Expression profiling of MSP1 gene involved in mega-gametophyte development in ploidy series of Guinea grass (*Panicum maximum* Jacq.)

ABSTRACT

Hybrid seeds are playing important role in increasing agricultural production. However, process of hybrid seed development involves high cost for most of the major crops. Under such situation apomixis can play crucial role in creation of one-time hybrid and maintain it for multiple generations. Apomixis and polyploidy are interrelated with each other and expression of apomixis completely relies on polyploidy. Guinea grass (*Panicum maximum* Jacq.) provides ideal system for research on apomixis and polyploidy because of its extensive diversity in morphological, cytological, biochemical, and molecular characteristics. The experiment was carried out in the eight-ploidy series i.e. 3x, 4x, 5x, 6x, 7x, 8x, 9x, 11x and one obligate sexual Guinea grass genotype. Considering role of MSP1 gene in apomeiosis, its expression was studied in guinea grass at three developmental stages of spikelet viz., pre-meiotic, meiotic and post-meiotic. Findings of this study highlight the complex regulation of MSP-1 expression during different developmental stages and across various ploidy levels in guinea grass. At pre-meiotic stage, except 4x, downregulation pattern of MSP1 was observed in all the ploidy levels. But opposite was observed in the post-meiotic stage.

Keywords: *Apomixis, Polyploidy, Expression analysis, RT-PCR, Guinea grass*

INTRODUCTION

High yielding varieties and intensive agronomic management practices played key role in bringing green revolution and feeding the high populous countries of the world (Ram et al., 2016). In present time, approximately 1/3rd of the seed supply comes from the commercial seed market, 1/3rd from government establishments and rest from the seeds preserved by farmers (Barcaccia and Albertini, 2013). Crop plants have undergone a journey of

introduction, selection and hybridization throughout of its history. Reproduction within flowering plants occurs by sexual and asexual pathways but most flowering plants rely on sexual reproduction, a process that promotes gene recombination among offspring (Van Dijk et al., 2016). Hybrid seed production playing important role in increased agricultural production and will remain important in future also. However, it involve high cost for major crop species like wheat and soybean (Whitford et al., 2013). In this situation apomixis can plays a crucial role in plant breeding and the preservation of hybrid seeds (Fiaz et al., 2021). In agriculture, apomixis allows the production of seeds containing maternal embryos. If the mother plant is well-adapted to a specific environment or purpose, the offspring will inherit those beneficial traits. Consequently, apomixis is highly desirable in agriculture due to its ability to maintain hybrid vigor (Niccolò et al., 2023). Apomixis has several benefits as it allows for one-time hybrid creation, enabling seed propagation across multiple generations, cutting hybrid seed production costs and empowering farmers to produce their own hybrid seeds.

Apomictic plants possess the ability to generate seeds genetically identical to the maternal plant (Mahlandt et al., 2023). Introducing apomixis into crop plants to perpetuate superior hybrids stands as a goal linked to comprehending the genes and pathways inherent in this process. Despite producing clonal seeds mirroring the maternal genotype, various pathways have evolved in creating these clonal seeds. Apomixis categorically splits into gametophytic or sporophytic types. In the sporophytic pathway, a clonal embryo emerges when a somatic cell or cells in the ovule take on the role of embryo initiators (Hand and Koltunow, 2014). Sporophytic apomicts rely on sexual reproduction to form functional endosperm for seed development, often resulting in multiple embryos within one seed—a mix of clonally derived and sexually derived ones. Gametophytic apomicts generate unreduced functional embryo sacs within the ovule, leading to clonal embryos. They further divide into aposporous, originating from nucellar cells, or diplosporous, arising from the megaspore mother cell. In gametophytic apomicts, the typical processes of meiosis and the fusion of egg and sperm

nuclei to form a zygote are replaced by developmental sequences involving apomeiosis and parthenogenesis (Hand and Koltunow, 2014).

