*Turk J
Field Crops
2021, 26(1), 111-116
DOI: 10.17557/tjfc.650908*

DEVELOPMENT OF JAPONICA TYPE CYTOPLASMIC MALE STERILE (CMS) RICE LINES FOR COMMERCIAL HYBRID RICE IN MEDITERRANEAN ECOLOGICAL CONDITION

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Received: 25.11.2019

ABSTRACT

Hybrid rice has an average 15% to 20% yield advantage over inbred lines. The accessibility of stable cytoplasmic male sterility system (CMS) is vital for commercial hybrid rice. The experiment was conducted to develop japonica type CMS lines of national rice varieties. National varieties were tested to determine as maintainer, CMS or restorer features via test cross. The test crosses were made by using 19 japonica type national rice varieties and 21 CMS lines and 1 restorer line. All national rice varieties which tested are determined maintainer features. In addition, 8 CMS introduction lines and 1 restorer lines adapted Mediterranean ecological condition for hybrid breeding process. Two maintainer were backcrossed for 6 generations and it was developed similar agronomic features and 100% spikelet sterile CMS lines. For production of CMS lines *Osmancik-97* and *Kiziltan,* it was employed wild abortive rice which is CMS *V20A*. Successive backcrossing of varieties succeeded to produce completely male sterile CMS lines. Wild abortive cytoplasm did not affect agronomic characteristic. *Osmancik-97* and *Kiziltan* were utilized as a recurrent parent in breeding program due to their popularity, and the new WA-CMS lines might be useful for hybrid rice breeding. *CMS-Osmancik-97* and *CMS-Kiziltan* are the first developed national CMS rice lines in Turkey.

Keywords: Breeding, cytoplasmic male sterility (CMS), hybrid rice, *Oryza sativa*, *Osmancik-97*

INTRODUCTION

It has been calculated that hybrid rice provides 15-20% yield advantage over conventional rice. The country with the highest hybrid rice production area compared to conventional rice area is China with 56%. After that, the countries with the highest hybrid production area are the USA, Vietnam, Bangladesh, Philippines, India, and Indonesia with 14.5%, 9.4, 6.8%, 4.3%,3.2%, and 0.5%, respectively (Barclay, 2010). Hybrids have been used efficaciously especially far East since 1975 however it is new tool for Europe. Initial step is to supply the male sterile (MS) lines to hybrid breeding framework. Genetic and non-genetic mechanisms are available to exploit for male sterility. Hybrid rice is produced by two or three lines breeding methods. Two lines method included thermo-sensitive genic male sterile line (TGMS) and Chemical Hybridizing Agents (CHA). Our previous studies focused on determine the better method for Mediterranean ecological condition. Sterility of male is specified 22-49% when sprayed gametocides in some cultivars. TGMS lines which TGMS1 and TGMS2 have 89-96% sterility in the uncontrolled field circumstance. CMS lines have been determined 100% male sterility (Unan et al., 2016). It was revealed that three lines breeding method is more stable for Mediterranean ecological condition. And also the most of hybrid rice varieties have been released subject to 3-line system, including R (restorer line), A (CMS line), and B (maintainer line). Three-line system utilize CMS lines especially wild abortive type CMS (WA-CMS) lines (Sattari et al., 2007).

Utilization of CMS framework might be fitting methodology for tradal hybrid rice. CMS frameworks are constrained by related of cytoplasm with nucleus gene (Kaul, 1997). Advancement of commercial hybrids utilizing CMS need a restorer R-line, a male sterile A-line, and a maintainer B-line. Principle attributes of these lines are A-line is male sterile, which are utilized as female line hybrid breed multiplying plot. Near-isogenic lines of A-line is B-line, which are expected to maintain A-line. B-line is considered as key line without that A-line could not be kept up (Ali et al., 2014). Fruitful utilization of hybrid breeds vigor in rice to a greatly related to the accessibility of regional developed restorer and CMS lines (Kumar et al., 1996). It was assessed CMS lines from Chinese which Zhen Shan97A and V20A alongside their maintainer, but the lines were not adapted Bangladesh ecological condition because of high susceptibility to diseases and insects (Julfiquar et al., 2002). Besides, plant breeders need wide scope of CMS lines to deliver wanted hybrids.

Consequently, it is important to move accessible CMS framework into local elite breeding lines. Thus, utilization of some CMS lines might be useful to ease the issue, and to create versatile, heterotic cross breeds (Ali et al., 2014).

The aim of this study is development of japonica type CMS lines for commercial hybrid breeding. In addition, second aim is to determine adaptable CMS-maintainer and restorer lines for Mediterranean ecological condition.

