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Research article

Protein expression profiling at different developmental stages of ploidy series of guinea grass (*Megathyrsus maximus*)

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Abstract

Guinea grass (*Megathyrsus maximus* (Jacq.) Simon & Jacobs.) exhibits wide ploidy variation and diverse reproductive behavior, making it a valuable model for studying protein expression dynamics during reproductive development. In the present study, protein expression profiles were comparatively analyzed across different developmental stages and ploidy levels of *M. maximus* using SDS-PAGE as a preliminary profiling approach. A diverse ploidy series (3x, 4x, 5x, 6x, 7x, 8x, 9x and 11x) derived from a 4x progenitor was evaluated at pre-meiotic, meiotic, and post-meiotic stages. Protein extraction from inflorescence tissue followed by SDS-PAGE analysis identified protein polymorphism across ploidy levels and developmental stages, revealing stage-specific and ploidy-dependent variations in protein expression. The meiotic stage exhibited the highest protein polymorphism, suggesting an active meiotic regulation, while the post-meiotic stage showed a decline in meiosis-specific proteins, indicating regulatory transitions in gamete maturation. Higher ploidy levels (7x, 8x, 9x and 11x) demonstrated greater protein polymorphism, suggesting ploidy-dependent regulation of meiosis-associated proteins. Cluster analysis, based on Jaccard's similarity coefficient, grouped samples according to similarity in protein banding patterns across developmental stages and ploidy levels. The findings provide a comparative description of protein expression variation associated with reproductive development and ploidy differences in guinea grass and establish a baseline for future studies employing advanced proteomic and functional validation approaches.

Keywords: Apomixis, Fodder grass, *Megathyrsus maximus,* Polyploidy, Protein profiling, Reproductive biology, Guinea grass

Introduction

Megathyrsus maximus (Jacq.) Simon & Jacobs, commonly known as guinea grass, is an important forage species widely cultivated in tropical and subtropical regions due to its high biomass yield, adaptability, and nutritional quality (Ram and Trivedi 2013; Savidan, 2000). The species exhibits extensive ploidy variation and displays both sexual reproduction and apomixis, making it an attractive system for investigating reproductive development in polyploid grasses (Barcaccia and Albertini, 2013). Similar reproductive behavior has also been reported in *Sehima nervosum* (Dwivedi *et al*., 2024). Sexual reproduction in plants follows the classical meiotic pathway, leading to the formation of genetically diverse offspring through fertilization (Koltunow *et al*., 2011). In contrast, apomixis is an asexual mode of reproduction that bypasses meiosis and fertilization, resulting in clonal progeny genetically identical to the maternal plant (Spillane *et al*., 2001). This phenomenon has significant implications for plant breeding, as it offers the potential to fix superior genotypes across generations (Koltunow and Grossniklaus, 2003). In *M. maximus*, apomixis is primarily aposporous in nature, where the embryo sac develops from a somatic cell in the ovule rather than a meiotic product (Kaushal *et al*., 2018). This process involves three key stages: (1) the initiation of apospory, where one or more somatic cells near the megaspore mother cell differentiate into aposporous initial cells; (2) parthenogenesis, where the egg cell develops into an embryo without fertilization; and (3) autonomous

or pseudogamous endosperm formation, which may require fertilization for seed viability (Hojsgaard *et al.,* 2008; Roy *et al.,* 2019). Despite its agricultural importance, the molecular mechanisms underlying reproductive development and apomixis in *M. maximus* remain incompletely understood (Ozias-Akins and van Dijk, 2007). Previous studies have explored cytological, biochemical, and molecular aspects of apomixis in grasses, with limited success in identifying consistent markers associated with reproductive mode (Dwivedi *et al.,* 2013).

Advances in proteomics and transcriptomics have provided new opportunities to examine developmental regulation at the molecular level; however, comprehensive protein expression studies across ploidy levels and reproductive stages in guinea grass are still scarce (Hand and Koltunow, 2014). Protein profiling techniques, such as sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), offer a simple and reproducible approach for preliminary assessment of protein expression patterns. Although SDS-PAGE does not provide protein identification or functional characterization, it remains useful for comparative analyses of protein banding profiles across developmental stages or genotypes (Sheoran *et al.,* 2007). In polyploid systems, such comparative profiling can reveal variation in protein expression patterns that may be associated with ploidy level or developmental transitions.

