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Research Note

A_5 cytoplasm: way to three way forage pearl millet hybrids

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Abstract
In the present study, two CMS lines *viz*., ICMA 03333 and ICMA 01777 based on A_5 cytoplasm were used. These A_5 lines were crossed with maintainers of different cytoplasmic sources during *kharif* 2016. The maintainer lines belong to A_1, A_4 and *Violaceum* cytoplasmic sources. The 15 F_1 hybrids developed from the crosses were found to be fully sterile and could be used as potential seed parents to develop three way forage hybrids.

Key words
Pearl millet, A_5 cytoplasm, Male sterility

Pearl millet commonly known as bajra is grown widely in the arid and semi arid tropical regions throughout the world. In the era of global warming, it's an important crop for diversification as it has the potential to withstand adverse agro climatic conditions where other crops like sorghum and maize fail to give economic yields. In India, it is grown in drier areas of central and western regions (Rajasthan, Maharashtra, Gujarat, Uttar Pradesh and Haryana) covering nearly about 90 per cent of the total area in these states. It covers an area of 7.5 million hectares with a production of 9.07 million tons and average productivity of 1305 kg/ha (Directorate of Millet Development, 2018) in the country. In the summer season, pearl millet provides a viable option as a green forage crop in northwestern India. In Punjab state, pearl millet is the second major fodder crop after sorghum and is grown on about 1.5 lakh hectare area (Anonymous, 2018). Pearl millet has outstanding biomass production power under low rainfall conditions which makes it an important summer growing forage crop for the rainfed conditions. It supplies green fodder in the lean period during the summer months of May to July and in combination with other fodder crops during the *kharif* season. In marginal to poor environments, the dry fodder and straw of bajra are also used to feed the livestock, particularly during the dry periods when green fodder is in limited availability. Moreover, pearl millet fodder has no antinutritional components like HCN content, so, it can be grazed, cut and fed at any growth stage, unlike sorghum. Thus, there is a need to produce forage hybrids for *kharif* and *summer* season. But the fodder crop requires three times more seed than the grain crop. Although, forage pearl millet varieties are a good option for resource poor farmers but the three way forage hybrids developed using male sterile hybrids as productive female parents after crossing with distant maintainers as male parents, would help in economic seed production owing to the higher seed yield capacity of F_1 sterile hybrids than a CMS line. The A_5 cytoplasmic male sterility system developed by Rai, (1995) offer a greater chance for developing genetically diverse male sterile hybrids using distantly related maintainer lines as about 99 per cent lines are maintainers of the A_5 CMS system (Rai *et al.,* 2000). The A_5 system is the consentient option for seed parent evolution, which would empower to generate male sterile F_{1s} with more ease due to the huge number of maintainers and the most stable male sterility (Rai *et al.,* 2009). A_5 CMS system is the most stable CMS system (Rai *et al.,* 2001 and Rai *et al.,* 2008) and having the highest frequency of maintainers in diverse composites (Rai *et al.,* 2006). Different male sterility based CMS lines has been used in different studies to experience heterosis (Jethva *et al*., 2012) and variability studies (Sandeep and Biradar, 2020). In the present study, efforts were made to identify the different maintainers to produce sterile F_{1S} which could be used as

potential seed parents of heterotic three way forage pearl millet hybrids to cater for the growing demand for fodder pearl millet.

The present study was conducted at Forage and Millet Research Farm, Department of Plant Breeding and Genetics, PAU, Ludhiana during *kharif* 2016 and *kharif* 2017. Two A_5 cytoplasms based CMS lines *viz.,* ICMA 03333 and ICMA 01777 were selected for the study. These two CMS lines were crossed with 15 different maintainers of various CMS sources other than A_5 (**Table 1**) during *kharif* 2016. These thirty F_{1S} generated were evaluated for male sterility/seed set during *kharif* 2017 to be used as parents for the development of high green forage yielding three way hybrids. During 2017, fresh crosses were also attempted to be tested in *kharif* 2018. The crosses were planted in the field in an unreplicated trial in single row plots of 2 m row length during *kharif* 2017 and *kharif* 2018. The spikes of five plants in each treatment were selfed with an imported parchment paper bag at the boot leaf stage and the same plants were used for recording the per cent seed set under bagging.

Table 1. List of inbred lines (maintainers of various CMS sources other than A_5)

Sr.No.	Inbred	Geographical Source	Cytoplasm
1	ICMA-04777B	ICRISAT, Hydrabad	A_4
2	PB-605B	PAU, Ludhiana	*Violaceum*
3	PB-274B	PAU, Ludhiana	A_4
4	ICMA-92777B	ICRISAT, Hydrabad	A_1
5	ICMA-92666B	ICRISAT, Hydrabad	A_1
6	ICMA-06999B	ICRISAT, Hydrabad	A_1
7	PB-220B	PAU, Ludhiana	A_1
8	ICMA-02999B	ICRISAT, Hydrabad	A_4
9	ICMA-91777B	ICRISAT, Hydrabad	A_1
10	ICMA-97111B	ICRISAT, Hydrabad	A_1
11	PB-111B	PAU, Ludhiana	A_1
12	ICMA-06555B	ICRISAT, Hydrabad	A_1
13	PB-263B	PAU, Ludhiana	A_4
14	ICMA-00444B	ICRISAT, Hydrabad	A_1
15	PB-543B	PAU, Ludhiana	A_1

The A_5 CMS lines ICMA 03333 and ICMA 01777 offered great success to the present study. The morphological characters of these two CMS lines are presented in **Table 2**.