Apomixis and polyploidy are interrelated phenomena, and complete expression of apomixis relies on polyploidy. Typically, naturally occurring apomictic species tend to be polyploids, whereby, usually diploids are sexually reproducing. However, the necessity for polyploidy along with apomixis has been challenged by the discovery of apomixis in diploid species (Voigt-Zielinski et al., 2012). The impact of polyploidy on individual components of apomixis remains unknown. Previously, it was believed that apomixis occurred exclusively in polyploid genotypes. However, the presence of apomixis in diploid species indicates that polyploidy is not an absolute requirement (Rodriguez-Leal and Vielle-Calzada, 2012). Understanding the influence of genome dosage on the expression of major reproductive traits is crucial for comprehending the complexities of sexual and apomictic seed production. Recent discoveries suggest that apomictic and sexual systems share common genes that exhibit heterochronic expression. Therefore, comparing the expression of genes involved in mega-gametophyte development between apomictic and sexual systems is of great importance, particularly in relation to meiotic reduction, embryo sac development, fertilization, and embryo and endosperm development (Kaushal et al., 2019).

Guinea grass (*Panicum maximum* Jacq.) is a robust, perennial forage grass known for its high biomass yield. Thriving in arid and semi-arid tropical regions, it serves as a valuable resource for both rangeland and cultivated crops, ideal for grazing and cut-and-carry systems alike (Kaushal et al., 2018). It provides an ideal model system for research on polyploidy and apomixis, owing to its extensive diversity in morphological, cytological, biochemical, and molecular characteristics (de Sousa et al., 2011). Natural forms of guinea grass are primarily apomictic tetraploids (2n = 4x = 32), although sexual diploids (2n = 16) and facultative hexaploids (2n = 48) have also been reported (Kaushal et al., 2018; Savidan, 2000).

Naturally occurring apospory commences with the emergence of aposporous initials (AIs) adjacent to the megaspore mother cell (MeMC) within the ovule (Bicknell and Koltunow, 2004). Similar to the MeMC, AIs are enlarged nucellar cells with the potential to mature into embryo sacs. However, unlike MeMC, they bypass meiosis, resulting in diploid embryo sacs instead of haploid ones. These diploid egg cells can give rise to embryos through parthenogenesis, wherein fertilization is not required, yielding offspring that mirror the genetic makeup of the maternal tissue (Barke et al., 2018). The precise cellular origin of AIs remains uncertain, but it was suggested that AIs originate from the same group of nucellar cells responsible for generating additional MeMCs when a genetic control, regulating MeMC numbers, is relaxed. The presence of this control mechanism was initially proposed through investigations into the maize mutant with multiple archaesporeal cells1 (mac1), which exhibited an excess of sporocytes in both the ovule and anther (Sheridan et al., 1999). Similar phenotypic characteristics were observed in the multiple sporocytes1 (msp1) mutant of rice (Nonomura et al., 2015). MSP1 exhibits close structural and functional similarities to EXS/EMS1 in Arabidopsis. Mutations in both EXS and EMS1 result in the production of additional sporocytes in the anther, as demonstrated by (Canales et al., 2002).

(Zhao et al., 2008) observed robust expression of MSP1 in the tapetum of the anther and across the ovule, excluding the MeMC. MSP1 exhibit widespread expression throughout the nucellus before meiosis. Interestingly, the primary MeMC does not seem to express the gene, and the additional MeMCs also do not demonstrate expression of MSP1 (Nonomura et al., 2003). This outcome implies a potential connection between the onset of meiosis and the cessation of MSP1 expression. It could be hypothesized that the cessation of MSP1 expression is either a result of entering meiosis or that the termination of their expression forms part of the signalling pathway leading to meiosis. The present study framed in keeping in the view of all mechanism involved in apomictic seed development in plants. In the present investigation candidate gene which is responsible for the apomeiosis was studied by their expression study in ploidy series of guinea grass.

MATERIALS AND METHODS

The study was carried out at ICAR-Indian Grassland and Fodder Research Institute, Jhansi, U.P., India (25°30'43"N and 78°32'02"E and 244 m above mean sea level) during the year 2019-2021. The experimental material includes the eight ploidy series Guinea grass i.e. 3x, 4x, 5x, 6x, 7x, 8x, 9x, 11x and one obligate sexual Guinea grass genotype SRP75. This ploidy series in Guinea grass was developed from a single progenitor 4x following a Hybridization-supplemented apomixis components partitioning (HAPA) approach (Kaushal et al., 2018; 2009) (Figure 1).