The present study aims to comparatively analyze protein-banding patterns across pre-meiotic, meiotic, and post-meiotic stages in a ploidy series of *M. maximus* using SDS-PAGE. By examining stage- and ploidy-dependent variation in protein expression profiles, this study provides a descriptive overview of proteomic variation during reproductive development in guinea grass. The findings are intended to serve as a baseline for future investigations employing advanced proteomic, molecular, and cytological approaches to elucidate the functional basis of reproductive development and apomixis in polyploid grasses.

Materials and Methods

Experimental site and planting material: The study was conducted at the ICAR–Indian Grassland and Fodder Research Institute, Jhansi (25°30′43″ N, 78°32′02″ E; 244 m AMSL), India, during 2019–2021. Guinea grass (*M. maximus*) seedlings representing different ploidy levels (3x, 4x, 5x, 6x, 7x, 8x, 9x and 11x) were developed from a 4x progenitor using the Hybridization-supplemented apomixis components partitioning (HAPA) approach (Kaushal *et al.,* 2018) and maintained in 30 L pots under controlled conditions.

Sample collection: Inflorescences were collected from each ploidy series at three reproductive stages: pre-meiotic (10% stigma exertion), meiotic (50%), and post-meiotic (100%), and immediately used for protein extraction.

Protein extraction & SDS-PAGE profiling: Protein extraction was carried out following the method of Tada *et al.* (2003). A 25 mg of fresh inflorescence tissue was homogenized in precooled extraction buffer (Tris-HCl 62.5 mM, SDS 2.5%, glycerol 10%, β-mercaptoethanol 5%; pH 6.8) and centrifuged at 13,000 rpm for 30 minutes at 4 °C. The supernatant containing total protein was collected and stored at –20°C.

SDS-PAGE was performed using standard vertical gel electrophoresis with 5% stacking and 12% resolving polyacrylamide gels following the standard protocol suggested by Laemmli (1970). Protein samples were mixed with loading buffer, denatured at 100°C for 3 minutes, and 15 µL were loaded into wells. Electrophoresis was carried out at 150 V constantly until the tracking dye reached the bottom of the gel. Gels were stained with Coomassie Brilliant Blue, destained to visualize clear protein bands, and documented using a gel documentation system (Neuhoff *et al.*, 1988).

***Scoring of the bands*:** Protein banding pattern was critically observed and bands were scored as present (1) or absent (0), considering only clear, bright and reproducible bands. Molecular weights were estimated using a pre-stained protein ladder (18.4–97.4 kDa). Band intensities were analyzed using GelAnalyzer software (version 23.1.1) (Lazar and Lazar, 2023).

Similarity matrix computation and cluster analysis: A binary data matrix was used to compute similarity coefficients based on the Jaccard similarity coefficient. A similarity matrix was constructed using the Jaccard similarity coefficient, which is appropriate for binary data. The Jaccard coefficient (J) was computed using the formula:

$$J = \frac{a}{a + b + c}$$

Where a = number of shared bands, b and c = bands present in only one of the two samples.

Hierarchical cluster analysis (HCA) was performed using the Unweighted Pair Group Method with Arithmetic Mean to group samples based on similarity. The relationships were visualized as a dendrogram, where branch lengths represent similarity levels (0.71–1.00). Analysis was carried out using NTSYS-pc (Rohlf, 2000).

Results and Discussion

***Protein polymorphism*:** SDS-PAGE analysis resolved nine reproducible protein bands with molecular weights ranging from 18.4 to 97.4 kDa across inflorescence samples

of guinea grass representing eight ploidy levels (3x, 4x, 5x, 6x, 7x, 8x, 9x, and 11x). Samples were collected at three developmental stages: pre-meiotic (~10% stigma exertion), meiotic (~50% stigma exertion), and post-meiotic (~100% stigma exertion) (Fig. 1). Three biological replicates per ploidy–stage combination (n = 72 total samples) and two technical replicates per biological replicate ensured analytical reproducibility. One-way ANOVA revealed highly significant main effects of developmental stage $p = 0.001$) and ploidy level $p < 0.01$) on normalized band-intensity profiles. Post-hoc Tukey's HSD test indicated significant differences between meiotic and pre-meiotic stages ($p < 0.01$) and between meiotic and post-meiotic stages ($p < 0.001$). Two-way ANOVA further detected a significant stage × ploidy interaction ($p = 0.01$), indicating that ploidy level modifies stage-specific protein expression patterns.

