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Research Article

AARI-Berseem 2024: A Climate-Smart and High-Nutrition Fodder Variety for Sustainable Livestock Production

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ABSTRACT

Adverse climatic conditions pose a rising threat to global food and fodder security. These challenges not only affect human populations but also livestock productivity. To address these challenges a new berseem variety "AARI Berseem-2024" has been developed by the Fodder Research Sub-Station, Ayub Agricultural Research Institute, Faisalabad. This variety is an outcome of composite selection from foundational parental population developed by pooling five high-performing accessions, four local BGP-09, BGP-17, BGP-29, BGP-37 and one exotic PI 468401 during 2013-2024. It proved its value in all yield trials and outperformed the current existing Berseem varieties. It produced 3.9% higher green fodder yield in comparison to check varieties Anmol Berseem and Berseem Agati during 2021-22 and 2022-23, in National Uniform Fodder Yield Trials. Furthermore, it has 22.3% dry matter, 19.24% crude protein, 21.8% crude fiber, 2.23% crude fat and 11.5% ash, and good palatability. The new variety showed better tolerance against Root Rot, Crown Rot and Stem Rot diseases. Agronomic studies revealed that it aligns more efficiently with current agronomic practices. It was approved by Variety Evaluation Committee during 2024 for general cultivation in Punjab. This offers a sustainable solution to fodder scarcity during lean periods in winter (December-January) and in summer (May-June) and unpredicted weather. Its climate resilience and high nutritional profile promises to boost livestock productivity and support livestock farming under changing climatic conditions. Its climate resilience and high nutritional profile promises to boost livestock productivity and support livestock farming under changing climatic conditions.

Keywords: AARI Berseem-2024, Climate Smart Fodder, Fodder Quality, High Forage yield, Livestock productivity

INTRODUCTION

Livestock is important sector of agricultural economy, contributing 60.84% to the agriculture value addition and 14.63% to the national GDP (Pakistan Economic Survey, 2024–25). Livestock in mixed farming often faces challenges due to the insufficient quantity and quality of forage. According to Habib *et al.* (2016), the annual feed requirements for livestock are met through a blend of crop residues (58.8%), green forages (23.8%), grazing (9.2%), and concentrates (8.2%). Nutritious green fodder is vital for both milch and draught animals, and its scarcity hampers livestock productivity and farm profitability (Khadda *et al.*, 2015). Forage crops are inevitable to the Livestock sector and reduce reliance on concentrates (Pereira *et al.,* 2022).

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Among these fodder crops, berseem (*Trifolium alexandrinum* L.) is most valued as it is commonly referred as "King of Fodder Crops" and rich in moisture (85.4%), crude protein (18–20%), essential nutrients and minerals like calcium and phosphorus (Khan *et al*.,2018). It enhances Livestock productivity and milking capacity (Balazadeh *et al*., 2021). It has rapid regrowth, high biomass yield, excellent palatability, digestibility. Its economic returns often exceed those of other forage crops by 30% to 70% depending on agronomic practices and local market rates (Sahu and Jha, 2022). It is a multi-cut, grown both as a sole crop and mixed with other fodder crops such as oats (*Avena sativa*) and barley (*Hordeum vulgare*) (Saad, 2012). Being leguminous forage crop, enhance soil fertility by contributing approximately 33–66 kg N ha^{-1} and this not only reduce the dependence on synthetic fertilizer but also encourage sustainable agriculture farming (Tufail *et al*., 2020). It is adaptable to a range of Agro-ecological zones and thrive well in loamy soils with neutral to slightly alkaline pH (Singha *et al*., 2020). It is a strategic forage crop due to low input requirement, restorative nature and sustainable agriculture production (Iannucci *et al*., 2001). It can remediate heavy metal contaminated soils and contributes to carbon sequestration (Ali *et al*., 2012).

The livestock sector faces growing challenges of climate change, land degradation, water scarcity, and inadequate feed resources. Feed scarcity both in quantity and quality has been cited as the major constraint to livestock productivity (Khan *et al*., 2019, Salma *et al*., 2020). Adverse climatic conditions reduce fodder quality, leading to reduced milk yield and composition (Ahmad *et al.,* 2024). Temperature fluctuations effect the yield, growth, and phenology of berseem and it perform best at 18–25 °C (Nand *et al*., 2018) below 10 °C or above 29 °C significantly hinder its germination and overall growth (Pal and Jain, 2023).

In current climate challenges, both the yield and quality of berseem are at risk and demand the climate-resilient, nutrient-rich, and high-yielding varieties. Despite its significance, limited efforts have been made to develop genotypes that combine heat tolerance with superior forage quality. Most existing varieties thrive only under optimal conditions and lack adaptability to climatic stress. To address this gap, the Fodder Research Sub-Station in Faisalabad has developed AARI Berseem-2024, a climate-smart variety characterized by high nutritional value and superior forage yield, recommended for general cultivation across Punjab.

