Ploidy Dependent Expression of Apomixis Components in Guinea Grass (*Panicum maximum* Jacq.)

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The XXIII International Grassland Congress (Sustainable use of Grassland Resources for Forage Production, Biodiversity and Environmental Protection) took place in New Delhi, India from November 20 through November 24, 2015.

Proceedings Editors: M. M. Roy, D. R. Malaviya, V. K. Yadav, Tejveer Singh, R. P. Sah, D. Vijay, and A. Radhakrishna

Published by Range Management Society of India

Presenter Information

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Paper ID: 1425
Theme 4. Biodiversity, conservation and genetic improvement of range and forage species
Sub-theme 4.1. Plant genetic resources and crop improvement

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Keywords: Apomixis components, Expression, Guinea, Ploidy

Introduction

Apomixis is an asexual method of reproduction through seeds. The potential of apomixis has been envisaged as "asexual revolution" by virtue of its capacity to fix hybrid vigour, a much desirable feature in breeding of agricultural crops. The genetic mechanism of apomixis regulation is complex and is believed to be largely affected by polyploidy (Nogler 1984). Expression of apomixis essentially contains three components, *viz*. apomeiosis (formation of unreduced egg cell), parthenogenesis (fertilization independent embryo development) and functional endosperm development (autonomous or psuedogamous). In contrast to previous reports, the evidence has now gathered that these three components can be functionally uncoupled and recombination is possible between these components (Kaushal, *et al*., 2008). Such recombinations lead to diversity in seed development pathways and also provide a mechanism to modify the ploidy levels. Uncoupling of apomeiosis from parthenogenesis may yield high frequency of triploids and haploids. Utilizing this partitioning principle we have generated a ploidy series following a Hybridization–supplemented Apomixis-components Partitioning Approach (HAPA) in guinea grass, a model crop for polyploidy and apomixis research, (Kaushal *et al*., 2009). From a single 4x (2n=32) progenitor, a ploidy series has been developed represented by 3x, 4x, 5x, 6x, 7x, 8x, 9x and 11x cytotypes. This ploidy series offers advantage of studying ploidy regulated gene expression. There have been sporadic reports on effect of polyploidy in expression of apomixis per se; however information on effect of polyploidy on individual apomixis components is not available. The guinea grass ploidy series with sequentially added monoploid genome doses has been used in present study to understand the effect of ploidy levels on phenotypic expression of partitioned apomixis components.

Materials and Methods

28 lines of guinea grass (*Panicum maximum* Jacq.) representing various ploidy levels *viz*. 3x (1 line), 4x (4 lines), 5x (3 lines), 6x (10 lines), 7x (1 line), 8x (2 lines) and 9x (7 lines), developed through HAPA were subjected to studies on phenotypic expression of apomixis and components. All the lines were characterized for their mode of reproduction following methyl salicylate mediated ovule clearing, whole mount observed under DIC microscopy following Young *et al*., (1979). Eight nucleated embryo sacs (ES) containing 2 synergids, 1 egg cell, 2 polar nuclei and 3 antipodals were considered as sexual ES, while aposporous ES were 4 nucleated (2 synergids, 1 egg cell and 1 polar nuclei) (Warmke, 1954). Plants with all sexual or aposporous ES were termed as obligate sexual or aposporous, respectively, while those with representation of both types of ES were considered as facultative.

The partitioning of apomixis components and their expression was estimated by Flow Cytometric Seed Screen (FCSS) following Kaushal *et al*., (2009). Proportion of seeds containing triploids (3n), arising through 2n+n hybridization (B_{III} hybrids) and/or haploids (M_I) arising through (n+0) development in a seed lot collected through self-pollination from each line individually were used as a measure to estimate occurrence of partitioned apomixis components *viz*., apomeiosis and parthenogenesis. Single seed FCSS was performed with individual mature seeds from all plants representing ploidy series.

Results and Discussion

Apomixis expression has been known to be influenced by ploidy levels (Carman 1997, Quarin *et al*., 2001). In view of the recent reports on recombination and independent existence of apomixis components, it becomes important to study the effect of ploidy on expression of individual apomixis components. One of the approaches used here was to use a ploidy series developed through HAPA in guinea grass. Recombination between apomixis and parthenogenesis components may allow uncoupling of these two traits and eventually the unreduced egg cell will lose the capacity of parthenogenesis and will require fertilization for development of embryo thereby yielding 3n embryos (B_{III}, 2n+n). Alternatively, a meiotic egg cell will acquire parthenogenetic capacity and will develop into a haploid (M_I, n+0). How far these uncoupling events are influenced by ploidy levels, were studied in this experiment.

