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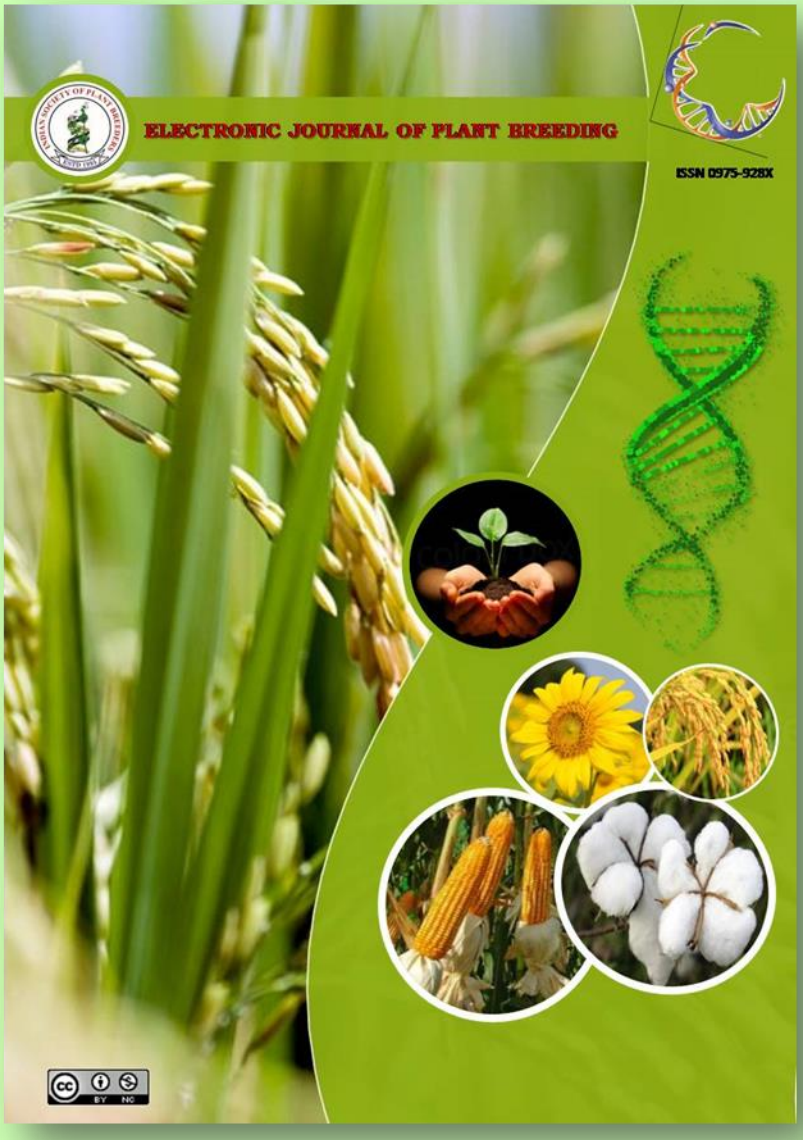

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Research Article

Multivariate analysis of hybrid performance and genetics of fertility restoration behavior in *rabi* sorghum hybrids

Krishnananda P. Ingle[*1], Santosh J. Gahukar[1], Mangesh P. Moharil[1], Pravin V. Jadhav[1], Rameshwar B. Ghorade[2], Vikram V. Kalpande[2]

[1]Biotechnology Centre, Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (MS), 444 104, India

[2]Sorghum Research Unit, Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (MS), 444 104, India

E-Mail*:* krisona369@gmail.com

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Abstract

The present investigation was undertaken with a view of expedition of putative restorers for the development of high yielding *rabi* sorghum hybrids. Three CMS line were tested with ten restorers in line × tester mating design to evaluate seed set per cent and fertility restoration behavior. Restorers AKRB-335-3, Rb-413-1, AKRB-428, AKRB-429, AKRB-430 and AKRB-431 exhibited high fertility restoration ability in the CMS lines and therefore, hybrids *viz*. AKMS 30A×AKRB-335-3, AKMS 30A×AKRB-428, AKMS 30A×Rb-413-1, AKMS 30A×AKRB-429, AKMS 30A×AKRB-430, AKMS 30A×AKRB-431, AKRMS 45A×AKRB-335-3, AKRMS 45A×AKRB-428 and AKRMS 45A×Rb-413-1 showed high seed set percentage with anther fertility rating (9.0). The inheritance of fertility restoration revealed that observed frequency fits well into Mendelian ration of 3:1 for seed setting indicates that fertility restoration is governed by single dominant gene. Cluster analysis differentiate thirty hybrids in three classes, fully fertile revealed that their corresponding restorers considered to be putative restorers having high potential of fertility restoration, partial fertile hybrids stated that some modifier genes has a vital role in restoration of fertility, whereas, sterile hybrids indicate that their restorers act as maintainer as it maintain the sterility of all three CMS line.