MATRIALS AND METHODS

The breeding for the development of berseem candidate line “AARI Berseem-2024” was started during 2013-2014 at Fodder Research Sub-Station, Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. This was developed by the composite selection breeding method. Composite selection sustains broad genetic diversity and more suited for traits influenced by multiple genes in cross-pollinated crops like berseem. The initial parental material involved four local and one exotic varieties. The local accessions were BGP-09, BGP-17, BGP-29, BGP-37 and the exotic one was PI 468401 originated from Portugal (Table 1 and Figure 1). These exotic lines were initially acclimatized to local conditions. During the Rabi 2013–14, seeds from the five selected accessions were pooled and the resulting composite population was sown over a 90 ft × 60 ft using the broadcasting method. Over the next two seasons (2013–14 and 2014–15), the population was allowed to undergo natural open pollination to enhance genetic variability. In the third season (2015–16), both open pollination and the first cycle of mass selection were conducted concurrently. From 2016–17 to 2017–18, mass selection continued for two more consecutive years to achieve uniformity. After finding uniformity, the candidate line (FB-01-2018) was evaluated in Preliminary Green Fodder Yield Trial at Fodder Research Sub Station during 2018-2019. During 2019-20, tested in Advance Green Fodder Yield Trial. During 2020-21, Zonal Green Fodder Yield Trial along with standard checks conducted at 3 locations Faisalabad, Sargodha, Bahawalpur. National Uniform Fodder Yield Trials were conducted throughout the country at different locations during 2021-22 and 2022-23. AARI-Berseem was also studied at Fodder Research Sub-Station, AARI, Faisalabad for consequential factors. Anmol Berseem and Berseem Agati were used as check varieties. All parameters were reconfirmed during the Distinctness, Uniformity, and Stability (DUS) testing conducted by the Federal Seed Certification and Registration Department (FSC&RD), Islamabad during 2022-23 and 2023-24 (Figure 2).

Agronomic Parameters

Agronomic trials like sowing date, seed rate and fertilizer requirement were determined during 2021-22 and 2022-23 at Fodder Research Sub Station, AARI, Faisalabad under the supervision of Forage Production Section (Agronomy), AARI, Faisalabad. The promising variety “AARI Berseem-2024” along with standard checks was sown across eight different sowing dates from 15th September to 30th December at 15-day intervals. Seven NPK fertilizer treatments (in kg/ha) were applied as follows: D1 (0-0-0), D2 (24-70-20), D3 (26-75-22), D4 (28-80-25), D5 (30-85-28), D6 (32.5-87.5-30), and D7 (35-90-35). The candidate line was also sown at four different seed rates 10 kg/ha, 15 kg/ha, 20 kg/ha, and 25 kg/ha to fix best suited seed rate.

Table 1. Characteristics of Parental Accessions incorporated in AARI Berseem-2024.

Genotype	Key Traits	Unique Features
BGP-09	Large, dark green leaves; Late maturity	Enhanced biomass due to prolonged vegetative growth
BGP-29	High seed yield; Uniform maturity; High crude protein; Excellent fall recovery	Ideal for consistent quality and extended forage availability
P1468401	Erect growth; Vigorous; Late maturity; White flower	Contributes to plant architecture and delayed senescence
BGP-37	Vigorous growth; High crude protein; High seed yield	Enhances nutritional value and productivity
BGP-17	High regrowth; White flowers; High 1000-seed weight	Supports rapid recovery and strong seed performance

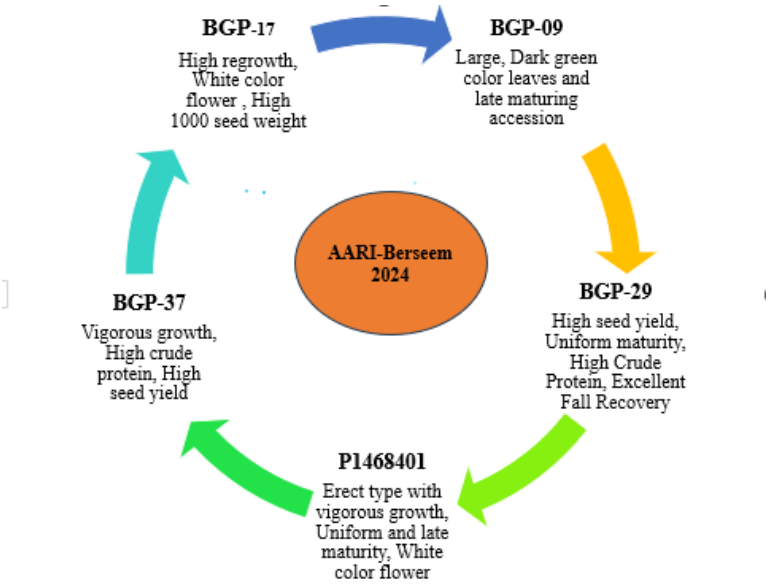

Figure 1. Characteristics of Parental Accessions incorporated in AARI Berseem-2024