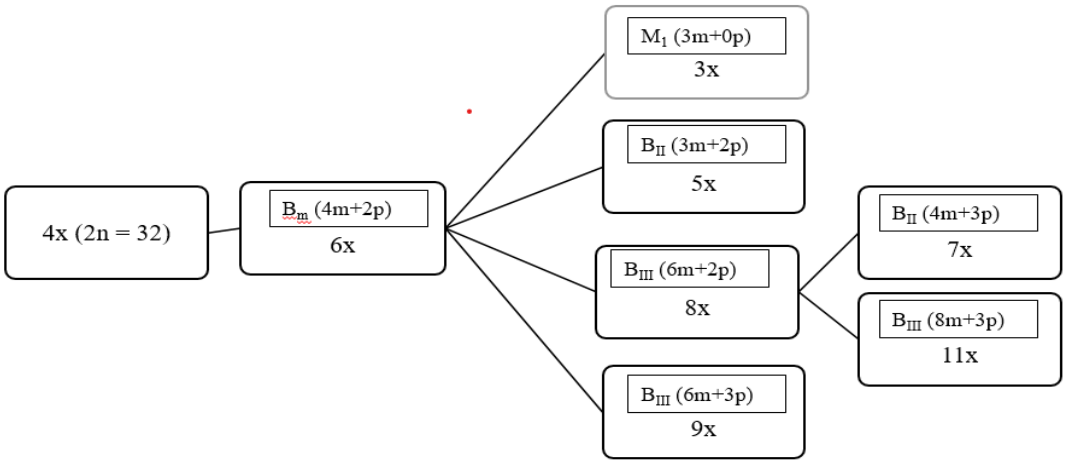

Figure 1: Scheme for generating a ploidy series. Various ploidies including 3x, 4x, 5x, 6x, 7x, 8x, 9x, and 11x were produced from a singular 4x progenitor using HAPA. The acquisition of plants with specific ploidy levels and their respective pathways of formation (M1, BII, or BIII) are illustrated. Maternal (m) and paternal (p) genomic contributions are indicated in parentheses (Kaushal et al., 2018).

For present study, individual rooted slip of each ploidy series was transplanted in separate plastic pot and replicated thrice. Recommended agronomic management practices were followed to raise the plants of different ploidy series of guinea grass.

RNA Isolation and cDNA Synthesis

Total RNA was extracted from spikelets of plants at three development stages viz., pre-meiotic, meiotic and post-meiotic using TRIZOL method (Rio et al., 2010). cDNA was synthesized from 1 µg of total RNA using Superscript II RNase H reverse transcriptase (Chromous Biotech, India).

Real-time RT-PCR analysis

Quantitative real-time PCR (qRT-PCR) amplifications were performed in 25µl final reaction volume containing 200 nM gene-specific primers, 2XqPCR Master Mix (Chromous Biotech, QCR 12), and 20 ng cDNA. Amplifications were performed in a Rotor-Gene Q thermocycler (Biorad) programmed as follows: 2 min at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C. A melting curve was produced at the end of the cycling period. The experiments were performed using three biological replicates and two technical replicates. Values were normalized using β-tubulin as an internal reference gene. The primers used for expression study of MSP1 gene were given in the table 1.

Table 1: Details of the primers used in the present study

Sr. No.	Name	Forward (5'-3')	Reverse (5'-3')
1.	MSP1	GTCTCTGACTTTGGCCTTGC	GCAGCTCCAGCATTACAACA
2.	β-tubulin	TTTGATTTCTGCCACCTGA	GGAACAGTAAGGGCACGGTA

RESULTS AND DISCUSSION

To understand the expression dynamics of apomixis controlling genes, we studied the differential expression patterns of MSP-1 gene in eight different ploidy series of guinea grass in comparison to the sexual line (SRP75) at three different stages of spikelet development; pre-meiotic, meiotic and post-meiotic (Figure 2 and Table 2). Expression analysis of MSP-1 at pre-meiotic stage revealed downregulation pattern for most of the ploidy levels of guinea grass (3x, 6x,7x, 8x,9x,11x) while significant upregulation (3.94-fold) was observed only in 4x plants. At meiotic stage, the expression of MSP-1 was significantly upregulated in 6x plants

(7.59-fold) and drastically downregulated in 4x (not detected) and 5x plants (not detected). At post-meiotic stage, the expression trend of MSP-1 was almost opposite as observed for pre-meiotic stage. The gene got upregulated in most of the ploidy series except for 4x and 5x where the expression got lowered.

