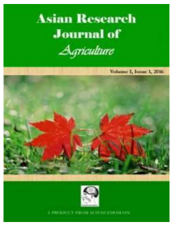

Asian Research Journal of Agriculture
4(3): 1-8, 2017; Article no.ARJA.31597
ISSN: 2456-561X

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Assessment of Genetic Combinations for Hybrid Rice in the Germplasm of Pakistan

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Authors' contributions

This work was carried out in collaboration between all authors. Author MR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MI and MA managed the analyses of the study. Authors TL, TB and ARK managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARJA/2017/31597
Editor(s):
(1) Afroz Alam, Department of Bioscience & Biotechnology, Banasthali University, Rajasthan, India.
Reviewers:
(1) Habu Saleh Hamisu, National Horticultural Research Institute, Kano State, Nigeria.
(2) Sasank Sekhar Chyau Patnaik, Central Rice Research Institute, Odisha, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/18892

Original Research Article

Received 16th January 2017
Accepted 25th April 2017
Published 3rd May 2017

ABSTRACT

An attractive approach is development of rice hybrids for the increase in crop productivity. A narrow genetic base and limited parental lines are the hindrance in the development of location specific hybrids. In this study 24 genotypes were crossed with 8 specific cytoplasmic sterile lines to check the heterosis for the development of hybrid rice and total 192 cross combinations were studies. Pollen fertility and spikelet fertility were the attributes on which the material was differentiated into restorers, partial restorers, partial maintainers and maintainers. Among the 192 test hybrids pollen parents with CMS lines were 14 behaved as restorers, restoring the fertility and 29 were maintaining the sterility while remaining were categorized as partial restorers and maintainers. The combination with local varieties basmati 515, super basmati, basmati 385, basmati 370 on the basis of restoring and maintainer fertility and sterility could be utilized in the development of hybrid rice in the country.

Keywords: Hybrid rice; restorer; maintainer; pollen fertility; spikelet sterility.

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1. INTRODUCTION

About half of the world population depends on rice (*Oryza sativa*) for their survival. Rice is cultivated 114 countries across the world and out of that 90% rice having share of Asia in production and consumption [1]. Rapid growing population demanding high yields in the major crops by the plant breeders. There is dire need to increase the productivity and production encouraged rice scientists to develop the hybrid rice in the tropics. Experience in China [2] and outside China, in IRRI [3], India [4] Vietnam [5], the Philippines [6], Bangladesh [7], and several other countries clearly indicates that hybrid rice technology offers to be the most viable, feasible and willingly adoptable one option to meet this challenge.

Amongst several genetic approaches being discovered to break the yield barrier in rice is to develop new hybrid by adopting the methodologies and practices of hybrid rice development. The commercial exploitation of heterosis has been made possible by the use of cytoplasmic male sterility and fertility restorer genes, however the adequate seed set was not produce male sterility genes. The use of cytoplasmic genetic male sterility attribute for the development of new hybrids in crops is possible only when effective maintainers and restorers are identified. Later selection and screening of top breeding lines as suitable and genetically diverse restorers and maintainers for specific cytoplasmic male sterile (CMS) lines is vital. The cytoplasmic sterile lines introduced from China are unstable to use in developing hybrid rice in other countries. Hence, it is imperative to classify maintainers and restorers from local germplasm for development of component lines in hybrid rice activities. Spikelet or pollen fertility or both have been used as an index to fix the restoration ability of the lines [8].

Furthermore, breeders required broad range of CMS lines to make desired hybrids. for that reason, it is compulsory to transfer available CMS system into local influential breeding lines. Hence, use of local CMS line would be helpful to ease this problem and to develop adaptable, heterotic hybrids. Two effectual maintainers for WA cytoplasmic source [9] identified and successfully converted into local lines through backcrossing. CMS genes from IR58025A and IR62829A into Basmati line 47456 in Pakistan were transferred [10] successfully. While working with 33 local aromatic genotypes of Bangladesh, a little work on identifying CMS in local aromatic rice varieties are also reported [11] who has been identified ten maintainers and four restorers of five exotic CMS lines.

So, the present study was assumed to categorize more restorer and maintainer lines from local aromatic and non-aromatic rice germplasm in contrast to exotic germplasm sources.

