



PLANT BREEDING &
GENETICS

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QS BERSEEM: A BREAKTHROUGH IN HIGH-YIELD AND CLIMATE-ADAPTED FORAGE PRODUCTION

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ABSTRACT

Food security is among one of the sustainable development goals. In the scenario of adverse climatic conditions, food security is becoming a global challenge that impacts not only human being but also livestock. Currently, the biggest concern of the farmers of the country is the lack of climate-adaptive fodder varieties and scarcity of green fodder during lean period. To address these critical challenges a new climate-resilient berseem variety "QS-Berseem" has been developed by the Fodder Research Sub-Station, Ayub Agricultural Research Institute, Faisalabad which have high green fodder yield, crude protein, palatability, digestibility and nutritional quality. This variety is an outcome of composite selection from foundational parental population developed by pooling five high-performing accessions, three local BGP-30, BGP-43, BGP-77, and two exotic PI 343166 43, PI 343166 during 2013-2024. It proved its value in all yield trials and outperformed the current existing berseem varieties. It produced 5.9% and 3.8% higher green fodder yield in comparison to check varieties Anmol Berseem and Berseem Agati during 2021-22 & 2022-23 respectively, in National Uniform Fodder Yield Trials. Furthermore, it has 22.3% dry matter, 17.94% crude protein, 23.1% crude fiber, 2.15% crude fat and 11.9% ash, and good palatability. The new variety showed better tolerance against Root Rot, Crown Rot & Stem Rot diseases. Agronomic studies revealed that it aligns more efficiently with current agronomic practices. So, this variety could be a promising choice for farmers as it is capable of overcoming fodder shortage during lean periods while contributing to enhanced milk and meat production, improved animal health and overall livestock sector particularly under changing climatic conditions.

KEYWORDS: *Trifolium alexandrinum*; climate smart; drought tolerant; forage yield; high nutrition; Pakistan

INTRODUCTION

Berseem (*Trifolium alexandrinum* L.) is a well-known leguminous crop among 240 species of *Trifolium* genus having $2n=2x=16$ chromosome. It is considered king of fodder due to its better nutritional profile, high palatability, and good digestibility (Arif *et al.*, 2020). Its center of origin is Syria and was introduced to Pakistan as a Rabi crop in 1904 (Sindh) and 1924 (Khyber Pakhtunkhwa) and currently grown on maximum area after Egypt (Singh *et al.*, 2020). Due to multiple cuts nature, it ensures prolonged fodder supply from November to May and is highly palatable and offers 62% total digestible nutrients. Its nutritional profile is 18-20% crude protein, 20-23% crud fiber, 10-12% dry matter and 8-10% ash contents (Yadav *et al.*, 2015; Rady *et al.*, 2022). Being a leguminous forage crop and symbiotic association with nitrogen-fixing bacteria it fixes atmospheric nitrogen and thereby

recovers soil fertility by adding 33-66 kg nitrogen per/ha (Tufail *et al.* 2020). Moreover, due to its high digestibility, palatability, succulent nature, high crude protein and total digestible nutrients, it invigorates lactation in milk animals and is known as a milk enhancer (Sourav *et al.*, 2023). In addition to forage, this crop is also cultivated for seed grains and as trap crop against various pests (Wagan *et al.*, 2015). Livestock is an important sector of the agriculture economy; it contributes 60.84 in value addition and 14.63 % to the national GDP (GoP 2023-2024). Forage crops are inevitable to the livestock industry as cost-effective sources of high-quality nutrition, essential for reducing dependence on concentrates and improving animal performance (Pereira *et al.*, 2022). The Livestock industry is imperiled by climate change, global warming, ever-increasing population and declining of forage crops areas (Salma *et*

al.,2020). Drought and heat stress reduce forage biomass and quality by increasing lignin content, lowering crude protein, and disrupting mineral balance (Seleiman et al., 2021; Khatun et al., 2021).