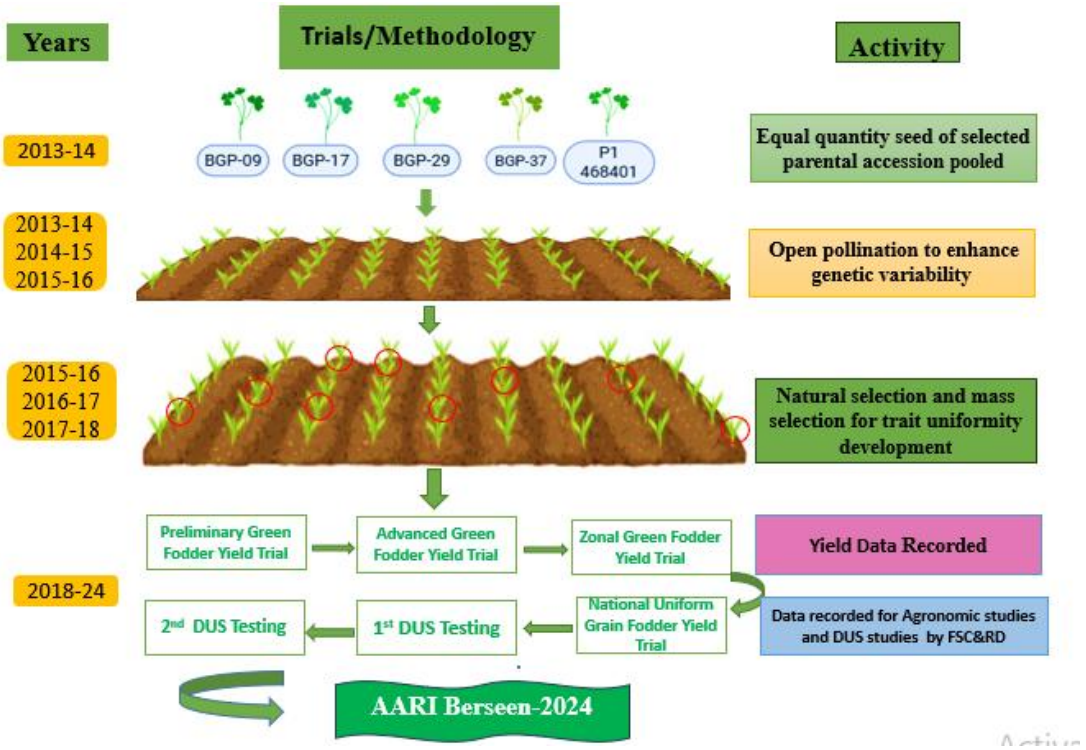

Figure 2. Diagrammatic sketch of AARI Berseem-2024 variety development process.

Pathological and Entomological Parameters

During the 2021–22 and 2022–23 growing seasons, the pathological traits of the promising line "AARI Berseem-2024" including resistance to root rot, stem rot, crown rot, and white mold were evaluated under artificial inoculation conditions at the Plant Pathology Research Institute, Faisalabad. Pathogen inoculum was prepared from cultured isolates and applied as a standardized spore to ensure uniform disease pressure. Post-inoculation high humidity was maintained to facilitate infection. Disease severity was scored 15–21 days after inoculation using a 1–9 scale as 1 indicated no visible symptoms and 9 represented severe infection or plant death. Concurrently, entomological parameters were assessed at the Entomological Section of the Ayub Agricultural Research Institute, Faisalabad.

Biochemical parameters

The proximate analysis of candidate line AARI BERSEEM-2024" was conducted by Biochemistry Section, AARI, Faisalabad using procedures recommended by AOAC (1990).

Palatability

Palatability was carried out at the Agronomy Forage Production Section, Faisalabad during 2022-23 using a standardized feeding trial with no-choice feeding method under controlled conditions. Animals of uniform breed and physiological status were offered equal quantities of fodder, and intake was recorded over a fixed period. It was calculated as

$$Palatability(\%) = \frac{Offered\ fodder - Leftover\ fodder}{Offered\ fodder} \times 100$$

DNA Fingerprinting

DNA fingerprinting of the promising variety FB-01-2018 was accompanied at the Department of Agricultural Biotechnology Research Institute, AARI, Faisalabad. DNA was extracted using the CTAB method and concentration and quality were assessed via Nanodrop spectrophotometry and confirmed through 0.8% agarose gel electrophoresis. A total of 50 Simple Sequence Repeat (SSR) markers including TassR and IGFRI series primers (e.g., TassR-66, TassR-68, IGFRI-SSR4, IGFRI-SSR11) were selected. 20 µL PCR reaction containing template DNA, PCR buffer, $MgCl_2$, dNTPs, forward and reverse primers and Taq DNA polymerase was used. The thermal cycling involved initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55–60°C for 30 seconds and extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes. PCR amplicons were separated on 3.5% agarose gels, stained with ethidium bromide and visualized under UV light. Fragment sizes were estimated using a 100 bp DNA ladder.

Statistical Analysis

Throughout the growth period data were subjected to analysis of variance (Steel *et al.,* 1997) and mean comparison test (Tukey, 1949). The candidate variety showed significant differences for all traits.

RESULTS AND DISCUSSION

Yield performance at station trials

The newly developed berseem variety AARI-Berseem was compared in preliminary and advanced fodder yield trials at Fodder Research Sub Station, Faisalabad during Rabi 2018-19 to 2019-20 in comparison with checks Anmol berseem and Berseem Agati. In both stational yield trials the promising line performed better than checks. In the preliminary yield trial, it gave 7.1 % higher green fodder yield (119.18 t/ha) as compared to check Anmol Berseem (112.37 t/ha) and Berseem Agati (110.32). In the advance yield trial, it gives 5.8 % high green fodder yield (120.34 t/ha) in comparison to check Anmol Berseem (117.4 t/ha) and Berseem Agati (109.9 t/ha).