Stage-specific banding patterns: Meiotic-stage samples exhibited six unique protein bands that were absent from pre- and post-meiotic profiles, accounting for 67% of the total observed polymorphism. These bands ranged from 25 to 68 kDa and showed the highest relative abundance (mean densitometry value 1.8 ± 0.3), particularly in higher ploidy levels (7x–11x). Pre-meiotic profiles showed the highest degree of conservation, with four bands (32, 45, 62, and 87 kDa) present across all ploidy levels, representing a 44% conservation rate. Post-meiotic samples showed a marked simplification of protein profiles, retaining only three bands with significantly reduced intensity (mean reduction of 62% relative to the meiotic stage; paired t-test, t = 7.43, $p < 0.001$).

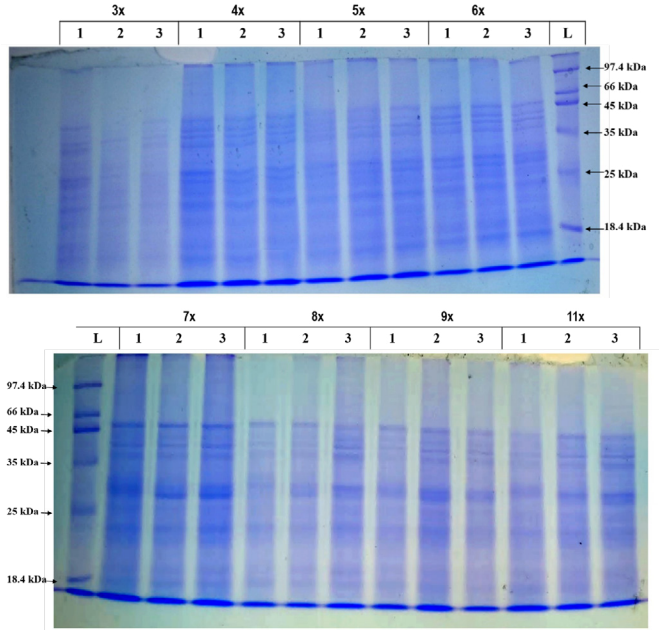

Fig 1. Protein polymorphism at various meiotic stages of the eight-ploidy series of guinea grass, where 3x, 4x, 5x, 6x, 7x, 8x, 9x and 11x indicate ploidy conditions, L indicates protein molecular weight marker, and 1, 2, 3 indicate pre-meiotic, meiotic and post-meiotic stages, respectively

The pre-meiotic stage exhibited a relatively conserved protein banding pattern with minimal variation in band number and intensity across ploidy levels, indicating stable expression profiles detectable by SDS–PAGE. In contrast, the meiotic stage showed an increased number of reproducible bands, reflecting higher observable protein polymorphism during this developmental phase. Post-meiotic samples displayed a reduced number of bands and lower overall intensity, indicating a simplification of detectable protein profiles following meiosis. During the post-meiotic stage, several protein bands disappeared or showed reduced intensity, suggesting down-regulation of meiosis-specific proteins and activation of post-meiotic regulatory pathways (Chen *et al.,* 2021). This increased protein complexity during meiosis may reflect activation of molecular pathways involved in chromosome pairing, synapsis, and homologous recombination (Kerim *et al.,* 2003). The subsequent reduction in band number and intensity in post-meiotic samples likely corresponds to gamete maturation and pollen development processes (Tidy *et al.,* 2022). These observations are restricted to differences in band presence and intensity and do not imply functional identity or mechanistic roles of the proteins resolved by SDS-PAGE, which provides separation based solely on molecular weight and abundance.