Adverse climate reduces fodder quality, impacting milk yield and composition. Nutritious forages like berseem boost milk production, while poor-quality feed like wheat straw limits it. Crude protein enhances milk protein, whereas high fiber and lignin lower digestibility and yield (Ahmad et al., 2024). Temperature fluctuations significantly impact the yield, development, and phenology of berseem, which thrives optimally between 18–25 °C (Nand et al., 2018). Recent studies suggest that temperatures below 10 °C or above 29 °C significantly reduce berseem germination and growth (Pal and Jain,2023).

Therefore, under current climate challenges, both the productivity and quality of berseem fodder are under threat and highlight the urgent need for climate-smart, highly nutritional, high forage yield varieties of Berseem. Despite its importance, limited research has focused on developing and evaluating berseem genotypes that combine heat tolerance with superior forage quality. Most existing varieties perform well under optimal conditions but lack resilience to diverse climates. To address this gap, the Fodder Research Sub-Station, Faisalabad, has developed QS-Berseem-2024, a newly released variety combining heat resistance and enhanced forage yield.

MATERIALS AND METHODS

The breeding for the development of the berseem

candidate line “QS Berseem-2024” was started during 2013-2014 at Fodder Research Sub-Station, Faisalabad. The candidate line was developed by utilizing the composite selection breeding method. The foundational parental material involved five high-performing accessions, comprising three local and two exotic varieties. The local accessions were BGP-30, BGP-43, BGP-77 and the exotic ones were PI 343166, PI 343166 (Fig. 1). These exotic lines were initially acclimatized to local conditions before their seeds were collected. During the Rabi season of 2013–14, seeds from the five selected accessions were pooled, and the resulting composite population was sown over a one-kanal area using the broadcasting method. This population was subjected to open pollination for three successive generations to promote natural recombination and enhance genetic variability. Natural cross-pollination was facilitated by field pollinators under open environmental conditions. To ensure the preservation of genetic diversity, a large number of vigorous and true-to-type plants were retained each generation, while off-type and undesirable plants were carefully rogued out before flowering. During 2015-16, the mass selection was employed for three years to obtain uniformity. Plants superior in plant height, leaves-to-stem ratio, leaf area, diseases/ insect resistance, and mainly high fodder yield were retained. After finding uniformity, the candidate line was evaluated in Preliminary Green Fodder Yield Trial at FRSS during 2018-2019. During 2019-20, tested in Advance Green Fodder Yield Trial. During 2020-21, Zonal Green Fodder Yield Trial conducted

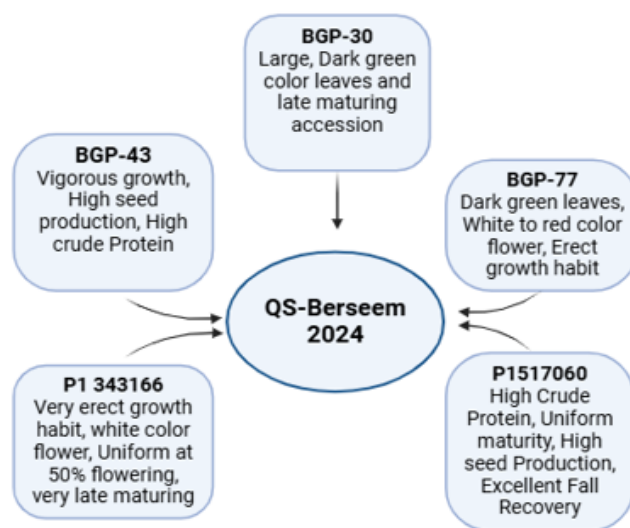


Fig. 1. Characteristics of parental accessions incorporated in QS-berseem 2024 (BGP-30, BGP-43, BGP-77, P1 343166, P1 517060)