Yield performance at zonal trials

The berseem variety AARI-Berseem was also accessed at three different locations of the Punjab province in zonal fodder yield Trials during Rabi 2020-21. The candidate line produced 8.8 % higher green fodder yield (122.6 t/ha) than checks Anmol Berseem (114.9 t/ha) and Berseem Agati (110.38 t/ha). It was recommended for inclusion in the National Uniform Fodder Yield Trial based on its superior performance in the Zonal trials.

Yield performance at national trials

The National Coordinator for Fodder at the Pakistan Agricultural Research Council (PARC) Islamabad conducted the National Uniform Fodder Yield Trial (NUFYT) during 2021-2022 and 2022-2023 to check its yield and adaptability at different agro-climatic zone. The candidate variety AARI Berseem-2024 produced 7.3 % more forage yield (154.47 t/ha) in 2021-22 and 7.2 % higher (125.08 t/ha) in 2022-23 in comparison to checks (Figure 3). It recorded green fodder yields 154.47 t/ha, consistently outperforming local checks like Anmol Berseem and Berseem Agati. These results are

highly promising, not only at the national level but also internationally, as global berseem yields typically range between 40 and 70 t/ha under standard agronomic practices (Abou El-Enain *et al.*, 2019). (See Supplementary Table 1 for details).

Agronomic parameters

Planting date trials

Trials on planting date were conducted at Fodder Research Sub Station, AARI, Faisalabad under the supervision of the Forage Production Section (Agronomy), AARI, Faisalabad, during 2021-22 and 2022-23 to fix specific agronomic requirements of the candidate variety AARI-Berseem. It was sown at eight different dates from 15th September to 30th December at 15-day intervals during 2021-22 and 2022-23. Sowing dates trial results demonstrated that AARI Berseem-2024 sown on 30th October gave a maximum green forage yield (102.5 t/ha) whereas produced a minimum green forage yield (56.12 t/ha) when planted on 15th September during 1st year of study 2021-22 (Figure 4).

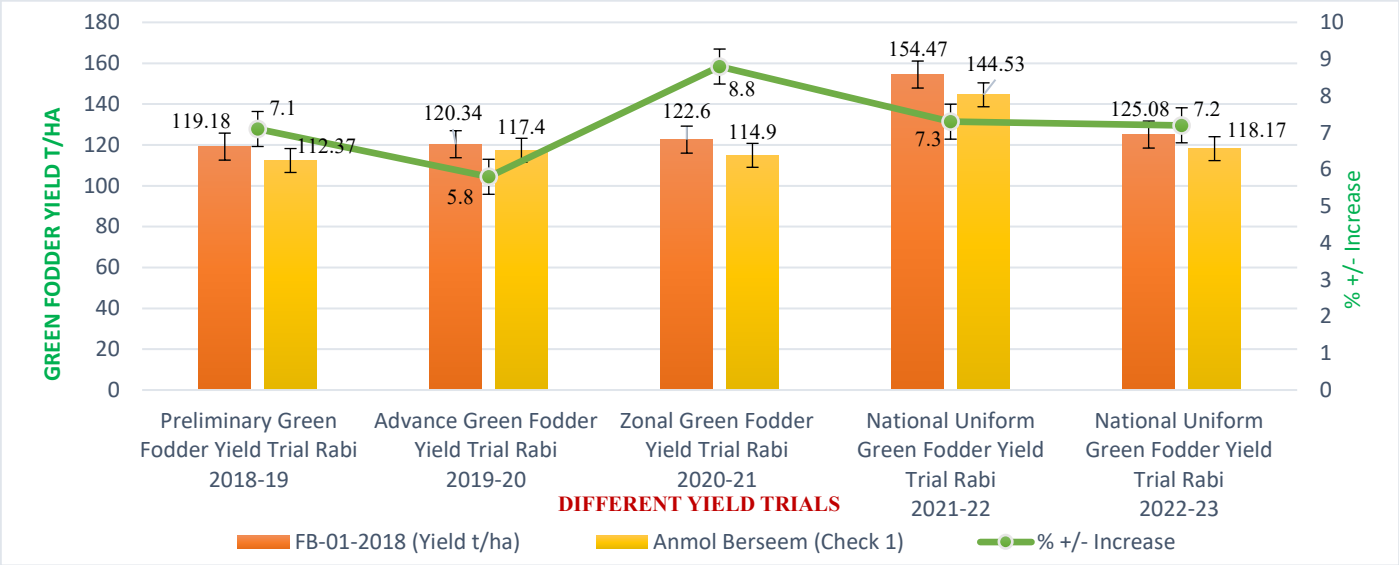

Figure 3. Green fodder yield (t/ha) of AARI Berseem-2024 (FB-01-2018) and check variety Anmol Berseem evaluated under different yield trials conducted during Rabi seasons from 2018 to 2023. Each bar represents the mean of three replications. Error bars indicate standard error of the mean (± SE). The green line indicates the percentage increase of AARI Berseem-2024 over the check variety in each trial.