2. MATERIALS AND METHODS

2.1 Plant Material Experimental Site

The study was carried out by evaluating the local and exotic germplasm in the experimental field of Rice Research Institute, Kala Shah Kaku in 2013 and 2014. Eight CMS lines IR58025A, IR70372A, SSMS2A, IR62829A, IR68902A, IR69616A, IR73322A, IR75596A having wild abortive cytoplasm were used as female parent in crossing scheme. Twenty-four aromatic and non-aromatic rice lines and varieties including Basmati 200, Basmati 515, KSK 133, KSK 483, KSK 484, Basmati Pak, Super Basmati, PS 2, Basmati 370, Shaheen Basmati, Punjab Mehran Basmati, 5015, FR 1, 4048, KSK 434, Pusa Basmati, Basmati 385, IR-6, LG-22, KSK 282, TN 1, KSK 432 were selected on yield and quality basis to use as pollen parent in hybridization programme. Line x tester mating design was used in this study.

2.2 Methodology

At flowering phase to facilitate hybridization, selected plants of CMS lines were transferred to pots previously filled with the soil. CMS panicles clipping of was completed in the evening and hand pollination practice was carried out in the next morning by dusting pollen from 24 selected local aromatic and non-aromatic rice genotypes. To avoid foreign pollen for contamination essential procedures were taken. The Bagging and tagging of clipped and crossed panicles were properly done with white papers. This Bagging after pollination sustained for 3-4 days to avoid unwanted pollination. After maturity, adequate spikelet of 192 crosses were collected from panicles. Properly dried seeds were kept in cold storage for further study. It involves the two nurseries source nursery and test cross nursery. During 2013 CMS lines were crossed with the inbred lines and the harvested seed was stored and used for further studies during 2014 in the experimental field (RCBD design) and transplanted in the row of 15 plants with plant spacing 22.5 cm on each side. All F1s along with their pollen parents were grown during 2014. Necessary cultural operations were carried out during the cropping periods as per standard for the proper plant growth and development.

2.3 Observations and Measurements

Randomly selected about 20 spikelets were collected from just emerged panicle of F_1 plant in a vial containing 70% ethanol for pollen viability test. At laboratory, one drop of 1% Iodine Potassium Iodide (IKI) stain was put on a glass slide. All anthers of 5-6 spikelets were then picked out with the help of small forceps and then placed on the stain of the slide. Anthers were crushed gently by using needle to release pollen grains. After removing debris, a cover slip was placed on crushed grains and pollen fertility status was observed under a compound microscope. The entire slide was scanned and pollen fertility was counted in three random fields. The pollen grains of F_{1s} were classified as follows based on the extent of pollen sterility.

Pollen fertility (%)	Category
0-5	Sterile (M)
50-90	Partial Maintanier (PM)
50-80	Partial Restorer(PR)
80-100	Restorer(R)

Only two characters like pollen sterility percentage and spikelet fertility percentage were recorded to identify restorers and maintainers of the CMS lines. The bellow mentioned formulae were used to estimate the spikelet fertility and pollen sterility (%).

Pollen sterility (%) = {(No. of sterile pollens X 100) / Total no of pollens}

Spikelet fertility (%) = {(Filled grains per panicle X 100) / Total spikelet per panicle}