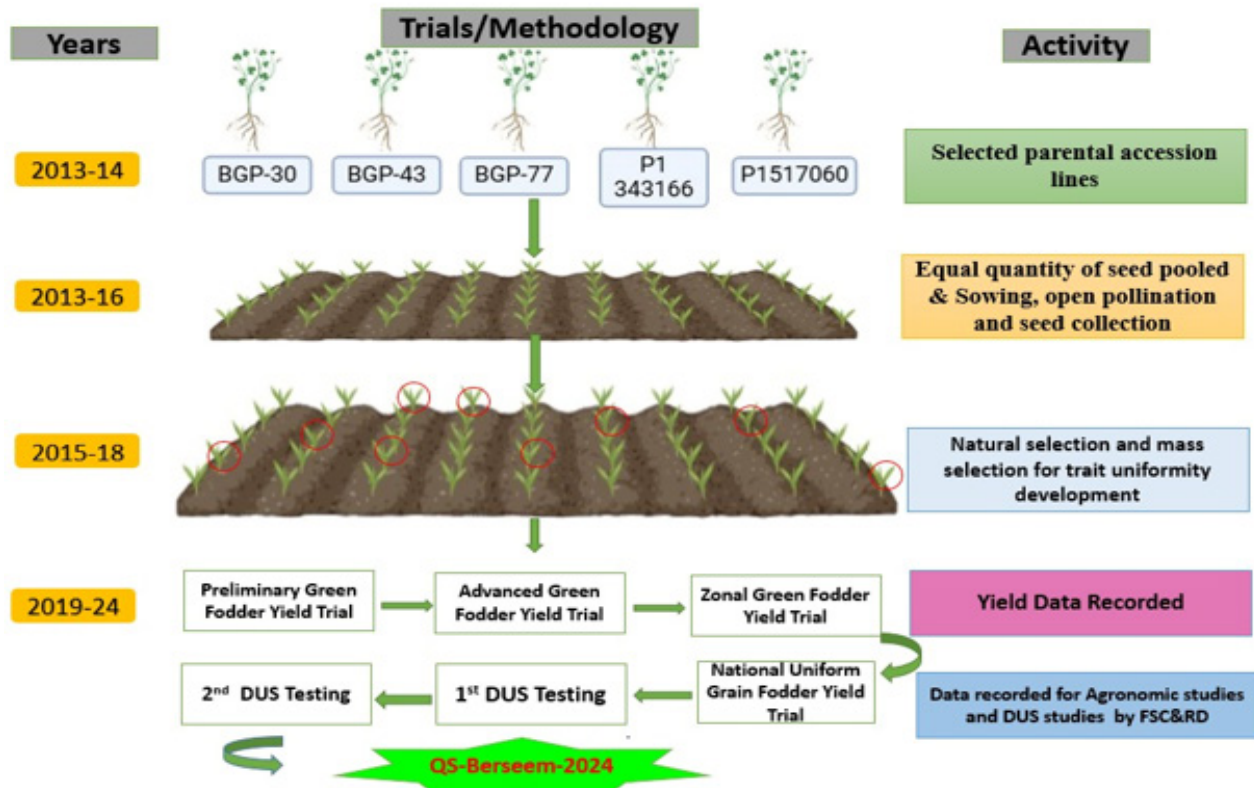


Fig. 2. Detail of QS-berseem variety development process

along with standard check replicated yield trials at 3 locations. National Uniform Fodder Yield Trials were conducted throughout the country at different locations during 2021-22 and 2022-23. During these trials, Anmol Berseem and Berseem Agati were used as check varieties due to their widespread cultivation and consistent performance under local conditions. Anmol Berseem is known for its excellent green fodder yield, while Berseem Agati is recognized for its resistance to certain diseases. QS Berseem was also studied at Fodder Research Sub-Station, AARI, Faisalabad for consequential factors. Anmol Berseem and Berseem Agati were used as checks. During DUS test by the Federal seed certification and Registration department, Islamabad (FSC & RD), all factors were reconfirmed. In all yield trials Randomized complete block design (RCBD) was used with 3 replications (Fig:2). **Agronomic parameters:** Agronomic requirements like sowing date, fertilizer dAgronomic requirements like sowing date, fertilizer dose and seed rate 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 “FB-02-2017” line as well as checks, were sown at 8 different sowing dates

15th Oct to 15th Nov after 15 days intervals and 8 different doses of NPK applied in kg/ha were; D1 (0-0-0), D2 (24-70-20), D3 (26-75-22), D4 (28-80-25) and D5 (30-85-28), D6 (32.5-87.5-30), 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.