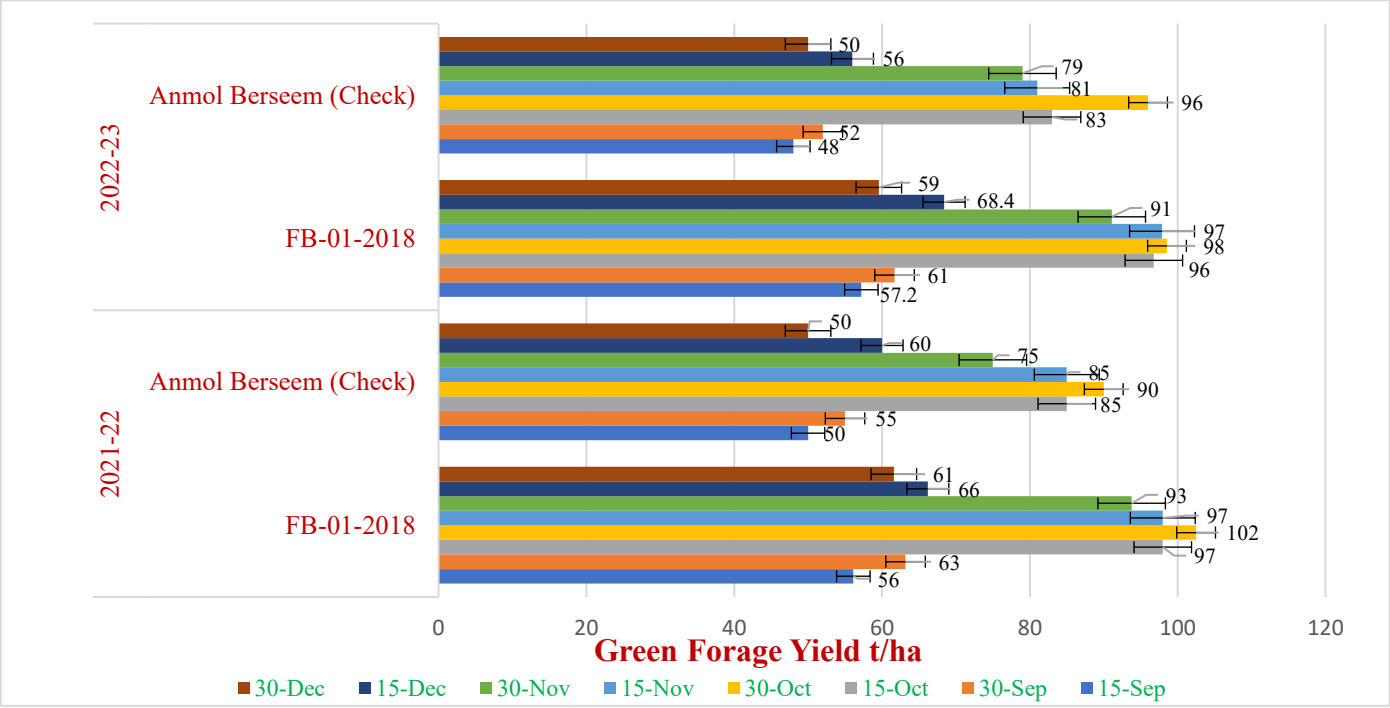

Figure 4. Green forage yield (t/ha) of FB-01-2018 and Anmol Berseem (Check) recorded at different planting dates during Rabi seasons of 2021–22 and 2022–23. Each bar represents the mean of three replications. Error bars (±SE) indicate standard error of the mean.

Likewise, during the 2nd year of study (2022-23) it produced a maximum green fodder yield (98.56 t/ha) when sown on 30th October and minimum (57.2 t/ha) on 15th September. On an overall basis, it was observed that the variety AARI-Berseem sown on 30th October produced significantly higher green forage yield than the crop sown before or after that date. This variety can be sown successfully from 15th October to 15th November, and our results were in accordance with (Din *et al*.,2014).

Seed rate trials

To find out the optimum seed rate of Berseem, a trial was conducted during 2021-22 and 2022-23 with seed rates of 10,15,20,25 kg/ha. The results (Figure 5) revealed that AARI Berseem-2024 produced maximum green fodder yield (97.98 t/ha) at 20 kg/ha seed rate. Seed rate is critical in the growth and development of berseem and highest seed yield detected for Agati Berseem-2002 at 20 kg/ha (Tufail *et al*., 2019).

Fertilizer dose trials

To find the accurate fertilizer requirement of AARI Berseem-2024 trial was conducted at Fodder Research Sub Station, ARRI, Faisalabad during 2021-22 and 2022-23. The results (Figure 6) revealed that it responded best to the fertilizer dose of 32.5-87.5-30 NPK resulting in a high green forage yield of 98.98 t/ha. Being a leguminous crop, an adequate quantity of phosphorus is required for better nodulation. More phosphorus and potassium are required as compared to cereals due to its dual-purpose plant growth and atmospheric nitrogen fixation. To improve nutritional quality and green forage yield P and K application is compulsory which increases milk production (Ayub *et al*., 2012).

Disease reaction

Response of Berseem variety AARI Berseem-2024 to the diseases was studied by Plant Pathology Research Institute, Faisalabad during 2021-22 and 2022-23. The results (Table 2) showed that it is moderately resistant to Root, Crown and Stem Rot and resistant to White Mold.