3. RESULTS AND DISCUSSION

3.1 Pollen Fertility

The pollen fertility percentage vary from 0.00 (Basmati 2000xIR73322A) to 95.7 (FR-1x IR69616A) when studies under microscope (Fig. 1). The magnitude of pollen fertility was very low (Table 1), the lines which are categorized as maintainer on sterility percentage can be further backcrossed with their respective F_1s to get completely sterile progenies for the development of new CMS lines. Sometime one pollen parent behaves as maintainer when used to cross with one CMS line and restorer with other CMS line. Tester line KSK-282 behaved as a maintainer when crossed with IR85025A (0.5% pollen fertility) and as restorer with the other CMS line IR70372A (81.6% pollen fertility) in Table 1. While the same tester acted as partial maintainer and partial restorer with other CMS lines IR62829A, IR73322A (Partial maintainer), IR68902A, IR69616A, IR75596A and SSMS2A (Partial restorer) respectively. Tester KSK-483, KSK-484, Super Hamalya, 5015, KSK 434 and Basmati 385 when crossed with eight specified CMS lines were behaved as restorers. Few other testers basmati 2000, Basmati 515 and super basmati were behaved as maintainer for the all CMS lines under study and few testers behaved as partial restorer and partial maintainer for the different CMS lines. The dissimilarities in performance of fertility restoration indicate that either the fertility-restoring genes are dissimilar or that their penetrance and expressivity diverse with the genotypes of the parents or the modifiers of female background. Different CMS lines of same cytoplasmic source were reported [12,13,14] having this kind of the differential reaction of the same genotype in restoring the fertility. The above said type of interaction is might be due to differential nuclear cytoplasmic interactions between the testers and CMS lines.

3.2 Spikelet Fertility

The spikelet's fertility was ranging from 0.0% (IR58025Ax Basmati 2000) to 98.3% (IR73322A). Similarly, Basmati 2000, Basmati 515 and Super Basmati exhibited the complete sterility for all the CMS lines while KSK-483, KSK-484, Super hamalya and 5015 showed more than 80% fertility with all CMS lines.IR58025A x KSK 133, 4048, FR-1, IR75596A x PS 2, IR62829A and IR73322A x Punjab Mehran Basmati were proved to be fertile combinations. While behavior of KSK 282 and other testers is due to differential penetrance and expressivity of fertility restoring gene with different CMS lines. The approved varieties Basmati 370 and Basmati 385 already showed the restoring behavior in previous studies.