MATERIALS AND METHODS

These experiments were conducted in rice experimental fields and plant growth chamber of the Trakya Agricultural Research Institute located in Edirne, Turkey (41°38′41″N, 26°35′46″E) between 2015 and 2019 during the summer season. Nineteen national japonica type rice variety, 1 restorer variety, 21 CMS lines and their corresponding maintainer lines was formed the material for the current examination. National varieties, restorer variety, A-lines, and B-lines lines were planted in two rows per plots having 10 hills per row with spacing of 20 × 20 cm apart. Field evaluation was conducted at experiment field, Edirne, Turkey between 2015-2019. *Osmancik-97* and *Kiziltan* varieties are among the famous Turkish varieties (Tatar and Gevrek, 2007; Gevrek, 2012) so they were utilized a recurrent parent for backcross process.

Spikelet fertility (%) = (Filled grains per panicle / Total spikelet per panicle) × 100

Spikelet sterility (%) = (Non-filled grains per panicle / Total spikelet per panicle) × 100

Observations were recorded on five randomly selected panicle isolated plants of each lines in each replication for various morphological and floral traits. Pedigree backcross method was utilized to breeding progress. The agronomic observation was evaluated by Standard Evaluation System for Rice (IRRI, 2014). Data were analyzed using analysis of variance (SAS, 2002) and means of treatment, and were compared based on the least significant difference test (LSD) at a 0.05 probability level.

RESULTS AND DISCUSSION

Evaluation of CMS lines for Mediterranean ecological condition

Currently developed and designated CMS lines and their maintainers were evaluated as CMS source in lowland ecology (IRRI, 1997). In Mediterranean ecological conditions, CMS lines having characteristic like early or moderate 50% flowering time and maturity, short height good panicle length. Twenty-one CMS and their maintainer lines were evaluated according to some agronomical characters both 2015 and 2016. *V20A, 15A, 23A, 31A, 76A, IR93559A, IR93560A,* and *IR93563A* were classified suitable in most of characteristics such as early flowering time, short height, and panicle length (Table 1). Flowering time observation revealed that early, moderate and, late flowering days, as 80-84 days, 86-88 days and, 98-135 days, respectively. Plant length ranged between 48-108 cm and, panicle length was between 13-16 cm. Result showed that existence of significant differences amongst lines for all the characteristics assessed. In different investigation significant varying was noted amongst the distinctive class or lines researched by Jain et al. (2017).

Identification of maintainer and restorer lines against V20A

It is the breeding nursery which F1s of cytoplasmic male sterile lines and test varieties from the source nursery are screened for spikelet fertility, and other agronomic characteristic to classify the possible restorers, maintainers, and heterotic hybrids (IRRI, 1997). Spikelet fertility of F_1s between *V20A* and 19 national rice and, F_1 between *V20A* and one restorer line (*IR50*) as a control were determined during of 2015-2016 for classification of restorer and maintainer lines. Spikelet sterility was 100% for all F_1s (*V20A* × 19 National Rice) and spikelet fertility was 90.6 for restorer control cross (*V20A* × *IR50*). Considering the results of two years, 19 F_1s were designated as completely sterile and it was estimated that the pollen parents carry maintainer genes. The 19 national rice varieties may be specified as maintainer lines against CMS lines (Table 2). The result of this experiment are somehow higher than the finding of Govinda Raj and Virmani (1989). Their results are as 24% restorer and 40% maintainer when tested 37 indica/japonica derivatives against CMS. And also our results are higher than results of Ali et al. (2014), they found 23 out of 129 crosses (15%) contributed straightforwardly to recognize restorers and maintainers. This distinction may be because of the hereditary foundation of the assessed genotypes.

Backcross breeding nursery

Backcross nursery is wherein the CMS framework from the accessible CMS lines is moved into the hereditary background of elit maintainer lines distinguished in the test-cross nursery by continuous backcrossing (IRRI, 1997). Test-cross is first cross between elite lines and CMS lines of an ideal cytosterility source to test their maintaining capacity. These elite lines which are classified as maintainer lines are repeatedly backcrossed up to six generations for complete transfer of cytosterility source (Figure 1). *Osmancik-97* and *Kiziltan* japonica varieties was successfully backcrossed with CMS *V20A* for six generations (Table 3). The similar results received that the other studies that the transfer by backcrossing into elite lines of the cytosterility system of CMS wild abortive (WA) lines *Er-Jiu-Nan 1A, V20A, V41A, and Zhen Shan 97A* (Yuan and Virmani, 1988; Leon et al., 1997).