Keywords

Anther fertility rating, cluster analysis, genetics of fertility restoration, seed set per cent

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop after wheat, maize, rice and barley, cultivated globally over 40 mha (Dandin *et al*., 2014; Praveen *et al*., 2015). Cytoplasmic male sterility is the nuclear-mitochondrial interaction, wherein, plant is unable to produce functional pollens leading to male abortion (Budar, 2003; Chase, 2007). CMS in the sorghum revolutionized the sorghum production through the subsequent exploitation of the A_1 (*milo*) cytoplasm system (Madugula *et al*., 2018). The (A_1) *milo* cytoplasm specifically explored for the commercial hybrid development programme in America, China, Australia and India because of the ease and stability of fertility restoration (Senthil *et al*., 1998; Reddy and Stenhouse, 1994). The genotype conversion with paired block crosses in the highly adaptable promising lines with A_1 (*milo*) cytoplasm have been well exploited but not with other cytoplasm. Secondly, majority of breeding lines act as restorers on A_1 (*milo*) cytoplasm (Senthil *et al*., 1998). Other cytoplasm sources in the sorghum are A_2 (Schertz and Ritchey, 1978), A_3 (Quinby, 1980) A_4 (Worstell *et al*., 1984), Indian A_4 (A_4M, A_4VZM, A_4G) (Rao *et al*., 1984), A_5, A_6, 9E (Webster and Singh, 1964) and KS cytoplasm (Ross and Hackerott, 1972). The efficient utilization of the CMS system leads the congregation of the desired characters into the hybrid parents (Mishra and Kumari, 2018).

The major constraint in *rabi* sorghum hybrid is fertility restoration (Prabhakar *et al*., 2014). Classical genetic studies indicate that modifier genes play an important role in fertility restoration (Maunder and Pickett, 1959; Erichsen and Ross, 1963; Miller and Pickett, 1964).The A_2 cytoplasm is the only acceptable alternative for A_1 cytoplasm for commercial exploitation of hybrids in sorghum (Moran and Rooney, 2003) but the genetic architecture of the fertility restoration in A_2 cytoplasm and other 'non-*milo'* cytoplasm is unknown and work in this sphere is quite limited (Jordan *et al*., 2011). Department of Employment, Economic development and Innovation (DEEDI), Queensland, Australia observed that lines having capacity to restore A_2 cytoplasm found typically also restorer in A_1 cytoplasm, whereas, lines that restored A_1 cytoplasm were often not restore in A_2 cytoplasm. Despite the availability of diverse

alternate CMS systems, hybrid vigor is exploited mainly using A_1 (*milo*) cytoplasm (Praveen *et al*., 2015).

The inheritance pattern of male fertility/sterility relies on the cytoplasm-genetic interactions (Senthil *et al*., 1998). Cytoplasmic genetic male sterility (CGMS) system discovery led the development of the parental line (restorers/R line) that carry dominant genes and restore fertility in hybrid cultivars and explored for the development of high yielding hybrids in *rabi* sorghum (Jordan *et al*., 2011). Fertility restoration trait associated with pollen fertility and seed set per cent. Thus, seed setting often considered to be a major reason for the low productivity (Ram *et al*., 2011). '*Rabi'* sorghum hybrids showed poor seed set as there is lack of appropriate hybrids with acceptable seed setting (Prabhakar *et al*., 2014). Therefore, the present investigation was conducted with an objective of exploration of the high yielding *rabi* sorghum hybrids with high genetic potential for fertility restoration in the CMS background through the identification of the potential restorers and the studied the genetics of fertility restoration.