Table 1. Pollen fertility of test hybrids in test cross nursery

Lines/Tester	IR58025A (L1)	IR62829A (L2)	IR73322A (L3)	IR68902A (L4)	IR69616A (L5)	IR75596A (L6)	IR70372A (L7)	SSMS2A (L8)
Basmati 2000 (T1)	0.0(M)	0.8(M)	0.1(M)	0.2(M)	0.3(M)	0.5(M)	0.4(M)	0.9(M)
KSK-133 (T2)	82.1(R)	68.5(PR)	80.7(R)	82.3(R)	92.6(R)	70.3(PR)	55.2(PR)	81.4(PR)
Basmati 515 (T3)	0.4(M)	2.5(M)	5.8(M)	0.6(M)	8.1(M)	2.5(M)	9.6(M)	0.6(M)
KSK-483(T4)	81.7(R)	84.5(R)	80.3(R)	91.3(R)	82.5(R)	80.7(R)	71.4(R)	82.2(R)
KSK-484(T5)	92.1(R)	84.6(R)	96.3(R)	93.2(R)	97.5(R)	90.3(R)	83.5(R)	87.1(R)
Basmati Pak(T6)	60.2(PR)	73.3(PR)	58.2(PR)	72.5(PR)	62.5(PR)	58.7(PR)	62.8(PR)	64.4(PR)
Super Basmati(T7)	0.5(M)	0.6(M)	0.3(M)	0.1(M)	0.4(M)	2.2(M)	0.5(M)	3.1(M)
PS-2(T8)	35.3(PM)	26.4(PM)	29.5(PM)	31.2(PM)	40.4(PM)	89.6(R)	41.3(PM)	22.2(PM)
Basmati 370(T9)	75.2(PR)	80.3(R)	78.5(PR)	55.5(PR)	69.7(PR)	77.3(PR)	72.3(PR)	58.1(PR)
Shaheen Basmati (T10)	21.6(PM)	32.4(PM)	40.4(PM)	28.3(PM)	40.5(PM)	29.4(PM)	41.8(PM)	27.2(PM)
SuperHamalya (T11)	85.5(R)	95.5(R)	93.1(R)	92.8(R)	94.6(R)	86.5(R)	90.6(R)	80.5(R)
Punjab Mehran Basmati (T12)	75.1(PR)	82.4(R)	95.1(R)	68.5(PR)	78.3(PR)	65.1(PR)	76.5(PR)	66.6(PR)
5015 (13)	91.2(R)	93.5(R)	98.3(R)	82.7(R)	95.4(R)	82.3(R)	80.1(R)	95.1(R)
FR-1(T14)	92.3(R)	75.2(PR)	35.5(PM)	45.6(PM)	95.7(R)	77.4(PR)	70.3(PR)	55.2(PR)
4048(T15)	95.1(R)	55.2(PR)	65.3(PR)	44.7(PM)	35.4(PM)	76.3(PR)	62.2(PR)	66.1(PR)
99404 (T16	0.2(M)	45.4(PM)	31.2(PM)	52.2(PR)	44.1(PM)	42.1(PM)	35.6(PM)	56.3(PR)
KSK 434 (T17)	92.2(R)	89.2(R)	95.3(R)	88.5(R)	92.6(R)	90.5(R)	92.3(R)	85.2(R)
Pusa Basmati(T18)	2.2(M)	45.5(PM)	35.2(PM)	42.2(PM)	32.1(PM)	24.2(PM)	33.3(PM)	0.3(PM)
Basmati 385 (T19)	91.5(R)	80.2(R)	85.3(R)	81.3(R)	93.3(R)	85.1(R)	88.2(R)	90.1(R)
IR-6 (T20)	43.7(PM)	5.6(M)	32.5(PM)	55.2(PM)	34.6(PM)	53.2(PR)	22.1(PM)	56.1(PR)
LG-22(T21)	52.4(PR)	58.6(PR)	77.1(PR)	63.1(PR)	65.3(PR)	57.2(PR)	61.6(PR)	66.2(PR)
KSK 282(T22)	75.3(PR)	82.1(R)	35.6(PM)	53.8(PR)	57.5(PR)	45.4(PM)	73.2(PR)	56.3(PR)
TN-1(T23)	56.6(PR)	66.5(PR)	68.3(PR)	51.6(PR)	61.5(PR)	35.2(PM)	91.5(R)	56.8(PR)
KSK 432 (T24)	0.5(M)	23.6(PM)	45.3(PM)	57.4(PR)	76.8(PR)	53.4(PR)	81.6(R)	23.4(PR)