Ploidy-dependent variation: Higher ploidy levels (7x–11x) accounted for 73% of the unique protein bands (n= 11), compared with 27% in lower ploidy levels (3x–6x; n = 4). Band-presence variability, quantified using binary presence/absence matrices, increased systematically with ploidy level ($\sigma^2 = 0.19$ for 3x–6x vs. $\sigma^2 = 0.42$ for 7x–11x). Specific molecular-weight classes exhibited ploidy bias: bands in the 25-40 kDa range were significantly enriched in higher ploidy levels (Fisher's exact test, $p = 0.008$), whereas bands in the 60 to 97 kDa range were more evenly distributed.

Higher ploidy levels (7x, 8x, 9x, and 11x) consistently exhibited greater protein polymorphism, including the presence of additional or unique bands, compared with lower ploidy levels (3x, 4x, and 5x). Several bands in higher ploidy samples also showed increased intensity, indicating higher protein abundance detectable by SDS–PAGE. This pattern may reflect ploidy-related variation in protein expression, potentially arising from gene-dosage effects or altered regulatory balance in polyploid genomes (Otto, 2007); however, functional inference cannot be made without protein identification.

The observed variation in protein banding patterns across developmental stages and ploidy levels indicates ploidy-dependent differences in the number and intensity of detectable proteins. Higher ploidy levels consistently showed a greater number of polymorphic

bands and increased variability in band presence compared with lower ploidy levels. These results suggest that polyploidization is associated with increased heterogeneity in protein profiles detectable by SDS-PAGE; however, no inference regarding protein function, regulation, or biological pathways can be made without protein identification. Similar increases in proteome complexity with increasing ploidy have been reported in descriptive protein profiling studies of polyploid plants (Otto, 2007; Zhang *et al.,* 2019). The presence of unique protein bands in higher ploidy levels may also indicate ploidy-dependent expression of proteins involved in chromosomal stability, DNA repair, and meiotic progression (Anatskaya and Vinogradov, 2022). Similarly, Dwivedi *et al.* (2013) reported the possible association of specific isozymes with ovarian tissues at different developmental stages in *Cenchrus ciliaris*.

Overall, SDS–PAGE analysis revealed reproducible and systematic variation in protein banding patterns across eight ploidy levels and three reproductive stages in guinea grass. The meiotic stage exhibited the highest number of polymorphic bands, while post-meiotic samples showed reduced band number and intensity. Higher ploidy levels displayed greater band polymorphism than lower ploidy levels. These findings provide a descriptive overview of stage- and ploidy-dependent variation in protein profiles and establish a baseline dataset for future studies employing protein identification and quantitative proteomic approaches.

Cluster analysis of protein polymorphism: The UPGMA dendrogram constructed using Jaccard's similarity coefficient summarizes the similarity of SDS-PAGE protein banding patterns among eight ploidy series of guinea grass across three reproductive stages: pre-meiotic, meiotic, and post-meiotic (Fig. 2). Importantly, this clustering reflects comparative similarity in protein profiles, rather than genetic relatedness or functional divergence, as SDS–PAGE resolves proteins solely on the basis of molecular weight and relative abundance.

The dendrogram resolved two major clusters (A and B), indicating clear differentiation in protein banding profiles among ploidy–stage combinations. This separation suggests that both developmental stage and ploidy level contribute to variation in detectable protein expression patterns, consistent with the stage- and ploidy-dependent polymorphism observed in the banding analysis. The similarity coefficient range (0.71–1.00) indicates moderate to high overlap among protein profiles, suggesting the presence of a conserved core proteome across ploidy levels derived from a common progenitor. At the same time, detectable divergence within the dendrogram highlights stage- and ploidy-dependent modulation of protein expression during reproductive development. Similar use of SDS-PAGE-based clustering to visualize developmental proteome shifts has been reported in grasses and other crop species (Kerim *et al.,* 2003; Sheoran *et al.,* 2009).

***Stage-wise grouping*:** Across several ploidy series, pre-meiotic and meiotic samples clustered closely, indicating relatively conserved protein expression during early reproductive development. This pattern suggests that entry into and progression through meiosis rely on a shared set of proteins that remain largely stable across ploidy levels. Conservation of early meiotic protein profiles has also been observed in cereal crops, where major proteomic reorganization occurs only after completion of chromosome segregation (Kerim *et al.,* 2003; Imin *et al.,* 2001). In contrast, post-meiotic samples frequently formed distinct sub-clusters, indicating increased divergence in protein banding patterns following meiosis. This divergence likely reflects stage-specific remodeling of the proteome associated with gametophyte differentiation and maturation, including the downregulation of meiosis-associated proteins and stabilization of a reduced functional proteome. Comparable post-meiotic proteome shifts have been documented during pollen development in rice and maize (Imin *et al.,* 2001; Tidy *et al.,* 2022).