Mode of female reproduction of different lines representing various ploidy levels is represented in Table 1. Lines representing 3x and 4x cytotypes were all obligate aposporous in female reproduction, 7x, 8x and 9x cytotypes were facultative aposporous, while 5x and 6x cytotypes were represented by both obligate and facultative reproduction behavior. These cytotypes showed wide range of variation in their expression of partitioned apomixis components. Range as high as 0-43% in B_{III} production and 4-21% in M_I production has been observed. This variation in expression of partitioned apomixis components can be attributed to genotypic differences within a ploidy level. No clear cut correlation could be observed between enhancements in frequency of B_{III} hybrids over ploidy raise; however, it was very evident that increase in ploidy had positively affected the expression of M_I seeds. As evident from Fig 1., it was noted that expression of parthenogenesis (M_I production) has increased from 6x till 9x in a linear fashion, however required a minimum level of ploidy to initiate the expression, as no M_I was observed in plants representing lower ploidies i.e. 3x, 4x and 5x.

The studies clearly indicated the effect of ploidy on phenotypic expression of apomixis component traits. While higher ploidies effect formation of both B_{III} and M_I hybrids, a minimal level of threshold ploidy was found to be essential for expression of partitioned parthenogenesis component. Ploidy regulated expression of key genes involved in reproductive pathway is underway to correlate phenotyping data with expression analysis in view to identify genes modulating in response to mode of reproduction and change in ploidy.

Table 1: Mode of female reproduction *vis-a-vis* ploidy level

Ploidy (x)	**2n=**	**No. plants**	**Mode of reproduction**		**Range (%)**		**Average (%)**	
			Obl Apo	**Fac Apo**	**B_{III}**	**M_I**	**B_{III}**	**M_I**
3x	24	1	1	-	20	0	20.0	0.0
4x	32	4	4	-	0-16	0	9.0	0.0
5x	40	3	2	1	0-42.9	0	15.4	0
6x	48	10	3	7	0-34.6	0-6.5	14.5	1.4
7x	56	1	-	1	29	3.2	29	3.2
8x	64	2	-	2	8.1-9.4	2.7-14.5	13.7	8.6
9x	72	7	-	7	6.7-23.9	4.0-21.2	14.2	10.5

Obl Apo: Obligate apomictic; Fac Apo: Facultative apomictic

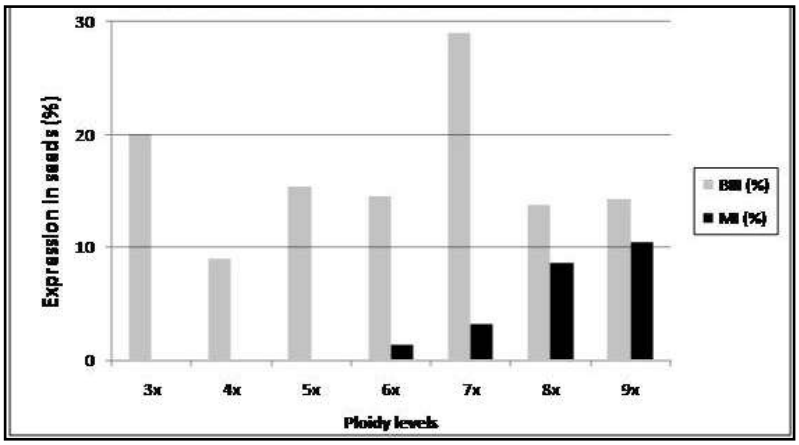

Fig. 1: Average expression of apomixis components over ploidy level

References

Carman, J. G. 1997. Asynchronous expression of duplicate genes in angiosperms may cause apomixis, bispory, tetraspory, and polyembryony. *Biol. J. Linn. Soc*. 61**:**51-94.

Kaushal, P., A. Agrawal, D. R. Malaviya, S. A. Siddiqui and A. K. Roy. 2009. Ploidy manipulation in guinea grass (*Panicum maximum* Jacq., Poaceae) utilizing a Hybridization-supplemented Apomixis-components Partitioning Approach (HAPA). *Plant Breed* 128: 295-303.

Kaushal, P., D. R. Malaviya, A. K. Roy, S. Pathak, A. Agrawal, A. Khare and S. A. Siddiqui. 20082 Reproductive pathways of seed development in apomictic guinea grass (*Panicum maximum* Jacq.) reveal uncoupling of apomixis components. *Euphytica* 174: 261–281.

Nogler, G. A. 1984. Gametophytic apomixis. In: *Embryology of Angioperms* (Ed. Johri BM). Springer, Berlin, pp: 475-518.

Quarin, C. L., Espinoza, F., E. J. Martinez, S. C. Pessino and O. A. Bovo. 2001. A rise of ploidy level induces the expression of apomixis in *Paspalum notatum*. *Sex plant Report* 13:243-249.

Warmke, H. E. 1954. Apomixis in *Panicum maximum*. *Am. J. Bot*. 41: 5-11.

Young, B. A., R. T. Sherwood, E. C. Bashaw. 1979. Cleared-pistil and thick sectioning techniques for detecting aposporous apomixis in grasses. *Can. J. Bot*. 57: 1668-1672.

Acknowledgement

Authors thankfully acknowledge the financial assistance (grant SR/S0/PS-117/2010) provided by the Department of Science and Technology, New Delhi, India.