R= Restorer, PR= Partial restorer, M= Maintainer, PM= Partial maintainer

Table 2. Spikelet fertility of test hybrids in test cross nursery

Lines/Tester	IR58025A (L1)	IR62829A (L2)	IR73322A (L3)	IR68902A (L4)	IR69616A (L5)	IR75596A (L6)	IR70372A (L7)	SSMS2A (L8)
Basmati 2000 (T1)	0.0(CS)	0.5(CS)	0.5(CS)	15.2(PS)	0.3(CS)	0.4(CS)	0.4(CS)	10.9(PS)
KSK-133 (T2)	82.1 (F)	77.5(PF)	81.2(F)	82.3(F)	92.5(F)	70.2(PF)	55.9(PF)	83.4(PF)
Basmati 515 (T3)	0.2(CS)	3.0(CS)	4.8(CS)	0.2(CS)	20.1(PS)	2.5(CS)	5.6(CS)	0.8(CS)
KSK-483(T4)	83.1(F)	89.5(F)	83.0(F)	90.4(F)	86.5(F)	80.7(F)	71.4(F)	86.2(F)
KSK-484(T5)	95.2(F)	83.2(F)	93.4(F)	92.5(F)	96.5(F)	90.8(F)	85.5(F)	88.4(F)
Basmati Pak(T6)	62.5(PF)	74.5(PF)	57.3(PF)	71.6(PF)	63.5(PF)	59.7(PF)	62.8(PF)	64.4(PF)
Super Basmati(T7)	0.5(CS)	0.7(CS)	0.5(CS)	0.8(CS)	0.2(CS)	2.3(CS)	0.5(CS)	2.1(CS)
PS-2(T8)	34.3(PS)	26.4(PS)	32.3(PS)	31.2(PS)	41.5(PS)	9PS0.6(F)	42.3(PS)	22.8(PS)
Basmati 370(T9)	76.2(PF)	85.9(F)	73.5(PF)	56.5(PF)	69.2(PF)	78.2(PF)	73.4(PF)	59.2(PF)
Shaheen Basmati (T10)	23.5(PS)	34.4(PS)	40.4(PS)	28.5(PS)	40.6(PS)	29.3(PS)	41.8(PS)	29.2(PS)
Super Hamalya (T11)	84.5(F)	95.5(F)	97.1(F)	92.8(F)	94.6(F)	79.2(PF)	90.6(F)	80.5(F)
Punjab Mehran Basmati(T12)	75.1(PF)	81.4(F)	95.1(F)	68.5(PF)	77.3(PF)	65.1(PF)	76.5(PF)	66.6(PF)
5015 (13)	91.2(F)	95.5(F)	98.3(F)	78.7(PF)	97.4(F)	82.3(F)	81.0(F)	94.1(F)
FR-1(T14)	92.3(F)	74.2(PF)	45.5(PF)	46.6(PS)	95.7(PF)	77.4(PF)	74.3(PF)	55.2(PF)
4048(T15)	96.3(F)	54.3(PF)	62.5(PF)	44.6(PS)	38.2(PF)	76.3(PF)	66.2(PF)	68.1(PF)
99404 (T16	0.8(CS)	48.2(PS)	36.4(PS)	53.6(PF)	46.5(PS)	45.5(PS)	36.5(PS)	57.3(PF)
KSK 434 (T17)	92.6(F)	90.8(F)	96.2(F)	87.5(F)	93.5(F)	92.0(F)	92.8(F)	86.2(F)
Pusa Basmati(T18)	2.5(CS)	46.6(PF)	35.7(PS)	42.9(PS)	33.1(PS)	23.2(PS)	32.4(PS)	23.4(PS)
Basmati 385 (T19)	91.1(F)	86.3(F)	84.1(F)	85.6(F)	93.6(F)	87.5(F)	86.2(F)	91.2(F)
IR-6 (T20)	47.7(PS)	1.7(CS)	36.5(PS)	55.8(PS)	32.6(PS)	54.2(PF)	23.1(PF)	58.2(PF)
LG-22(T21)	58.2(PF)	36.6(PS)	76.5(PF)	64.2(PF)	66.4(PF)	55.4(PF)	62.5(PF)	66.6(PF)
KSK 282(T22)	77.2(PF)	81.2(F)	35.6(PS)	53.8(PF)	57.5(PF)	48.4(PS)	73.7(PF)	45.3(PS)
TN-1(T23)	58.7(PF)	69.6(PF)	62.7(PF)	49.7(PS)	61.6(PF)	36.3(PS)	92.0(F)	55.8(PF)
KSK 432 (T24)	0.3(CS)	24.5(PS)	47.4(PS)	58.4(PF)	77.8(PF)	58.4(PF)	86.6(F)	23.8(PF)

F= Fertile, PF=Partial fertile, CS=Complete sterile, PS=Partial sterile

3.3 Restorers and Maintainers

The potential restorer and maintainer identified presented in the Table 3. The maintainer line could be utilized in the backcrossing for the development of new CMS lines. The lines which were identified as restorer can be used to make new hybrids with desirable morphological traits with different CMS line. There was illustration that the sorting of tester based on the pollen fertility did not correlate with the sorting based on the spikelet fertility by Murugan and Ganesan [14]. For example, the Basmati 2000 was behaving as partial maintainer with IR68902A in spikelet fertility and maintainer in case of pollen fertility and Basmati 515 was expressing the partial fertility and sterility both in spikelet fertility and pollen fertility respectively. So non-correlation between pollen fertility and spikelet fertility was claimed by Murugan and Ganesan [14].

4. DISCUSSION

For the development of three-line rice hybrids with high yield, the basic step is to identify parental lines as maintainers possessing recessive fertility restorer gene or genes and restorers with dominant fertility restorer genes or gene from local or exotic germplasm resources. Wild abortive cytoplasmic source extensive research work on identification of restorers and maintainers and the inheritance of fertility restoration has been done [15,16,17]. Those CMS lines developed from the WA sources have been showed the most stability in terms of complete pollen sterility in the different sources of cyto sterility [17]. Different studies presented by [18,19] have suggested greater frequency of restorers identified than maintainers for WA-CMS lines, while on the other side more frequency of maintainers identified was observed than restorers in studies [20]. The lines identified