Quality characteristics

Nutritional quality parameters of green forage berseem variety AARI Berseem-2024 in comparison to checks Anmol Berseem were tested at Biochemistry Section, AARI, Faisalabad. An important parameter to estimate nutritional value of fodder is crude protein. Proximate analysis of green fodder presented in (Figure 7) illustrated that AARI Berseem-2024 variety has 22.3% dry matter, 19.24% crude protein, 21.8% crude fiber, 2.23% crude fat and 11.5% ash. The crude protein (19.24%) is significantly higher than that of Anmol Berseem (check). Protein in feed provides carbon skeletons that support the growth of cellulolytic bacteria that enhance carbohydrate digestion. This leads to increased production of fatty acids such as acetic and butyric acid, which are absorbed into the bloodstream and serve as precursors for milk fat synthesis in the udder (Rizqan *et al.,* 2024). These findings categorize AARI Berseem-2024 as a nutrients rich forage crop and aligned with sustainable livestock nutrition programs.

Climate smartness under drought and heat stress

AARI Berseem-2024 unveiled strong adaptability to drought and heat stress based on multi-season trials conducted under staggered sowing dates. During early sowing on 15th September when average day temperatures exceeded 35°C and relative humidity dropped below 40%, the variety sustained green fodder yields ranging from 56.12 to 57.2 t/ha. This indicates a notable tolerance to early-season heat and moisture deficit conditions. In contrast, sowing on 30th October under moderate temperatures (25–30°C) and improved soil moisture availability resulted in peak yields of up to 102.5 t/ha. These results confirm the variety's resilience across a range of climatic stresses. Moreover, its sustained performance under low fertility soils demonstrates efficient nutrient utilization. Disease resistance against root rot, crown rot, and white mold further enhances its climate-smart profile, making AARI Berseem-2024 a reliable choice for sustainable fodder production under climate change.

DNA fingerprinting

In accordance with the Plant Breeder's Rights Act of 2016, DNA fingerprinting of the promising variety FB-01-2018 was accompanied at the Department of Agricultural Biotechnology Research Institute, AARI, Faisalabad. DNA was extracted using the CTAB method and DNA concentration and quality were assessed via Nanodrop spectrophotometry and confirmed through 0.8% agarose gel electrophoresis. A total of 50 Simple Sequence Repeat (SSR) markers including TassR and IGFRI series primers were selected. Marker selection was based on the following criteria high polymorphism information content (PIC). Each SSR marker was validated for consistency, clarity of banding pattern, and amplification efficiency through PCR optimization and gel electrophoresis prior to the full-scale fingerprinting assay. The molecular analysis included two check varieties (Sandal Berseem and Anmol Berseem) and followed the SOPs laid out by the Ministry of Agriculture, Punjab. The details of SSR markers used for DNA fingerprinting are given in Supplementary File 1.

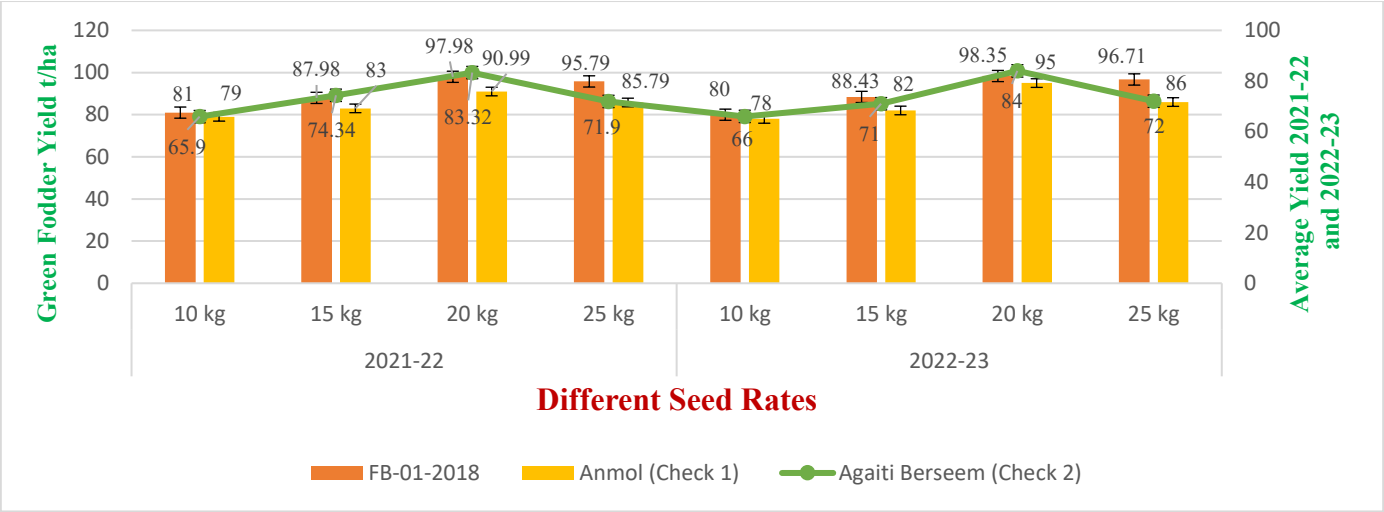

Figure 5. Effect of different seed rate on green fodder yield of AARI BERSEEM-2024 (candidate variety), Anmol Berseem (Check 1), and Agaiti Berseem (Check 2) during the Rabi seasons of 2021–22 and 2022–23. Each bar represents the mean of three replications. Error bars indicate the standard error of the mean (±SE).