Table 1. Evaluation of agronomic traits and adaptability of CMS lines in Mediterranean Ecological Condition in 2015 and 2016

Order	CMS and Maintainer Lines	Flowering Days (Day)	Flowering Days Situation	Plant Length (cm)	Panicle Length (cm)
1	V20A	81 w	Early	51.2 w	14.6 m
2	V20B	80 x	Early	52.8 w	14.8 lm
3	15A	83 t	Early	53.6 w	15.6 jm
4	15B	82 u	Early	54.8 w	15.8 im
5	31A	88 q	Moderate	72.2 im	20.4 ai
6	31B	86 s	Moderate	73.4 hl	21.0 ag
7	23A	87 r	Moderate	65,6 qv	16,6 gm
8	23B	86 s	Moderate	66.8 ou	17.4 fm
9	76A	83 u	Early	61,8 v	18,2 dm
10	76B	81 w	Early	63.0 tv	18.8 cm
11	IR58025A	135 a	Very Late	64,0 sv	17,4 fm
12	IR58025B	132 b	Very Late	65,2 rv	18.4 dm
13	IR62829A	120 l	Very Late	69.8 lp	18.0 em
14	IR62829B	117 n	Very Late	71.0 jn	18.8 cm
15	IR68886A	121 k	Very Late	69.6 lq	19.8 ak
16	IR68886B	117 n	Very Late	70.8 ko	20.6 ah
17	IR69625A	125 g	Very Late	68.6 mr	19.4 al
18	IR69625B	123 i	Very Late	69.8 lp	20.2 aj
19	IR73793A	124 h	Very Late	79.0 cf	23.6 ab
20	IR73793B	122 j	Very Late	80.2 cd	24.4 a
21	IR75596A	120 l	Very Late	75.4 fi	21.4 af
22	IR75596B	118 m	Very Late	76.6 dh	22.4 ae
23	IR75606A	127 e	Very Late	85.2 ab	22.4 ae
24	IR75606B	125 g	Very Late	86.4 a	23.4 ac
25	IR78369A	126 f	Very Late	75.0 fj	19.0 bm
26	IR78369B	124 h	Very Late	75.8 ei	19.6 ak
27	IR79156A	127 e	Very Late	78.6 cg	21.6 af
28	IR79156B	125 i	Very Late	79.8 ce	22.8 ad
29	IR80151A	130 c	Very Late	63.2 tv	15.8 im
30	IR80151B	128 d	Very Late	64.4 sv	17.0 fm
31	IR80156A	126 f	Very Late	73.4 hl	19.0 bm
32	IR80156B	123 g	Very Late	74.8 gk	20.2 aj
33	IR80564A	123 i	Very Late	81.2 bc	20.4 ai
34	IR80564B	120 l	Very Late	82.4 ac	21.4 af
35	IR93559A	84 s	Early	62.8 uv	15.4 km
36	IR93559B	82 v	Early	64.0 sv	16.6 gm
37	IR93560A	81 w	Early	63.0 tv	16.0 hm
38	IR93560B	80 x	Early	64.2 sv	17.2 fm
39	IR93562A	100 o	Late	65.0 rv	17.6 fm
40	IR93562B	98 p	Late	66.4 pu	19.0 bm
41	IR93563A	88 q	Moderate	67.0 nt	18.8 cm
42	IR93563B	86 s	Moderate	67.8 ns	19.6 bk
F Ratio of lines		12160.3**		34.4**	8.7**
CV%		0.4		4.6	9.9
LSD		0.5		4.0	4.8

*: P < 0.05, **: P < 0.01, ns: nonsignificant, values followed by the same letter are not statistically significantly different.

Table 2. Sterility maintenance of 19 national rice varieties and fertility restoration ability one restorer rice against V20A CMS line, in 2015 and 2016, and status remarks