Table 2: Expression of MSP1 gene in different ploidy series of guinea grass

Sampling stage	*3x*	*4x*	*5x*	*6x*	*7x*	*8x*	*9x*	*11x*
Control	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Pre-Meiotic	0.30	3.94	1.32	0.12	0.04	0.63	0.27	0.08
Meiotic	0.78	0.01	0.00	7.59	0.93	1.43	1.08	0.80
Post- Meiotic	75.85	0.37	0.31	10.30	48.00	13.13	22.78	11.08

Plants have developed versatile strategies to achieve successful propagation, adaptation, and reproduction. Gymnosperms, angiosperms, and certain ferns exhibit heterospory, generating two types of spore mother cells (PMCs and MMCs), which develop into microspores and megaspores, respectively (Wang and Bai, 2019). The evolutionary transition of heterospory evolved from the homospory entails flowering plants adopting distinct developmental pathways for sporogenesis and gametogenesis in the anther and ovule. The genes or mutations affecting reproduction in both male and female organs may serve a fundamental role in germ cell initiation, differentiation, sporogenesis, and gametogenesis, shared between homosporous and heterosporous plants (Schiefthaler et al., 1999; Yang et al., 1999).

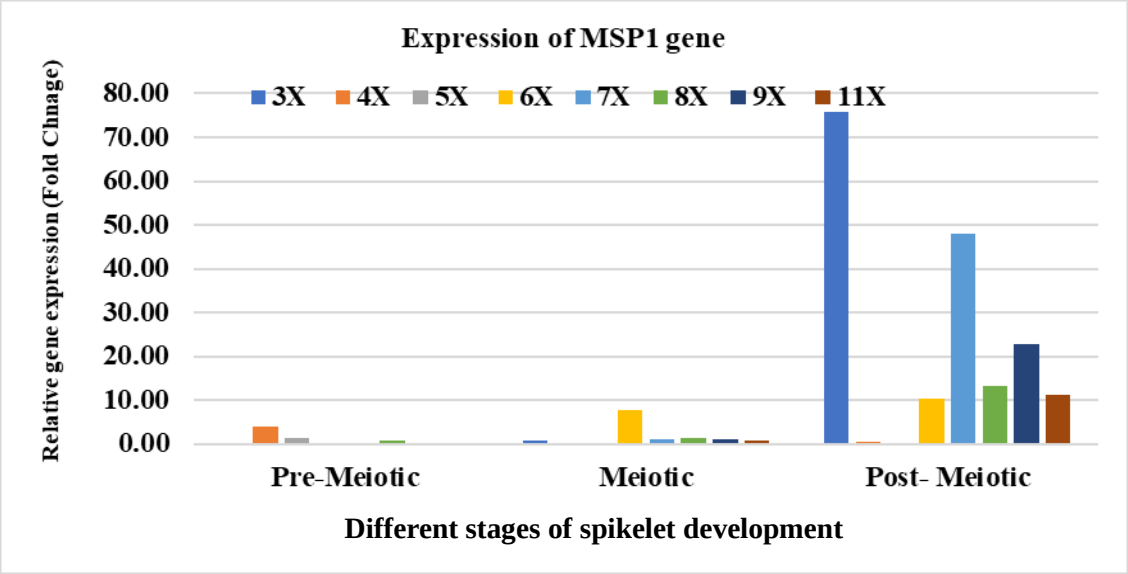

Figure 2: Differential expression patterns of MSP-1 gene in eight different ploidy series of guinea grass in comparison to the sexual line (SRP75) at three different stages of spikelet development; pre-meiotic, meiotic and post-meiotic.