Pathological and entomological parameters: During the 2021-22 and 2022-23, pathological traits (resistance against root rot, stem rot and crown rot, white mold) of promising line FB-02-2017 were ascertained under artificial inoculation by Plant Pathology Research Institute, Faisalabad, simultaneously entomological parameters (aphids, armyworms, leafhoppers) were determined at Entomological section of Ayub Agricultural Research Institute, Faisalabad. Disease severity was assessed using a standard 0–9 visual scoring scale based on the percentage of infected plant tissue.

Biochemical parameters: The proximate analysis of candidate line FB-02-2017 was conducted by Bio Chemistry Section, AARI, Faisalabad using procedures recommended by AOAC (1990).

Palatability: Palatability was carried out at the Agronomy Forage Production Section, Faisalabad

during 2022-23.

STATISTICAL ANALYSIS:

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Throughout the growth period analysis of variance (Steel *et al.*, 1997) and mean comparison test (Tukey, 1949) at a 5% significance level were applied to all the collected data for forage yield traits to find out the genetic variations among the recorded traits.

RESULTS AND DISCUSSION

Yield performance at station trial: The newly developed berseem variety QS-Berseem was compared in preliminary and advanced fodder yield trials at Fodder Research Sub Station, Faisalabad during 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 15.6% higher green fodder yield (128.52 t/ha) as compared to check Anmol Berseem (112.37 t/ha) and Berseem Agati (110.32). In the advance yield trial, it gives 12.9% high green fodder yield (127.8 t/ha) in comparison to check Anmol Berseem (117.4 t/ha) and Berseem Agati (109.9 t/ha).

Yield performance at zonal trial: The berseem variety QS-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 a 9.7% higher green fodder yield (123.23 t/ha) than checks Anmol Berseem (114.9 t/ha) and Berseem Agati (110.38). It was endorsed to the National uniform fodder yield trial due to its better performance in the

Zonal trial.

Yield performance at national trial: The National Coordinator for Fodder at the National Agriculture Research Center (NARC) Islamabad conducted the National Uniform Fodder Yield Trial (NUFYT) during 2021-2022 & 2022-2023 to check its yield and adaptability at different agroclimatic zone. The candidate variety QS-Berseem produced 2.5% more forage yield (152.44 t/ha) in 2021-22 and 2.4% higher (73.17 t/ha) in 2022-23 in comparison to checks (Fig. 3). The new variety QS-Berseem approved by Punjab Seed Council based on high forage yield, high dry matter, high crude protein, high palatability and digestibility and resistant to insect/pest and diseases. Its green fodder yield ranged from 123.23 to 152.44 t/ha, with consistent superiority over local checks such as Anmol Berseem and Berseem Agati. These results are not only promising at the national level but also stand out when compared to international yield. Globally, green fodder yields of berseem typically range from 40 to 70 t/ha under standard agronomic practices (Abou El-Enain *et al.*, 2019).

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 QS-Berseem. It was sown at eight different dates from 1st September to 31 December at 15-day intervals during 2021-22 and 2022-23. Sowing dates trial results are demonstrated in figures 4 and 5. QS-Berseem sown on 30th October