Variety Name	Type	Sterility (%) 2015	Sterility (%) 2016	Status
IR50	Indica	9.0 hi	9.9 g	Restorer
V20A	Wild Abortive	100 a	100 a	CMS
V20A × IR50 (F_1)	Indica	9.2 gi	10.1 f	Hybrid
Osmancik-97 × V20A (BC3 and BC4)	Japonica	100 a	100 a	CMS
Osmancik-97	Japonica	9.3 fh	9.6 I	Maintainer
V20A × Kiziltan (BC3 and BC4)	Japonica	100 a	100 a	CMS
Kiziltan	Japonica	8.8 ij	8.8 j	Maintainer
V20A × Halilbey (BC2 and BC3)	Japonica	100 a	100 a	CMS
Halilbey	Japonica	9.3 fh	9.7 hi	Maintainer
V20A × Tosyagunesi (BC2 and BC3)	Japonica	100 a	100 a	CMS
Tosyagunesi	Japonica	8.5 j	6.8 k	Maintainer
V20A × Gonen (BC2 and BC3)	Japonica	100 a	100 a	CMS
Gonen	Japonica	6.3 l	9.7 hi	Maintainer
V20A × Ece (BC2 and BC3)	Japonica	100 a	100 a	CMS
Ece	Japonica	10.1 c	11.6 e	Maintainer
V20A × Pasali (BC2 and BC3)	Japonica	100 a	100 a	CMS
Pasali	Japonica	6.3 m	9.8 gh	Maintainer
V20A × Beser (BC2 and BC3)	Japonica	100 a	100 a	CMS
Beser	Japonica	9.7 de	10.1 f	Maintainer
V20A × Edirne (BC2 and BC3)	Japonica	100 a	100 a	CMS
Edirne	Japonica	6.3 l	9.8 gh	Maintainer
V20A × Duragan (BC2 and BC3)	Japonica	100 a	100 a	CMS
Duragan	Japonica	9.4 eg	12.7 c	Maintainer
V20A × Kale (BC2 and BC3)	Japonica	100 a	100 a	CMS
Kale	Japonica	7.8 k	8.7 j	Maintainer
V20A × IMIHalilbey (BC2 and BC3)	Japonica	100 a	100 a	CMS
IMIHalilbey	Japonica	10.1 c	12.1 d	Maintainer
V20A × IMIOsmancik (BC2 and BC3)	Japonica	100 a	100 a	CMS
IMIOsmancik	Japonica	9.8 cd	10.2 f	Maintainer
V20A × Hamzadere (BC2 and BC3)	Japonica	100 a	100 a	CMS
Hamzadere	Japonica	7.7 k	8.7 j	Maintainer
V20A × HalilbeyPi40 (BC2 and BC3)	Japonica	100 a	100 a	CMS
HalilbeyPi40	Japonica	9.5 df	9.9 g	Maintainer
V20A × OsmancikPi40 (BC2 and BC3)	Japonica	100 a	100 a	CMS
OsmancikPi40	Japonica	9.5 df	9.8 gh	Maintainer
V20A × Tunca (BC2 and BC3)	Japonica	100 a	100 a	CMS
Tunca	Japonica	18.9 b	20.5 b	Maintainer
V20A × Yildiz (BC1 and BC2)	Japonica	100 a	100 a	CMS
Yildiz	Japonica	NA	NA	Maintainer
V20A × Boyabatkalesi (BC1 and BC2)	Japonica	100 a	100 a	CMS
Boyabatkalesi	Japonica	NA	NA	Maintainer
F Ratio of lines		143682**	1573390**	
CV (%)		0.4	0.1	
LSD		0.4	0.1	

*: P < 0.05, **: P < 0.01, ns: nonsignificant, values followed by the same letter are not statistically significantly different.