Materials and Methods

Genetically diverse ten parents (Restorers) were deliberately selected to cross with three CMS line (Table 1) at Sorghum Research Unit (SRU), Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra state, India The crosses were made based on line × tester mating design for obtaining F_0 seeds during *rabi* 2014-2015, F_1 seeds during 2015-2016 and F_2 seeds (selected crosses) during *rabi* season 2016-2017. The experiment was laid out in randomized block design with three replicates using thirty crosses, thirteen parents and two standard checks. Each genotype was planted in a single row of 3 m length with 30 cm spacing between rows and 15 cm within rows. The recommended package and practices was followed as per recommendation.

Prior to flowering 5 heads from each plot were covered with brown paper bags to exclude foreign pollen contamination. All self pollinated panicles per replication were harvested at physiological maturity. Total grain number was assessed because grain production indicates viable pollen. For each branch total number of florets, the number of viable (seed filled) and non-viable (unfilled) floret was counted. Seed set percent was calculated as the number of viable florets as a percentage of the total number of florets and the percentage of seed set was calculated as per procedure modified from (Kishan and Borikar, 1989; Jordan *et al*., 2011; Sinha *et al*., 2013) enlisted in Table 2.

Five heads of each F_1 hybrids from three replicates were covered with brown paper bags prior to anthesis and anther fertility rating was scored qualitatively. DEEDI (Department of Employment, Economic Development and Innovation, Queensland, Australia) scored it as 1-9 rating which relies on the phenotypic evaluation of anther size, color and morphology (Jordan *et al*., 2011). The experiment was conducted at the experimental farm of Biotechnology centre, Department of Agricultural Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra state, India. Seeds were harvested from selected fully fertile CMS × Restorer F_1s grown during *rabi* 2016-2017 as F_2 population (~225 plants per population). Finally, the data were subjected to chi-square analysis to know the inheritance pattern and genetics of fertility restoration on A_1 cytoplasm (Dandin *et al*., 2014).The seed set per cent value of F_1 hybrids was subjected to DARwin version 5.0 (Perrier *et al*., 2003; Perrier and Jacquemoud-Collet, 2006) and Dendroscope (Huson and Scornavacca, 2012) software to differentiate the hybrids in different clusters.

Results and Discussion

Seed set per cent revealed highly significant difference among the crosses suggesting the presence of considerable genetic variation. The seed set value varied from 0% -100% (Table 3).

Amongst all tested hybrids, nine hybrids (cross combinations) showed variable expression and exhibited high seed set percentage with anther fertility rating (9.0). The class of fully fertile hybrids include AKMS 30A×AKRB-335-3, AKMS 30A×AKRB-428, AKMS 30A×Rb-413-1, AKMS 30A×AKRB-429, AKMS 30A×AKRB-430, AKMS 30A×AKRB-431, AKRMS 45A×AKRB-335-3, AKRMS 45A×AKRB-428 and AKRMS 45A×Rb-413-1. The class of partially restored hybrid was heterogeneous and include AKMS 30A×RS 585, AKMS 30A×AKR 354, AKMS 30A× SLR 24, AKRMS 45A×RS 585, AKRMS 45A×AKR 354, AKRMS 45A×SLR 24, AKRMS 45A ×AKRB-429, AKRMS 45A×AKRB-430, AKRMS 45A×AKRB-431, AKMS 66-2A× RS 585, AKMS 66-2A×AKR 354, AKMS 66-2A×AKRB-335-3, AKMS 66-2A× SLR 24, AKMS 66-2A×AKRB 428, AKMS 66-2A×Rb-413-1, AKMS 66-2A×AKRB-429, AKMS 66-2A×AKRB-430 and AKMS 66-2A×AKRB-431 with anther fertility rating (7), whereas, three hybrids, AKMS 30A×RB 324, AKRMS 45A×RB 324 and AKRMS 66-2A×RB 324 found completely sterile with no seed setting under bagged conditions having (5) anther fertility rating (Table 3).

The seed set per cent depends on the compatibility of the cytoplasm of male gamete with the nuclear background of the female gamete. The extreme variation was observed in different cross combinations this could be due to differential interaction of the nuclear background of the restorers with the A_1 cytoplasm CMS lines (Amiribehazadi and Satyavathi, 2012). Hybrids with no seed set considered as completely sterile and assume that their corresponding restorers are maintainer. This finding support the statement of Shalini *et al*. (2015), that the cross combination with null seed set indicated that their counterpart act as maintainer just maintaining the sterility of CMS line.