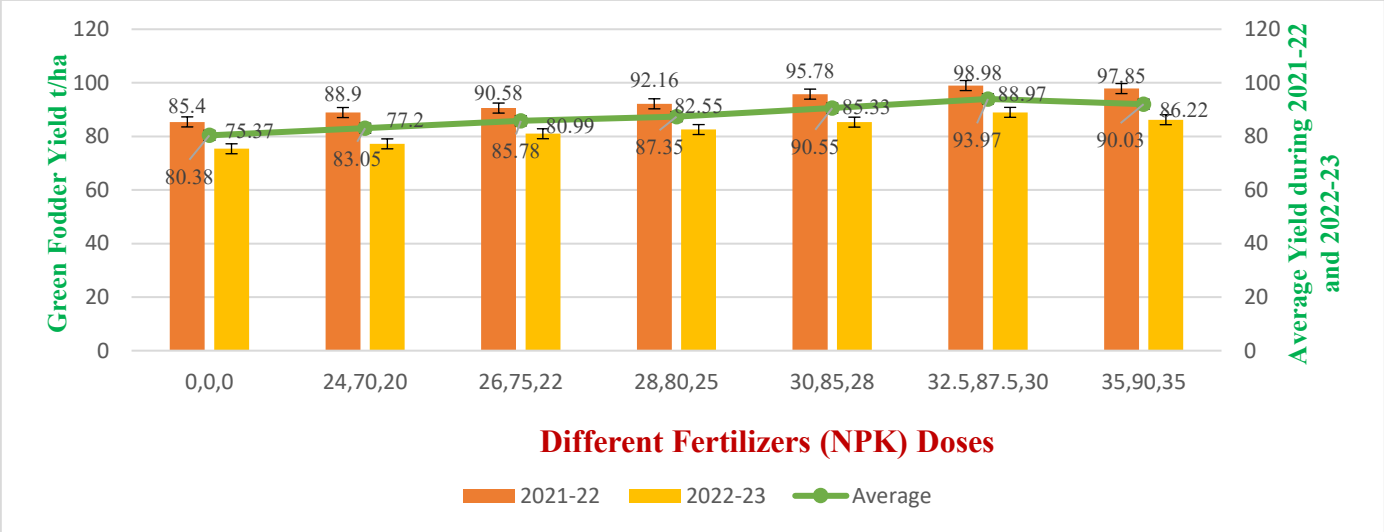

Figure 6. Effect of different NPK fertilizer doses on green fodder yield of AARI BERSEEM-2024 (candidate variety), Anmol Berseem (Check 1), and Agaiti Berseem (Check 2) during the Rabi seasons of 2021–22 and 2022–23. Each bar represents the mean of three replications. Error bars indicate the standard error of the mean (±SE).

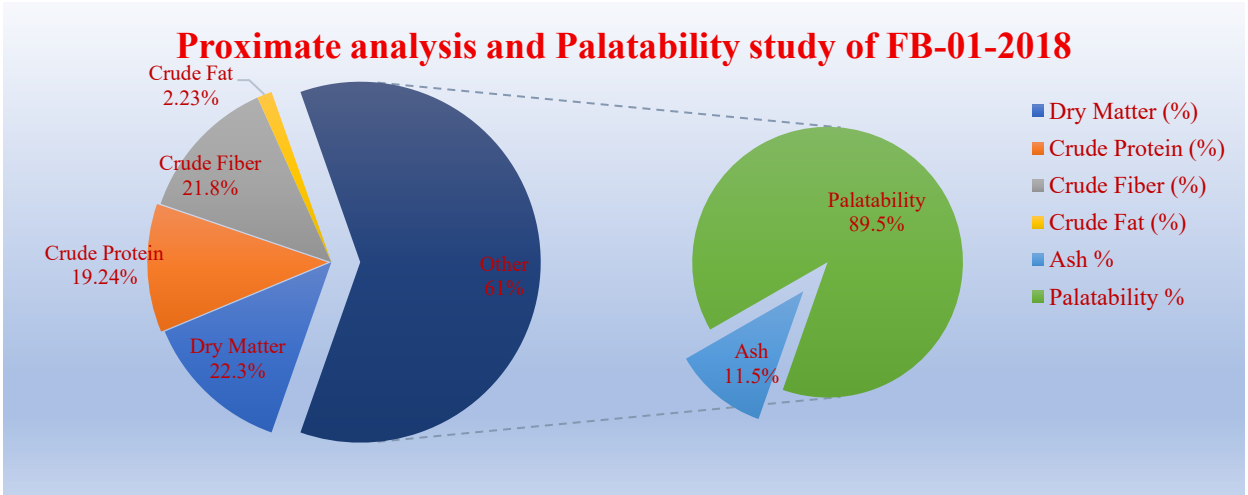

Figure 7. Proximate analysis and Palatability study of AARI BERSEEM-2024 (2022-23). Carried out at Biochemistry Section, Ayub Agricultural Research Institute, Faisalabad.