Diploid series-specific trends: The 4x ploidy series showed close clustering of pre-meiotic, meiotic, and post-meiotic stages, indicating relatively stable protein expression across reproductive development. Such stability is consistent with observations that intermediate ploidy levels often exhibit balanced gene dosage and reduced expression variability compared with higher polyploids, although this cannot be directly tested using SDS–PAGE alone (Parisod *et al.,* 2010; Han *et al.,* 2025).

Lower ploidy levels (3x and 5x) clustered tightly within their respective developmental stages, indicating relatively consistent protein banding profiles across pre-meiotic, meiotic, and post-meiotic stages. In contrast, higher ploidy levels, particularly 8x and 9x, exhibited greater separation of post-meiotic samples from earlier stages, reflecting increased variability in protein banding patterns during later reproductive development. The similarity coefficient range (0.71-1.00) indicates moderate to high overlap among protein profiles across all ploidy levels, suggesting the presence of a conserved core set of proteins despite detectable stage- and ploidy-dependent variation.

The observed increase in post-meiotic protein profile variability in higher ploidy levels is consistent with known effects of polyploidy on regulatory coordination and gene dosage balance, which can influence protein abundance and expression heterogeneity during developmental transitions (Ramsey and Schemske, 2002; Comai, 2005; Otto, 2007). Pre-meiotic stages remained comparatively conserved across ploidy levels, supporting previous observations that basic reproductive processes are

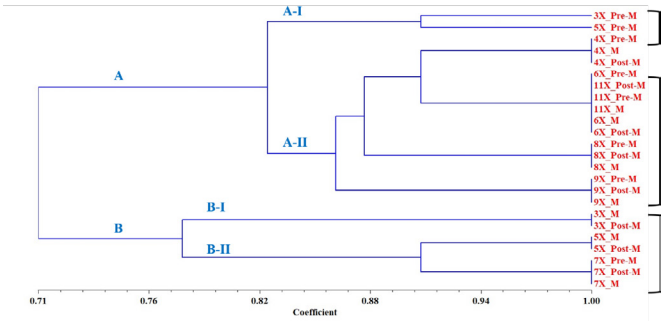

Fig 2. UPGMA dendrogram based on Jaccard's similarity coefficient showing similarity in SDS–PAGE protein banding patterns among eight ploidy series of guinea grass across three reproductive stages [pre-meiotic (Pre-M), meiotic (M), and post-meiotic (Post-M)].

maintained through conserved regulatory frameworks in polyploid plants (Parisod *et al.*, 2010; Renny-Byfield and Wendel, 2014). Although SDS-PAGE does not allow functional or genetic inference, the reproducible post-meiotic divergence observed in higher ploidy series highlights this stage as a key point of proteomic variation and a suitable target for future studies employing protein identification and quantitative approaches, with potential relevance for breeding and improvement of guinea grass (Jank *et al.*, 2011).

Conclusion

The present study provides a comparative analysis of protein banding patterns across reproductive stages and ploidy levels in guinea grass using SDS-PAGE. Distinct stage- and ploidy-dependent variations in protein banding profiles were observed, with relatively higher polymorphism during the meiotic stage and greater diversity in higher ploidy levels compared with lower ploidy levels. The clustering of samples based on protein banding patterns reflected similarities in expression profiles across developmental stages and ploidy levels rather than genetic relatedness or functional divergence. While the findings are descriptive in nature, they establish a baseline overview of protein expression variation associated with reproductive development and polyploidy in guinea grass. Given the limitations of SDS-PAGE, protein identification and functional characterization are not possible using this approach. Future studies integrating protein identification using LC-MS/MS, quantitative proteomics, transcriptomic analysis and cytological validation are required to further investigate the biological relevance of the observed variations and to better understand reproductive regulation in polyploid grasses.

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