The segregation of fertile and sterile plants in all F_2 population derived from selected nine crosses fitted well with a monogenic ratio of 3:1, χ^2 (0.05 and 01 df=1)=3.84. The F_2 data from the present investigation suggest that a single dominant gene is responsible for fertility restoration of male sterile cytoplasm (Table 4). Reddy *et al*. (2004) and Dandin *et al*. (2014) also observed that single dominant gene action in one cross for the restoration on maldandi cytoplasm. The differential fertility restoration relies on the parental lines, with some populations exhibiting a distinct bi modal distribution, whereas, others having a broad continuum of restoration phenotype. This variation reflects the segregation of a series of partial fertility restoration genes, with the number of modifier genes (Jordan *et al*., 2011). However, Yadav *et al*. (2010), suggested more likelihood of a single gene control of male sterility and fertility restoration.

The observation of seed set per cent recorded on a hybrid represents the restoration ability of a pollen parent. This seed set vary from 0% - 100%. Kishan and Borikar (1989) elaborate the fertility restoration in sorghum and concluded that, those hybrids showing >80% seed set were broadly grouped as restorers and these restorers were classified into different categories based on their restoration ability. The genotypes which set seed above 80%-90% were categorized as potential restorers which restore fertility fully, those are in the range of 10%-80% considered as partial restorers, whereas, hybrids showing 0% seed set grouped as maintainer and no restoration was observed. The restoration categorized based on seed set per cent are presented in Table 5.

In the present investigation, ten restorers were evaluated for their restoration ability on three cytoplasm male sterile lines based on seed set per cent. Thus, restorers AKRB 335-3, AKRB 428, Rb-413-1 restore fertility in both CMS lines (AKMS 30A and AKRMS 45A), whereas, restorers AKRB 429, AKRB 430 and AKRB 431 restore fertility only in AKMS 30A and act as partial restorer for AKRMS 45A and AKRMS 66-2A. Restorer RS 585, AKR 354 and SLR 24 acts as partial restorer for three CMS lines (AKMS 30A, AKRMS 45A and AKRMS 66-2A) (Table 5). This clearly suggests the impact of nuclear and cytoplasm interactions which affect the fertility status. These findings support the statement of Bharaj *et al*. (1991) that restorer line found to restore completely under a particular CMS line may restore partially under another CMS line possessing the same CMS source and suggested that this differential fertility restoration behavior of the restorers indicates the complexity of the fertility restoration in inheritance.

Restorer RB 324 could be a maintainer line maintains the sterility of three CMS lines (Table 5). This shows the possibility of utilization of that particular combination and development of new male sterile lines in the future in different cytoplasm backgrounds. Similar findings also reported by Shalini *et al*. (2015). The fertility restoration genes and their modifiers are under strong differential selection in hybrid breeding programs with selection for complete restoration of fertility in CMS hybrids by male parents and complete sterility in male sterile cytoplasm in female parents. Hence, the identification of restorers and maintainers for A_1 cytoplasm opens up new avenues in development of hybrids in *rabi* sorghum.

Neighbour joining cluster analysis differentiates thirty *rabi* sorghum hybrids into three major groups, A, B and C (Fig. 1). Group A comprises nine hybrids which are fully fertile (indicated by color green in figure) releasing anthers in a variable range with high amount of pollen load and therefore having high seed set per cent. Group B, comprised of three hybrids which did not release anthers and therefore, did not release pollens and hence no seed set observed, considered them completely sterile and maintaining the sterility of three CMS line (AKMS 30A, AKRMS 45A and AKRMS 66-2A) (indicated by color pink in figure). Most of the hybrids, eighteen, were clustered together forming a solitary group C which includes partially restored hybrids and hence partial seed set observed (indicated by color blue in figure).

It was observed that thirty hybrids formed distinct group from each other so the genotypes studied in the present investigation can be said genetically

diverse. Ganapathy *et al*. (2012) reported that the hybrids grouped in same cluster has narrow genetic base with more genotypic similarity. In this study, eighteen hybrids were completely sterile which indicates that the sorghum breeding pool is relatively free of *rf* genes, whereas partially restored hybrid stated the presence of minor *rf* genes/modifier genes highly influenced the crosses and fully restored hybrids indicate the presence of single dominant gene having role in fertility restoration. This findings support the statement of the Weider *et al*. (2009).