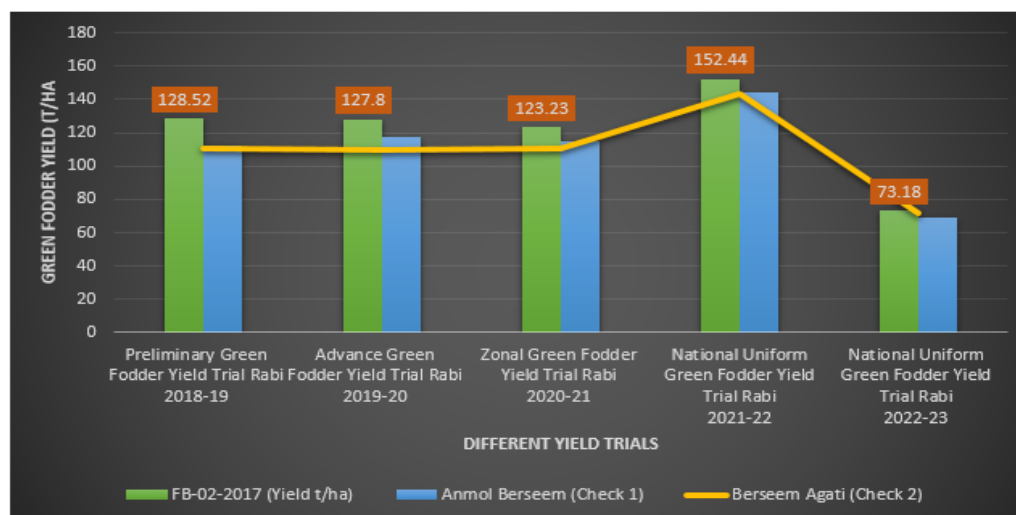


Fig. 3: Yield analysis of FB-02-2017, Anmol berseem, Berseem agati

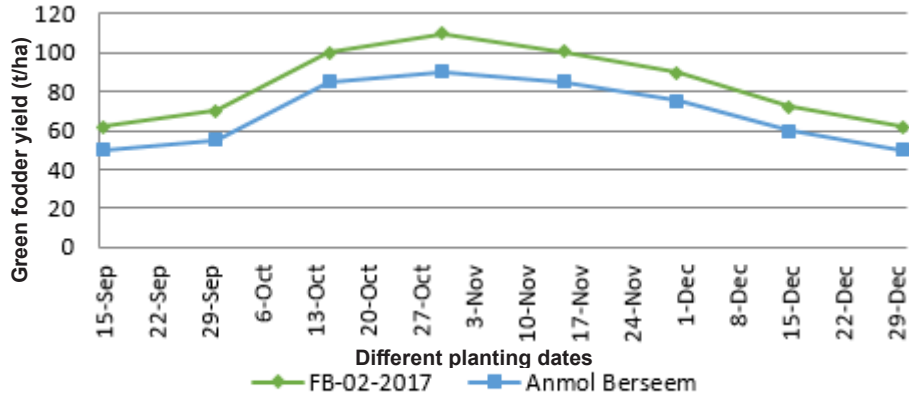


Fig. 4. Planting date trial of FB-02-2017(2022-23)

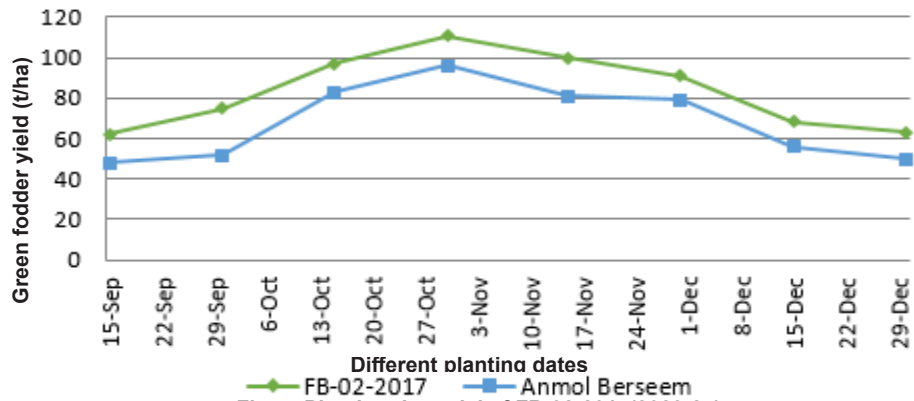


Fig. 5. Planting date trial of FB-02-2017(2023-24)