The molecular mechanism of apomixis is a complicated process; however the exact expression analysis study gives insights the mechanism of apomictic seed development in plants. Over the last ten years, numerous candidate genes associated with apomixis have been pinpointed (Hand and Koltunow, 2014; Rodriguez-Leal and Vielle-Calzada, 2012). However, the mechanisms through which these genes interplay within sexual reproduction networks, leading to alterations in expression patterns, remain predominantly elusive.

MSP1 (Multicopy Suppressor of IRA1) is a gene found in *Arabidopsis thaliana*, a model plant species. It has been studied for its involvement in various cellular processes, including responses to stress and growth regulation. MSP1 encoded a Leu-rich repeat receptor–like protein kinase. In our study, we have observed that the expression level of MSP1 gene in pre-meiotic stage was much lower as compared to post-meiotic stage. This is in accordance with the earlier finding that the expression pattern of MSP1, coupled with the phenotype exhibited by the loss-of-function mutant, suggests that this gene functions within a signaling pathway to inhibit adjacent cells from undergoing sporogenesis in rice

(Nonomura et al., 2003) found multiple MMC–like cells in pre-meiotic stage of flower tissue in MSP1 mutant rice. The findings indicate that the function of MSP1 suppresses the transformation of neighboring cells into male and female sporocytes.

Our findings indicate a genetic linkage between apomixis and MSP1 gene, with its presence correlating with the down-regulation of its function throughout all stages of apomictic seed formation in *P. maximum*. Furthermore, the inactivation of this gene in rice and Arabidopsis leads to endosperm development failure and the arrest of embryo development at early stages (Nonomura et al., 2003). In the MSP1 mutant, the excess sporogenous cells underwent differentiation into sporocytes and proceeded with meiosis, suggesting that a primary pathway of sporogenesis remains unaffected by the MSP1 mutation. This process leading to the formation of an abnormal embryo sac structure characterized by internal cell walls and varying quantities of supernumerary nuclei. The majority of nucellar cells are typically utilized for embryo sac development. However, in the mutant ovule, nucellar cells persisted, although some were transformed into MMCs. These cells may retain the capability to sustain female sporogenesis and gametogenesis. Thus, it is believed that the main role of the MSP1 gene is confined to the early stages of sporogenesis. The disarray observed in the embryo sac is likely a secondary consequence, as indicated by the gene's expression pattern during early sporogenesis. (Siena et al., 2014) also observed the expression of PnTgs1-like is notably elevated in ovules of sexual plants across various developmental stages, spanning from premeiosis to maturity. Notably, areas exhibiting distinct expression patterns include nucellar cells, which serve as the locus for aposporous initials differentiation in apomictic genotypes.

A comprehensive search across various databases has revealed striking similarities between the entire sequence of the presumed MSP1 protein and several Ser/Thr kinases known to possess an LRR domain as a receptor. Notably, EMS1 and EXS, derived from allelic mutations within the same gene in Arabidopsis, exhibit the highest degree of sequence identity with MSP1 presumed kinase domain, sharing 63.8% identity (176 out of

276 residues). This discovery is particularly significant as it aligns with observed phenotypic resemblances in anther development between Arabidopsis and rice mutants. These resemblances include an excessive number of microsporocytes and abnormalities in anther wall formation, notably the absence of tapetal cells, as previously documented (Sorensen et al., 2002; Zhao et al., 2008).

CONCLUSION

We conducted a study to examine the expression of the apomeiotic gene MSP1 using quantitative real-time PCR in guinea grass. Our aim was to analyze the impact of ploidy on the expression of this apomictic gene. Importantly, this study represents our inaugural investigation into the expression of the MSP1 gene across the ploidy series of guinea grass. The research involved the selection of an appropriate gene associated with apomeiosis and subsequent expression analysis. We compared the MSP1 gene expression in flowers of different ploidies at differnt develpomenatl satges of apomictic/facultative guinea plants to those of sexual counterparts. Our findings demonstrate that the low expression of the MSP1 gene during the pre-meiotic stage of flower development which may leads to the formation of multiple sporocytes. Thus, this study would be useful to deregulate the apomixis trait by genome editing technique.