The present investigation revealed that nine hybrids showed >80%-90% seed set percentage and hence considered as fully fertile and therefore, their corresponding restorers AKRB 335-3, AKR 354, Rb-413-1, AKRB 428, AKRB 429, AKRB 430 and AKRB 431 considered as putative restorers which restore good fertility in CMS lines and could be incorporated in further breeding programme for the development of high yielding *rabi* sorghum hybrids. The restorer RB 324 considered as maintainer and maintaining the sterility of all three CMS lines used in present study. The diverse set of the hybrids based on neighbor joining method could be used further for the differential gene expression profiling. The current study firstly brings out the successful development of hybrid combinations on A_1 cytoplasmic background. Secondly it also brings out the possibility of use of RB 324 in the development of new male sterile lines in sorghum. Thirdly the genetics of fertility restoration study revealed the presence of single dominant gene in action for fertility restoration trait. Furthermore appropriate combinations of CMS hybrids and fertile pollinators could lead a significant gain in yield.

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Table 1. **Genotypes used during present investigation**

SN	Cytoplasm	CMS (Male sterile line)	Restorers (Male parent)
1	A_1 (*milo*)	AKMS-30A, AKRMS-45A, AKRMS-66-2A	RS-585, AKR-354, AKRB-335-3, SLR-24, AKRB-428, RB-324, Rb-413-1, AKRB-429, AKRB-430, AKRB-431

Table 2. ***Per cent*** **seed set and fertility restoration behavior**

SN	Per cent (%) seed set	Restoration behavior
1	>80%-90%	Strong restoration (SR)/ fully fertile hybrids
2	40%-80%	Partial restoration (PR)/ partial fertile hybrids
3	10%-40%	Low restoration (LR)/ partial fertile hybrids
4	0.5%-10%	Very Low restoration (VLR)/ partial sterile hybrids
5	0%	Maintainer (M)

Table 3. ***Per cent*** **seed set and anther fertility rating observed in different cross combinations involving A_1 cytoplasm of sorghum**

Parents	RS 585	AKR 354	AKRB 335-3	SLR 24	AKRB 428	RB 324	Rb-413-1	AKRB 429	AKRB 430	AKRB 431
AKMS 30A	60 (7)* (PR)	75 (7)* (PR)	100 (9)* (SR)	60 (6)* (PR)	100 (9)* (SR)	0 (5)* (M)	100 (9)* (SR)	100 (9)* (SR)	100 (9)* (SR)	100 (9)* (SR)
AKRMS 45A	50 (7)* (PR)	60 (7)* (PR)	100 (9)* (SR)	55 (7)* (PR)	100 (9)* (SR)	0 (5)* (M)	100 (9)* (SR)	55 (7)* (PR)	80 (7)* (PR)	60 (7)* (PR)
AKRMS 66-2A	50 (7)* (PR)	50 (7)* (PR)	60 (7)* (PR)	60 (7)* (PR)	60 (7)* (PR)	0 (5)* (M)	50 (7)* (PR)	60 (7)* (PR)	60 (7)* (PR)	60 (7)* (PR)

***Anther fertility rating**:
5- sterile medium anthers, colored, dehiscence pore absent, 7-Partial fertile anthers, 9- Plump colored and fertile anthers

Per cent **seed set Range: 0-100%**:
> 80-90% - Fully fertile (SR), 40%-80% - Partial fertile (PR), 0% - Maintainer (M), SR: strong restoration, PR: partial restoration,
M: Maintainer