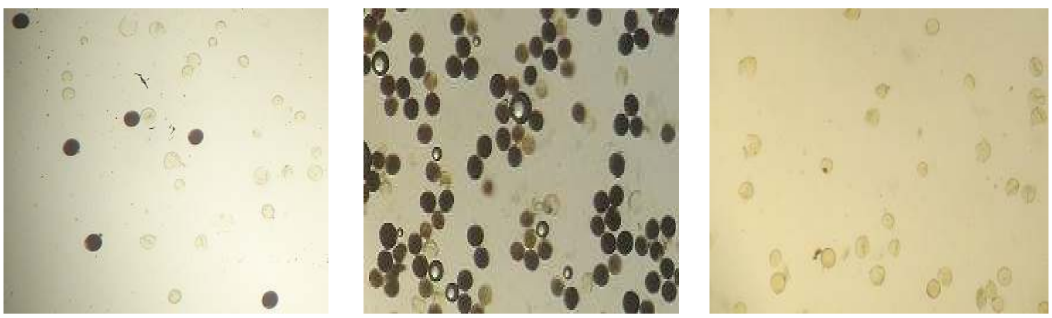

Fig. 1. (a) Partial fertile/sterile (b) Fertile (c) Sterile

Table 3. Potential maintainers and restorers for eight CMS lines

CMS lines	Restorer	Maintainer
IR58025A	KSK 133, KSK 483, KSK 484, Super hamalya, 5015, FR-1, 4048, Basmati 385	Basmati 2000,Basmati 515, Super Basmati, 99404, Pusa Basmati, KSK 432
IR62829A	KSK 483, KSK 484, Super Hamalya, Basmati 370 ,Punjab Mehran Basmati, 5015, Basmati 385, KSK 282	IR-6, Basmati 2000, Basmati 2000, Super Basmati
IR73322A	Punjab Mehran Basmati, Basmati 385, KSK 434, Super Hamalya, 5015, KSK 133, KSK 483, KSK 484	Basmati 515, Super Basmati
IR68902A	Super Hamalya, 5015, Basmati 385, KSK 133, KSK483, KSK484	Basmati 2000,Basmati 515,Basmati 2000
IR69616A	FR-1, Super hamalya, 5015, Basmati 385, KSK 133, KSK483, KSK484	Super Basmati
IR75596A	PS 2, Basmati 385, 5015, KSK 434, KSK 483, KSK 484, Super Hamalya	Basmati 2000, Super Basmati
IR70372A	TN 1, KSK 432, Super hamalya, KSK 483, KSK 484, 5015	Super basmati, Basmati 515, Basmati 2000
SSMS2A	Basmati 385, Super Hamalya, KSK 434, 5015, KSK 483, KSK484	Pusa Basmati, Super Basmati, Basmati 515

as restorers were indica type. The distribution of restorers for WA system is mostly high among improved indica cultivars. In the present study, among 192 test hybrids raised through crossing with 5 CMS lines, the number of restorers (14) were more than that of maintainers (29). Furthermore, the relatively little higher frequency of restorers among the lines indicates that with respect to fertility restoration genes lines are closer to restorer gene pool. Since heterosis is often realized in terms of genetic distance, the distinct gene pools of maintainers and restorers can hasten hybrid rice breeding. Among the KSK 133, KSK 483, KSK 484, 5015, FR 1, TN-1, Basmati 385 and Super hamalya could be utilized for the breeding program in the new hybrid developments. Effective diversified maintainer lines could come out to incorporate specialty traits in hybrids. Specific traits evolved from landraces into restorer and maintainer lines could hasten specific trait related hybrid rice development. It was observed that the frequency of maintainers among the elite breeding lines is rather low. Furthermore, even if some of the lines are identified as maintainers, they are susceptible to various biotic and abiotic stresses. So, there is dire need to combine desirable traits through recombination breeding. The maintainer lines identified could be converted into new CMS lines through backcross approach and utilized for new potential hybrid rice development with desirable traits.

5. CONCLUSION

Hybrid rice development technology is very much important to combat the poverty in the world as adapted by the China. In the identified heterotic combination few basmati lines and non-basmati lines were found maintainer ad restorers to for the development of hybrid in the country. These can also be utilized for the further new heterotic combinations development as well.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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