REFERENCES

Barcaccia G, Albertini E. Apomixis in plant reproduction: A novel perspective on an old dilemma. Plant Reprod. 2013;26:159–79. PMid:23852378. doi: https://doi.org/10.1007/s00497-013-0222-y.

Barke BH, Daubert M, Hörandl E. Establishment of apomixis in diploid F2 hybrids and inheritance of apospory from F1 to F2 hybrids of the ranunculus auricomus complex. Front Plant Sci. 2018;9:1–12. doi: https://doi.org/10.3389/fpls.2018.01111.

Bicknell RA, Koltunow AM. Understanding apomixis: Recent advances and remaining conundrums. Plant Cell. 2004;16:228–45. PMid:15131250. doi: https://doi.org/10.1105/tpc.017921.

Canales C, Bhatt AM, Scott R, Dickinson H. EXS, a putative LRR receptor kinase, regulates male germline cell number and tapetal identity and promotes seed development in Arabidopsis. Curr Biol. 2002;12:1718–27. PMid:12401166. doi:

https://doi.org/10.1016/S0960-9822(02)01151-X.

Van Dijk PJ, Rigola D, Schauer SE. Plant breeding: Surprisingly, less sex is better. Curr Biol. 2016;26:R122–4. PMid:26859270. doi: https://doi.org/10.1016/j.cub.2015.12.010.

Fiaz S, Wang X, Younas A, Alharthi B, Riaz A, Ali H. Apomixis and strategies to induce apomixis to preserve hybrid vigor for multiple generations. GM Crop Food. 2021;12:57–70. PMid:32877304. doi: https://doi.org/10.1080/21645698.2020.1808423.

Hand ML, Koltunow AMG. The genetic control of apomixis: Asexual seed formation. Genetics. 2014;197:441–50. PMid:24939990. doi: https://doi.org/10.1534/genetics.114.163105.

Kaushal P, Agrawal A, Malaviya DR, Siddiqui SA, Roy AK. Ploidy manipulation in guinea grass (Panicum maximum Jacq., Poaceae) utilizing a Hybridization-supplemented Apomixis-components Partitioning Approach (HAPA). Plant Breed. 2009;128:295–303. doi: https://doi.org/10.1111/j.1439-0523.2008.01567.x.

Kaushal P, Dwivedi KK, Radhakrishna A, Saxena S, Paul S, Srivastava MK, Baig MJ, Roy AK, Malaviya DR. Ploidy dependent expression of apomixis and its components in guinea grass (Panicum maximum Jacq.). Euphytica. 2018;214. doi: https://doi.org/10.1007/s10681-018-2232-1.

Kaushal P, Dwivedi KK, Radhakrishna A, Srivastava MK, Kumar V, Roy AK, Malaviya DR. Partitioning apomixis components to understand and utilize gametophytic apomixis. Front Plant Sci. 2019;10:1–17. doi: https://doi.org/10.3389/fpls.2019.00256.

Mahlandt A, Singh DK, Mercier R. Engineering apomixis in crops. Theor Appl Genet. 2023;136:1–18. PMid:37199785. doi: https://doi.org/10.1007/s00122-023-04357-3.

Niccolò T, Anderson AW, Emidio A. Apomixis: oh, what a tangled web we have! Planta. 2023;257:1–19. PMid:37000270. doi: https://doi.org/10.1007/s00425-023-04124-0.

Nonomura AK, Miyoshi K, Eiguchi M, Suzuki T, Miyao A, Hirochika H, Kurata N. The MSP1 Gene Is Necessary to Restrict the Number of Cells Entering into Male and Female Sporogenesis and to Initiate Anther Wall Formation in Rice. Plant Cell. 2003;15:1728–39. doi: https://doi.org/10.1105/tpc.012401.der.

Nonomura AK, Miyoshi K, Eiguchi M, Suzuki T, Miyao A, Hirochika H, Kurata N, The S, Cell P, Aug N. The MSP1 Gene Is Necessary to Restrict the Number of Cells Entering into Male and Female Sporogenesis and to Initiate Anther Wall Formation in Rice Published by : American Society of Plant Biologists (ASPB) Linked references are available on JSTOR for t. 2015;15:1728–39. doi: https://doi.org/10.1105/tpc.012401.der.