Table 4. Segregation pattern of F_2 population based on A_1 cytoplasm

S.N	Crosses	Population	No. of plants	No. of fertile plants	No. of sterile plants	Fertility/ sterility	χ^2 ratio
1	AKMS 30A × AKRB 335-3	F_2	205	145	60	2.417	1.992
2	AKMS 30A × AKRB 428	F_2	135	100	35	2.857	0.123
3	AKMS 30A × Rb-413-1	F_2	160	125	35	3.571	3.333
4	AKMS 30A × AKRB 429	F_2	135	100	35	2.857	0.123
5	AKMS 30A × AKRB 430	F_2	202	152	50	3.040	0.003
6	AKMS 30A × AKRB 431	F_2	190	135	55	2.454	3.157
7	AKMS 45A × AKRB 335-3	F_2	200	150	50	3.000	0.004
8	AKMS 45A × AKRB 428	F_2	198	150	48	3.125	1.220
9	AKMS 45A × Rb-413-1	F_2	200	152	48	3.166	1.280

Note: χ^2 0.05, 1: 3.84

Table 5. Classification of the sorghum restorers on A_1 cytoplasm background

Restorer	Complete restorer	Partial restorer	Maintainer
RS 585	-	AKMS 30A , AKRMS 45A, AKRMS 66-2A	None
AKR 354	-	AKMS 30A , AKRMS 45A, AKRMS 66-2A	-
AKRB-335-3	AKMS 30A , AKRMS 45A	AKRMS 66-2A	-
SLR-24	-	AKMS 30A , AKRMS 45A, AKRMS 66-2A	-
AKRB-428	AKMS 30A , AKRMS 45A	AKRMS 66-2A	-
RB-324	-	-	AKMS 30A, AKRMS 45A, AKRMS 66-2A
Rb-413-1	AKMS 30A , AKRMS 45A	AKRMS 66-2A	-
AKRB-429	AKMS 30A	AKRMS 45A, AKRMS 66-2A	-
AKRB-430	AKMS 30A	AKRMS 45A, AKRMS 66-2A	-
AKRB-431	AKMS 30A (A_1)	AKRMS 45A, AKRMS 66-2A	-