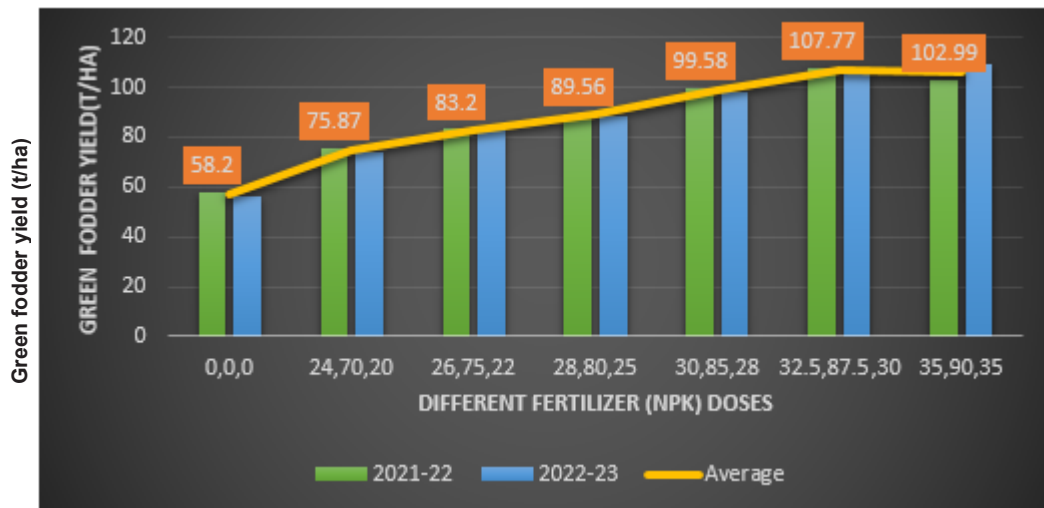


Fig. 6. Effect of different NPK fertilizer doses on green fodder yield of FB-02-2017

gave a maximum green forage yield (110.6 t/ha) whereas produced a minimum green forage yield (59.1 t/ha) when planted on 15 September. This variety can be grown successfully on 30th October based on favorable agro-environmental conditions at that time, which supported ideal seed germination, seedling establishment, and vigorous early growth and our results were in accordance with (Din *et al.*,2014).

Fertilizer dose trials: To find the accurate fertilizer

requirement of QS-Berseem trial was conducted at Fodder Research Sub Station, ARRI, Faisalabad during 2021-22 and 2022-23. The results (Fig. 6) revealed that the promising line responded best to the fertilizer dose of 32.5-87.5-30 NPK resulting in a high green forage yield of 106.94 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-