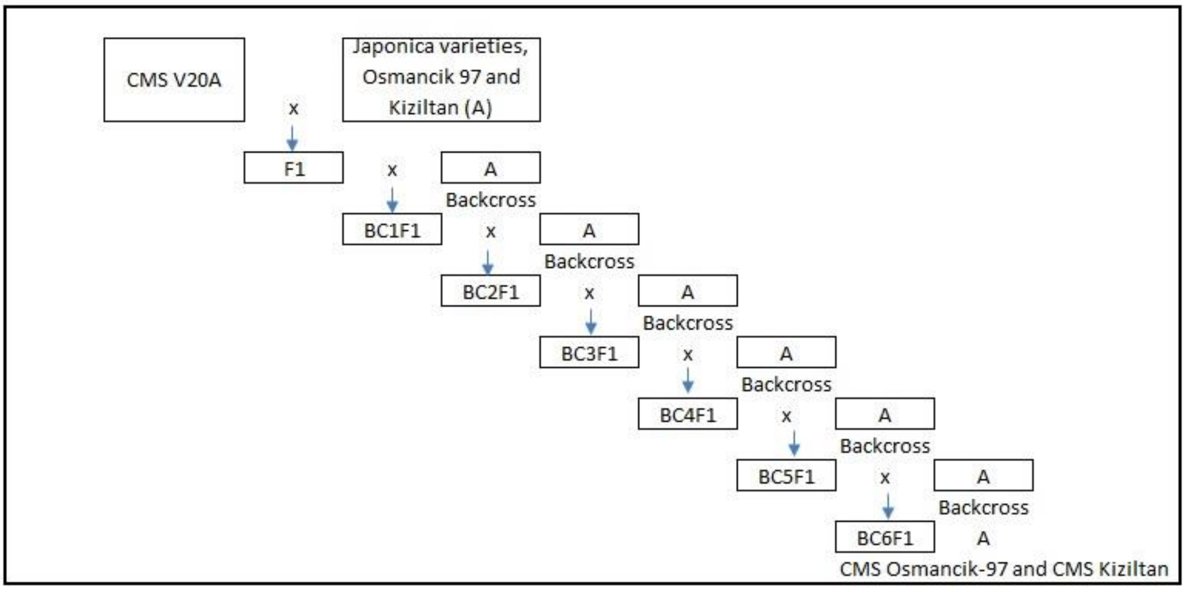

Figure 1. Flowchart of breeding method of new CMS lines

Table 3. Seed setting rates (%) of backcrossed lines and one hybrid in 2017 and 2018

Initial Female Parent	Recurrent Polen Parent	2017 Backcross	2017 Spikelet Fertility[a] (%)	2018 Backcross	2018 Spikelet Fertility[a] (%)
V20A	Osmancik-97	BC_5F_1	0.0b	BC_6F_1	0.0b
V20A	Kiziltan	BC_5F_1	0.0b	BC_6F_1	0.0b
31A	Osmancik-97	BC_5F_1	0.0b	BC_6F_1	0.0b
31A	Kiziltan	BC_5F_1	0.0b	BC_6F_1	0.0b
V20A	IR50 (Restorer)	F_1	90.3a	F_1	91.2a
F Ratio of lines			6115**		2772**
CV (%)			4.94		7.44
LSD			1.68		2.51

[a]Average spikelet fertility of three plants, each containing three bagged panicles. *: P < 0.05, **: P < 0.01, ns: nonsignificant, values followed by the same letter are not statistically significantly different.

Agronomic characteristics were compared among *Osmancik-97*, *CMS-Osmancik-97*, *Kiziltan,* and *CMS-Kiziltan* in 2019. The experiment plants were transferred in the rice field at a dispersing of 20 cm between plants inside columns and 20 cm between lines. It was not found significant differences between the CMS lines and their maintainer lines in plant length, panicle length, flowering time and anthesis time (Table 4). National rice varieties had a similar agronomic features the other reports (Unan et al., 2013; Sezer et al., 2016)

Table 4. Comparison of some agronomic features between CMS lines and maintainer lines in 2019

Properties	Osmancik-97	CMS-Osmancik-97	F Ratio	CV (%)	LSD	Kiziltan	CMS-Kiziltan	F Ratio	CV (%)	LSD
Plant Length (cm)	100.3	98.4	10.83	0.7	ns	85.4	84.3	3.63	0.8	ns
Panicle Length (cm)	16.4	16.2	4.92	0.9	ns	15.3	15.2	2.28	0.7	ns
Panicle Exsertion rate (%)	100.0a	86.4b	13872 **	0.2	0.47	100.0	85.3	10282 **	0.2	0.60
Flowering time (day)	85.0b	86.3a	16.00	0.5	ns	83.3b	84.7a	16.00	0.5	ns
Sterility (%)	9.5b	100.0a	98283 **	0.6	1.2	8.8b	100.0a	6238 **	2.6	4.90
Anthesis time	11.30-13.30	11.30-13.30	-	-	-	11.30-13.30	11.30-13.30	-	-	-

*: P < 0.05, **: P < 0.01, ns: nonsignificant, values followed by the same letter are not statistically significantly different.

CONCLUSION

It was developed japonica type CMS lines of *Osmancik-97* and *Kiziltan*, which did not set any seeds in temperate climate region. The *CMS-Osmancik-97* and *CMS-Kiziltan* might be useful for commercial hybrid rice breeding. Eight introduction source CMS lines (*V20A, 15A, 23A, 31A, 76A, IR93559A, IR93560A*, and *IR93563A*) and one restorer line (*IR 50*) are useful for hybrid rice breeding programs ins Mediterranean ecological condition. New japonica type CMS lines have characteristics such as short height, early maturity, good panicle exsertion and panicle length which are a significant influence on commercial hybrid rice production.

ACKNOWLEDGMENT

This research was supported by Republic of Turkey, Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies (Project No: TAGEM/TBAD/16/A12/P04/001). This article selected in the Turkey 13th National and 1st International Field Crops Conference in 2019.

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