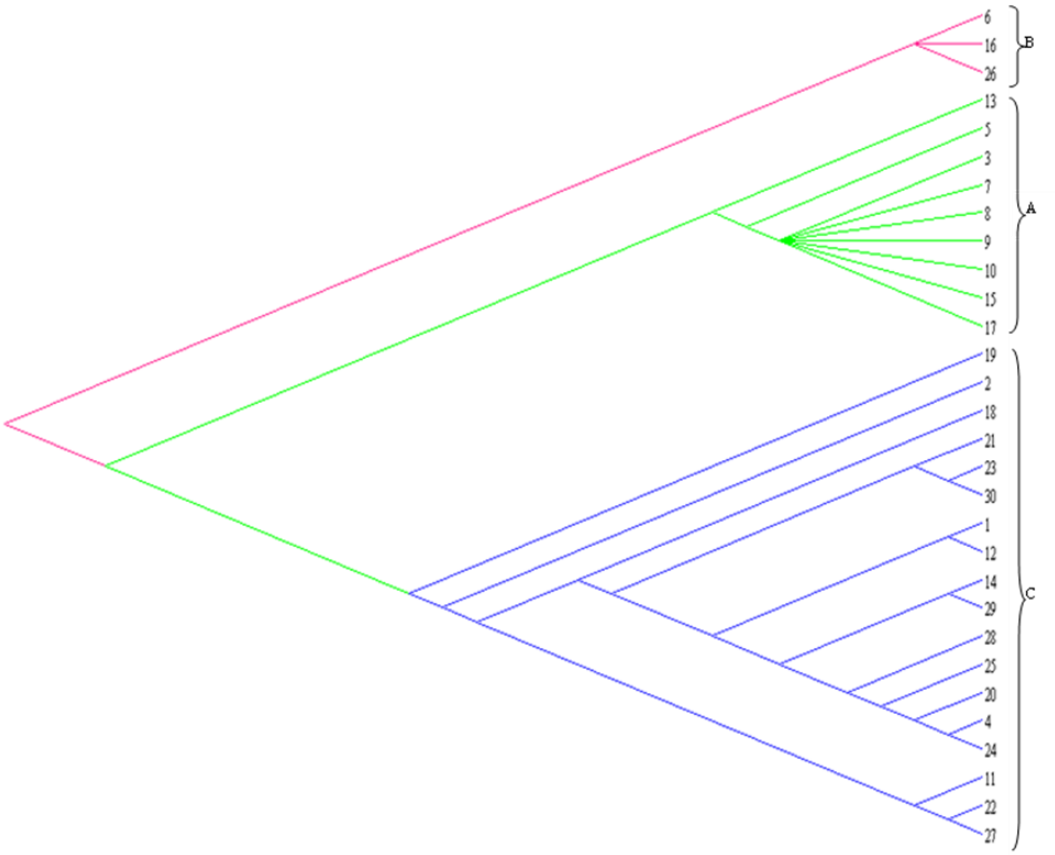
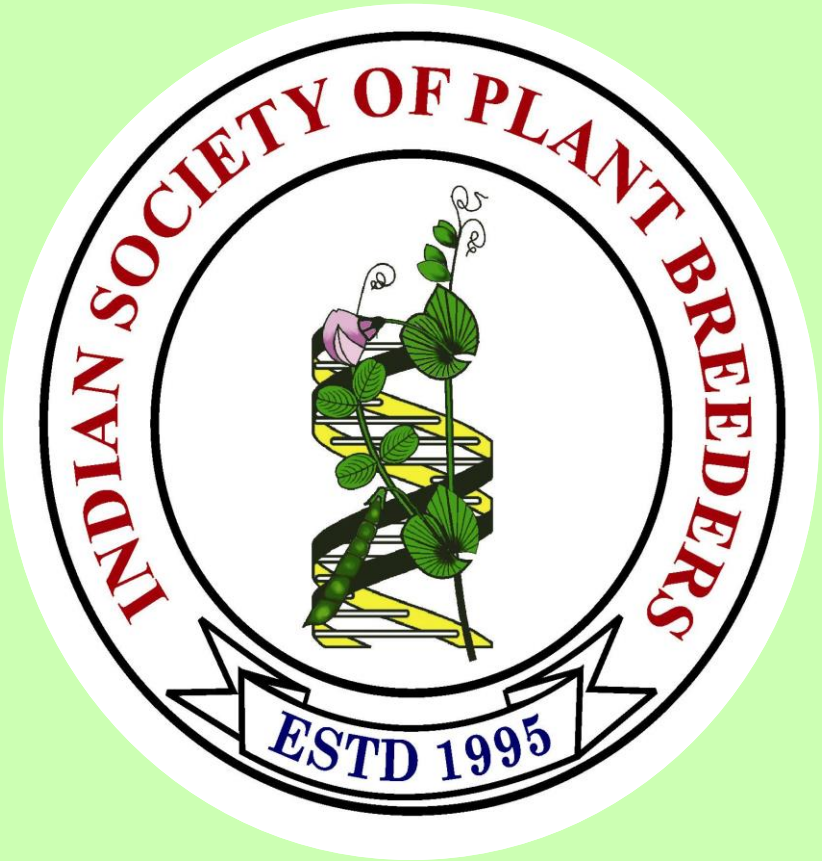


Fig. 1. Cluster analysis grouped the hybrids in three different clusters. The different working groups are identified by specific colors (green for high seed set/fully fertile hybrids, pink for no seed set/fully sterile hybrids, blue for partial seed set/partial fertile hybrids)

Sterile hybrids: AKMS 30A×RB 324, AKRMS 45A×RB 324, AKRMS 66-2A×RB 324
Fully fertile hybrids: AKMS 30A×AKR-335-3, AKMS 30A×AKRB-428, AKMS 30A×Rb-413-1, AKMS 30A×AKRB-429, AKMS 30A×AKRB-430, AKMS 30A×AKRB-431, AKRMS 45A×AKRB-335-3, AKRMS 45A×AKRB-428, AKRMS 45A×Rb-413-1
Partial fertile hybrids: AKMS 30A×RS 585, AKMS 30A×AKR 354, AKMS 30A× SLR 24, AKRMS 45A×RS 585, AKRMS 45A ×AKR 354, AKRMS 45A×SLR 24, AKRMS 45A×AKRB-429, AKRMS 45A×AKRB-430, AKRMS 45A ×AKRB-431, AKRMS 66-2A×RS 585, AKRMS 66-2A×AKR 354, AKRMS 66-2A×AKR-335-3, AKRMS 66-2A×SLR 24, AKRMS 66-2A×AKRB-428, AKRMS 66-2A×Rb-413-1, AKRMS 66-2A ×AKRB-429, AKRMS 66-2A×AKRB-430, AKRMS 66-2A×AKRB-431



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