The genetic relationships among the entries were estimated using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering algorithm to develop a Cultivar Identification Diagram (CID). The genetic similarity coefficients among the genotypes ranged from 0.10 to 1.00, indicating a broad range of genetic diversity. The CID (Figure 8) highlighted significant dissimilarity of 55% with Sandal Berseem and 59% with Anmol Berseem. These results highlight the genetic uniqueness of FB-01-2018 and support its distinctness, uniformity, and stability (DUS), a key requirement for variety registration and potential protection under the Plant Breeder's Rights (Figure 9). The minutes of Variety Evaluation Committee (VEC) are given in the supplementary material (Supplementary File 2) which recommended this variety in 2024.

Table 2. Response of FB-01-2018 and Anmol Berseem (Check) to different diseases.

	Root Rot	Stem and Crown Rot	White Mold
FB-01-2018	MR	MR	R
Anmol Berseem (Check)	MR	MR	MR

Carried out by Pathological Research Institute, AARI, Faisalabad.

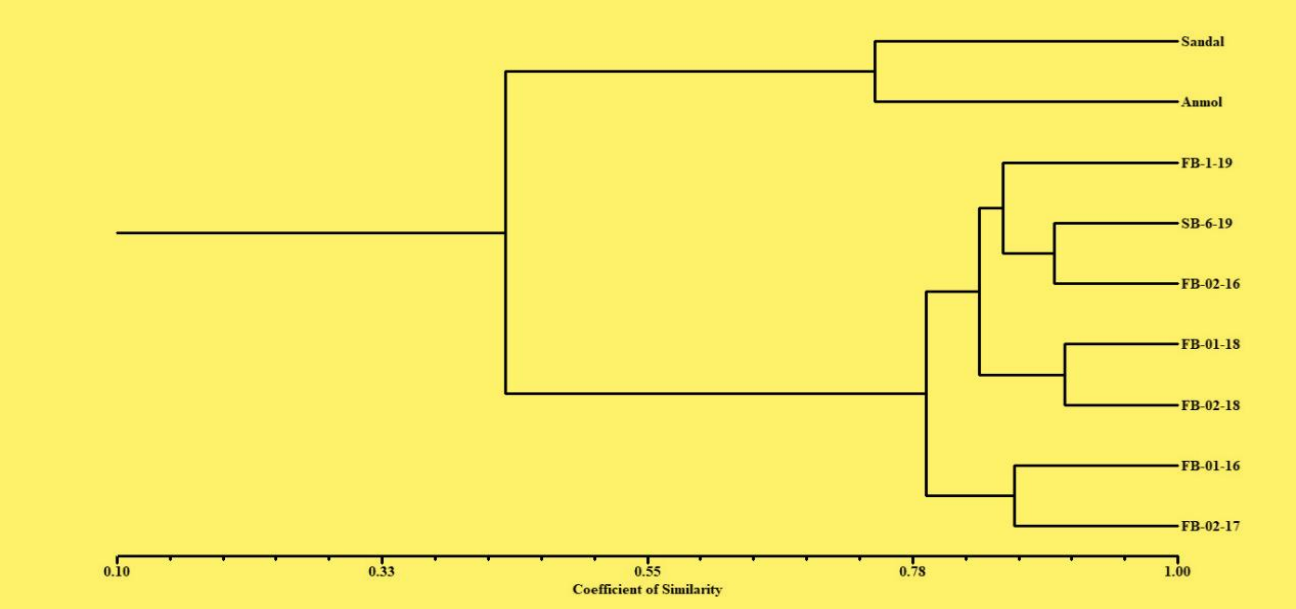

Figure 8. DNA Fingerprinting highlighted significant divergence of FB-01-2018 from the standard cultivars Sandal Berseem and Anmol Berseem.

Figure 9. Field view of FB-01-18 (AARI Berseem-2024) at flowering stage at Fodder Research Sub-Station, AARI.

CONCLUSION

AARI Berseem-2024 was developed by the Fodder Research Sub Station, ARRI, Faisalabad. It gives a higher green fodder yield than already approved commercial varieties Anmol Berseem and Berseem Agati. It has 22.3% dry matter, 19.24% crude protein,21.8% crude fiber, 2.23% crude fat and 11.5% ash, also has good palatability. It is moderately resistant to Root Rot, Crown Rot and Stem Rot diseases and resistant to White Mold. Due to its superior forage yield, high nutritional value and more adaptability to Agro-ecological zone, it is poised to replace the existing berseem varieties and is a game changer for livestock. It ensures better growth, health, and milk production in milch animals. Based on superior traits, availability for extended time, and adaptability to diverse climates, it ensures an uninterrupted supply of fodder during lean periods and ultimately enhance milk and meat production. However, the widespread adoption of this variety depends significantly on the availability of quality seeds. Strengthening the seed supply chain is essential to fully realize the commercial potential of AARI-Berseem 2024. Moreover, continued multi location monitoring under varying climatic conditions is recommended to ensure its long-term stability and adaptability.

AUTHOR CONTRIBUTIONS

Qamar Shakil: Supervised the research, Amna Kanwal: Writing – original draft, Zohab Asif: Writing – review & editing, Asma Parveen: Writing – original draft, Muhammad Humaiyon: Assisted in data collection, Javed Iqbal: Writing – review & editing, Khalid Hussain: Proof read the manuscript, Asif Ali Khan: Variety approval documentation, Zulfiqar Ali: Variety approval documentation, Mujtaba Ali: Data Analysis, Shakra Jamil: DNA Fingerprinting, Shamsa Kanwal: DNA Fingerprinting.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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