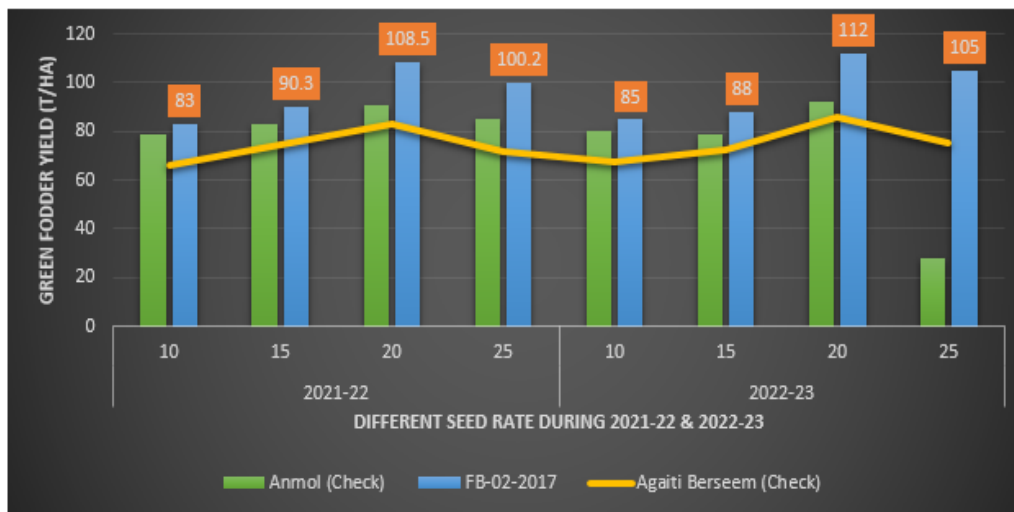


Fig. 7. Effect of different seed rate on green fodder yield of FB-02-2017

Proximate analysis and palatability study of FB-02-2017

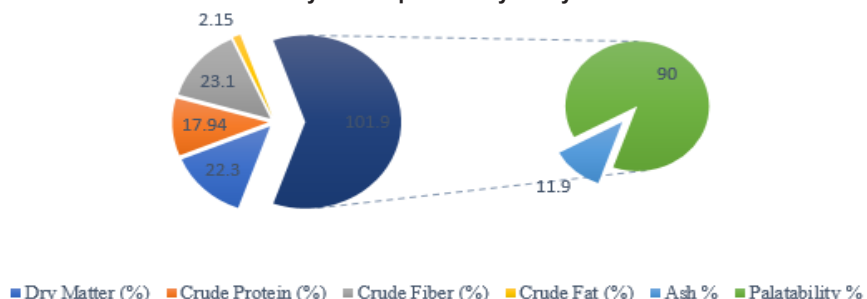


Fig. 8. Proximate analysis and palatability study of FB-02-2017 (2022-23)

Table No.1. Response of FB-02-2017 and Anmol berseem (Check) to different diseases

Sr No.	Variety name	Root rot	Stem and crown rot	White mold
1	FB-02-2017	MR	MR	R
2		MR	MR	R
3	Anmol berseem (Check)	MR	MR	MR
4		MR	MR	MR

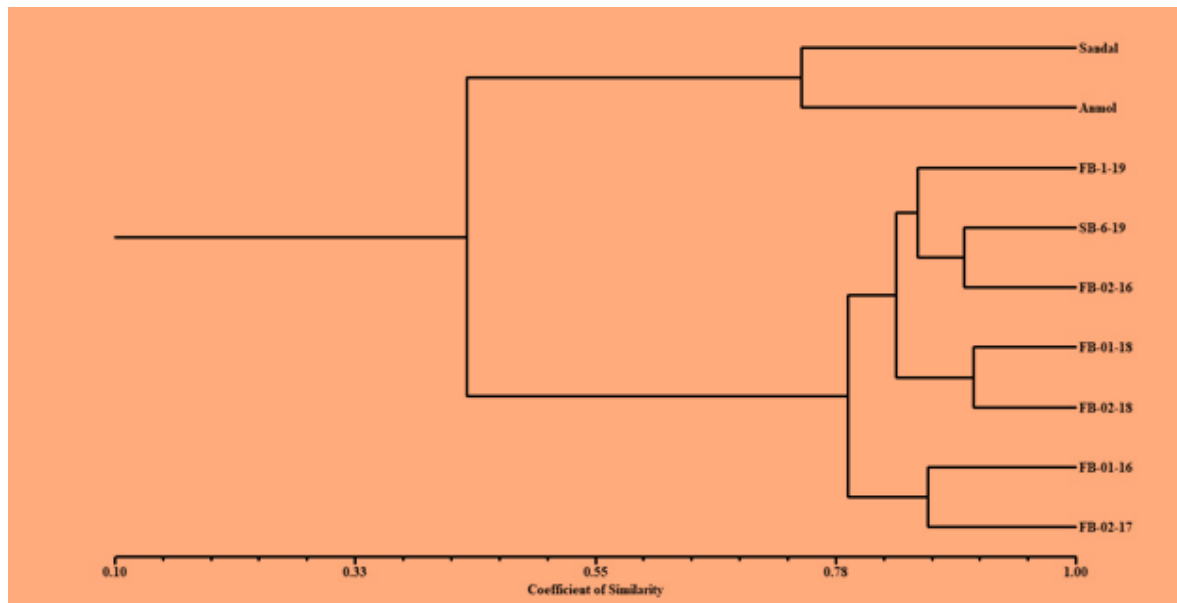


Fig. 9. DNA fingerprinting of FB-02-2017 from the standard cultivars Sandal berseem and Anmol berseem

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).

