaves, high nutritive value, lower cost of production, and could overcome the green fodder and scarcity/lean period.

5. CONCLUSIONS

The study is based on the development of silage-type sorghum that has been evaluated for biomass yield over multiple harvests while assessing regrowth vigor, biomass composition, and biotic stress resistance. In this present study, we screened and characterize the sorghum germplasm for adaptive climatic conditions and also integrate multicut features via breeding practices of over 10 years. The novelty of this variety is that it is open-pollinated but yields comparably to a popular hybrid (Pak Sudex), which might allow farmers to save their own seed. The line developed is the first multicut sorghum genotype which has high palatability and is also eco-friendly. Our data suggested that the genotype has the potential to overcome fodder scarcity worldwide, especially during lean fodder period. Due to the reduced need for irrigation and fertilizer, forage sorghum grown in systems with legumes offers dairy animals nutrient-rich feed that is also very cost-effective. To feed animals when green forage is scarce, sorghum fodder can also be kept as hay or silage. Therefore, it is imperative to utilize this crop to its full capacity in order to assure a sustainable supply of animal feed and, ultimately, to ensure human food security.

6. ACKNOWLEDGEMENT

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