The paramount features that contribute to the commercial viability of any male sterility system are complete male sterility of A line and a high level of male fertility restoration of their hybrids (Rai *et al.,* 2001). The study confirmed the reports of non availability of suitable restorers of A_5 cytoplasm as also reported by Rai *et al.* (2006). The CMS lines ICMA 03333 and ICMA 01777 based on A_5 cytoplasm were crossed with the 15 different maintainers (B lines) of different cytoplasmic backgrounds i.e. A_1, A_4, *Violaceum* (**Table 1**). The success of this study was based on the fact that all the maintainers of different cytoplasmic sources used in the present study when crossed with A_5 CMS lines were able to maintain the sterility of A lines. This was confirmed by bagging the individual's panicles of A × B cross and seed set under bag was recorded (**Table 3**). It was found that there was no seed set under the bag and confirmed that these crosses were completely sterile. This may be ascribed to the facts that different cytoplasmic backgrounds of the female parents showed variable interactions of nuclear and cytoplasmic organelle genomes (Amiribehzadi and Tara Satyavathi, 2012). The sterility of F_{1S} from A_5 cytoplasm with different nuclear background maintainers is therefore expected. So, this sterile F_{1S} were further used for the development of three way forage hybrids.

Table 2. Morphological characters (mean value) of A_5 CMS lines used in study

Genotype	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Panicle length (cm)	Panicle diameter (cm)
ICMA 03333	53-61	31-32	2.4-2.6	15.3-18.2	2.6-3.2
ICMB 03333	59-66	33-36	2.6-3.1	17.4-19.0	2.6-3.1
ICMA 01777	108-118	46-50	4.2-4.6	18.7-20.4	3.9-4.3
ICMB 01777	106-112	44-47	4.2-4.7	19.3-22.3	4.5-4.7

Table 3. Per cent seed set under bagging in F_1 hybrids using different maintainers in kharif 2017 and kharif 2018

Cross	Seed set under bagging (%)	Cross	Seed set under bagging (%)
ICMA 03333A (A5) × ICMA-04777B (A_4)	0	ICMA 01777A (A5) × ICMA-04777B (A_4)	0
ICMA 03333A (A5) × PB-605B (vio)	0	ICMA 01777A (A5) × PB-605B (vio)	0
ICMA 03333A (A5) × PB-274B (A_4)	0	ICMA 01777A × PB-274B (A_4)	0
ICMA 03333A (A5) × ICMA-92777B (A_1)	0	ICMA 01777A (A5) × ICMA-92777B (A_1)	0
ICMA 03333A (A5) × ICMA-92666B (A_1)	0	ICMA 01777A (A5) × ICMA-92666B (A_1)	0
ICMA 03333A (A5) × ICMA-06999B (A_1)	0	ICMA 01777A (A5) × ICMA-06999B(A_1)	0
ICMA 03333A (A5) × PB-220B (A_1)	0	ICMA 01777A (A5) × PB-220B(A_1)	0
ICMA 03333A (A5) × ICMA-02999B (A_4)	0	ICMA 01777A (A5) × ICMA-02999B (A_4)	0
ICMA 03333A (A5) × ICMA-91777B (A_1)	0	ICMA 01777A (A5) × ICMA-91777B (A_1)	0
ICMA 03333A (A5) × ICMA-97111B (A_1)	0	ICMA 01777A (A5) × ICMA-97111B (A_1)	0
ICMA 03333A (A5) × PB-111B (A_1)	0	ICMA 01777A (A5) × PB-111B (A_1)	0
ICMA 03333A (A5) × ICMA-06555B(A_1)	0	ICMA 01777A (A5) × ICMA-06555B(A_1)	0
ICMA 03333A (A5) × PB-263B (A_4)	0	ICMA 01777A (A5) × PB-263B (A_4)	0
ICMA 03333A (A5) × ICMA-0444B(A_1)	0	ICMA 01777A (A5) × ICMA-0444B (A_1)	0
ICMA 03333A (A5) × PB-543B (A_1)	0	ICMA 01777A (A5) × PB-543B (A_1)	0

The present study, therefore brings out the possibility of the development of sterile F_1 hybrids using A_5 cytoplasmic based CMS lines. These F_1 hybrids would be used as potential female parents with high seed yield to economise the seed production as regards to higher seed rate of fodder pearl millet than grain pearl millet.

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