Seed rate trials: To find out the optimum seed rate of QS-Berseem, a trial was conducted during 2021-22 and 2022-23 with seed rates of 10,15,20,25 kg/ha. The results (Fig. 7) revealed that QS-Berseem produced maximum green fodder yield (108.5 t/ha) at 20 kg/ha seed rate. While higher seed rates can boost early biomass and ground coverage, they also lead to higher input costs, quality deterioration, and increased disease risk. An optimum seed rate (20 kg/ha) offers the best balance between yield, quality, and cost-effectiveness. 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).

Disease reaction: Response of Berseem variety QS-Berseem to the diseases was studied by Plant Pathology Research Institute, Faisalabad during 2021-22 and 2022-23 under both artificial inoculation and natural field conditions. The results (Table No.1) showed that it is moderately resistant to Root, Crown & Stem Rot and resistant to White Mold. In plant pathology Resistant (R) variety shows minimal to no symptoms under both artificial inoculation and natural conditions, with disease incidence typically less than 10–15% and no significant impact on yield or plant vigor. Moderately Resistant (MR) variety shows limited disease symptoms, with disease incidence ranging from 16–30%, and some reduction in vigor or yield may be observed under high disease pressure, especially

in conducive environments.

Quality characteristics: Nutritional quality parameters of green forage berseem variety QS-Berseem were tested at Bio Chemistry Section, AARI, Faisalabad. An important parameter to estimate nutritional value of fodder is crude protein. Proximate analysis of green fodder presented in (Fig. 8) illustrated that QS-Berseem variety has 22.3% dry matter, 17.94% crude protein,23.1% crude fiber%, 2.15% crude fat and 11.9% ash higher than the check Anmol Berseem. QS-Berseem crude protein (17.94%), compares to alfalfa (16–20%), a leading high-protein forage. Moreover, QS-Berseem has a lower crude fiber content (23.1%) than alfalfa (25–30%), suggesting better digestibility. This optimal balance of high protein and moderate fiber enhances feed efficiency and supports higher milk yields in dairy animals (Maurice *et al.*, 1985), making QS-Berseem a valuable choice for improving livestock productivity.

DNA fingerprinting: In accordance with the Plant Breeder’s Rights Act of 2016, DNA fingerprinting of the promising variety FB-02-2017 was accompanied at the Department of Agricultural Biotechnology, Agricultural Research Institute, AARI, Faisalabad. A total of 50 polymorphic Simple Sequence Repeat (SSR) markers were selected from a pool of pre-screened SSRs known to be highly informative for berseem and related forage legumes. 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 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 (Fig. 9) highlighted significant dissimilarity of 54% with Sandal Berseem and 59% with Anmol Berseem. These results highlight the genetic uniqueness of FB-02-2017 and support its distinctness, uniformity, and stability (DUS), a key requirement for variety registration and potential protection under the Plant Breeder's Rights.

CONCLUSION

QS-Berseem a heat resilient, drought tolerant and highly nutritional variety was developed by Fodder Research Sub Station, ARRI, Faisalabad. It was approved by the Punjab Seed Council in 2024 for general cultivation all over the Punjab. It gives a higher green fodder yield than already approved commercial varieties Anmol Berseem and Berseem Agati. It has 22.3% dry matter, 17.94% crude protein, 23.1% crude fiber, 2.15% crude fat and 11.9% ash, also has good palatability. It is moderately resistant to Root Rot, Crown Rot & Stem Rot diseases and resistant to White Mold. Due to its superior forage yield, high nutritional value, heat resilience, drought tolerance and more adaptable 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 and livestock productivity. However, the widespread adoption of this variety depends significantly on the availability of quality seed. To address this, seed multiplication programs are being initiated through public-private partnership to ensure timely and large-scale dissemination to farming communities. Strengthening the seed supply chain is essential to fully realize the commercial potential of QS-Berseem.

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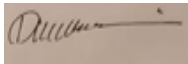
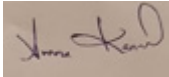
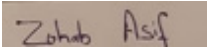
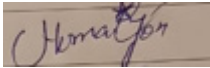
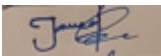



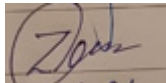
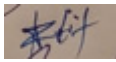
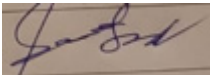
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