Ram A, Kumar D, Anand A, Singh N, Prasad D. Relative efficiency of sulphur sources at varying rate in aerobic rice (Oryza sativa)-wheat (Triticum aestivum) cropping system. Indian J Agric Sci. 2016;86.

Rio DC, Ares M, Hannon GJ, Nilsen TW. Purification of RNA using TRIzol (TRI Reagent). Cold Spring Harb Protoc. 2010;5:2010–3. doi: https://doi.org/10.1101/pdb.prot5439.

Rodriguez-Leal D, Vielle-Calzada JP. Regulation of apomixis: Learning from sexual experience. Curr Opin Plant Biol. 2012;15:549–55. PMid:23000434. doi: https://doi.org/10.1016/j.pbi.2012.09.005.

Savidan Y. Apomixis: Genetics and Breeding. In: Janick J, editor. Plant Breed. Rev., vol. 18, John Wiley & Sons, Ltd; 2000, p. 267–320. doi: https://doi.org/10.1002/9780470650073.ch9.

Schiefthaler U, Balasubramanian S, Sieber P, Chevalier D, Wisman E, Schneitz K. Molecular

analysis of NOZZLE, a gene involved in pattern formation and early sporogenesis during sex organ development in Arabidopsis thaliana. Proc Natl Acad Sci U S A. 1999;96:11664–9. PMid:10500234. doi: https://doi.org/10.1073/pnas.96.20.11664.

Sheridan WF, Golubeva EA, Abrhamova LI, Golubovskaya IN. The mac1 mutation alters the developmental fate of the hypodermal cells and their cellular progeny in the maize anther. Genetics. 1999;153:933–41. PMid:10511568. doi: https://doi.org/10.1093/genetics/153.2.933.

Siena LA, Ortiz JPA, Leblanc O, Pessino S. PnTgs1-like expression during reproductive development supports a role for RNA methyltransferases in the aposporous pathway. BMC Plant Biol. 2014;14:1–10. PMid:25404464. doi: https://doi.org/10.1186/s12870-014-0297-0.

Sorensen A, Guerineau F, Canales-Holzeis C, Dickinson HG, Scott RJ. A novel extinction screen in Arabidopsis thaliana identifies mutant plants defective in early microsporangial development. Plant J. 2002;29:581–94. PMid:11874571. doi: https://doi.org/10.1046/j.0960-7412.2001.01240.x.

de Sousa ACB, Jank L, de Campos T, Sforça DA, Zucchi MI, de Souza AP. Molecular Diversity and Genetic Structure of Guineagrass (Panicum maximum Jacq.), a Tropical Pasture Grass. Trop Plant Biol. 2011;4:185–202. doi: https://doi.org/10.1007/s12042-011-9081-6.

Voigt-Zielinski ML, Piwczyński M, Sharbel TF. Differential effects of polyploidy and diploidy on fitness of apomictic Boechera. Sex Plant Reprod. 2012;25:97–109. PMid:22367230. doi: https://doi.org/10.1007/s00497-012-0181-8.

Wang X, Bai SN. Key innovations in transition from homospory to heterospory. Plant Signal Behav. 2019;14:1–4. PMid:30892985. doi: https://doi.org/10.1080/15592324.2019.1596010.

Whitford R, Fleury D, Reif JC, Garcia M, Okada T, Korzun V, Langridge P. Hybrid breeding in wheat: Technologies to improve hybrid wheat seed production. J Exp Bot. 2013;64:5411–28. PMid:24179097. doi: https://doi.org/10.1093/jxb/ert333.

Yang W-C, Ye D, XU J, Sundaresan V. The SPOROCYTELESS gene of sporogenesis and encodes a novel of Arabidopsis is required for initiation nuclear protein. Gene Dev. 1999;13:2108–17. PMid:27541731. .

Zhao X, De Palma J, Oane R, Gamuyao R, Luo M, Chaudhury A, Hervé P, Xue Q, Bennett J. OsTDL1A binds to the LRR domain of rice receptor kinase MSP1, and is required to limit sporocyte numbers. Plant J. 2008;54:375–87. PMid:18248596. doi: https://doi.org/10.1111/j.1365-313X.2008.03426.x.