Vegetos

Vol. 18 (1 & 2) : 127 – 133 (2005)

Studies on the Effect of Gamma-Rays on the Chromosomal Behaviour during Meiosis in Barley (*Hordeum Vulgare* L.)

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Seeds of Barely variety Lakhan and K 1155 were treated with 25kR, 20kR and 40kR of gamma-rays. The mutation frequency increased gradually up to moderate dosages of gamma rays and the highest frequency rate was found in the variety K 1155 being huskless. Reduced chiasmata frequency per cell was observed with mutagens on barley of both the tested cultivars. Some of the plants in mutants population of 40kR dose of gamma-rays were found to be abnormal aberrations in chromosomal behaviour in metaphase I. In Anaphase I the chromosomal behaviour in both the cultivars were irregular, delayed separation, laggards etc. at higher dosages than that of lower dosages.

Keywords: Barley cultivar—Lakhan & K 1155, gamma-rays, chromosomal behaviour, meiosis.

INTRODUCTION

Conventional methods of plant improvement like selection, hybridization and induced polyploidy have not caused any major breakthrough primarily for want of genetic variation within the crop. The narrow genetic base of species is attributed to its number of small chromosomes with a lot of hetrochromatin, low chiasmata frequency and high selfing rate. However, the present advances in genetic plant breeding through mutagenesis have caused a greater change with remarkable positive impact to obtain new gene with higher productivity due to mutagenesis. The potential physical mutagens like gamma-rays cause severe reshuffling of genetic material and induced variation of various kind in crop plants (John, 1995). In order to induce variability and utilize useful mutations directly for efficient breeding, a systematic study has been taken to analyse the chromosomal behaviours in meiosis of barley following gamma-irradiation.

MATERIALS AND METHODS

Dry uniform with 10% moisture of each cultivar of barley (*H. vulgare*) varieties Lakhan and K1155 were exposed to 25kR, 30kR and 40kR gamma-rays from 60_{CO} source. Meiotic studies were made in pollen mother cells (PMCs) from young spikes of suitable size. The most suitable time for collection of the spike was found to be between 8:30 A.M. and 11:00 A.M. The buds of suitable size were directly fixed in 1:3 acetic-alcohol or in carnoy's fluid (absolute alcohol 6 parts : chloroform 3 parts : glacial acetic acid 1 part). After 18 hours of fixation, the spikes were washed in 70% alcohol and stored in the same grade on alcohol at 10 degrees celsius. The fixed buds were crushed in a drop of 2% aceto-orcein solution. The cytological analysis were made from temporary slides and suitable cells were photogaphed on 35mm film with olympus-PM-6 microphographic camera at 50 x 12 x and 100 x 12 x magnification.

RESULTS AND DISCUSSION

The cytological observation particularly chromosomal behaviour during meiosis with different treatments cultivars are discussed separately.

LAKHAN

25 kR

The plant raised from seeds exposed to 25kR gamma-rays showd some irregularities during meiotic division and chromosomes were normal in great majority of PMCs. The chiasmata frequency per PMCs at this dose was adversely affected (Table 1 & 2). More than 60% of PMCs showed normal orientation of bivalents at the equator (Plate 1—Fig. 1, 2, 3 & 4). However, in some PMCs the univalents and even one or more bivalents did not orient on the equatorial plate. Separation of chromosomes was normal in 75% of PMCs with distribution of 7:7 while 25% PMCs showed abnormalities at anaphase I which was observed at delayed separation with laggards.

30kR

At this dose meiosis in pollen mother cells of mutants plants was to be disturbed with good degree of stickiness (Table 1 & 2). The frequencies of univalents and bivalents were greater than 25kR dosages. The chromosome oriented on equatorial plate in majority of PMCs (Plate I—Fig. 5, 6, 7, 8, 9 & 10). At anaphase I there was equal separation of chromosomes at both the poles in about 69% while in 31% PMCs abnormalities like delayed separation of chromosomes and the occurrence of laggards and unequal distribution of chromosomes at the poles were observed.

40kR

At this dose meiosis was greatly distributed and the pairing of chromosomes was very irregular with the occurrence of univalents, bivalents and polyvalent at diakinesis. The frequency of PMCs with univalents at 40kR dose was greater than dose at lower dosages (Table 1 & 2). The chiasmata frequency at this stage per PMCs was 5.7 (Plate 1—Fig. 11, 12 & 13). Metaphase I appeared to be much disturbed in some PMCs and univalents, polyvalents and bivalents were found to be away from the equator. At anaphase I in some of the PMCs delayed separation of chromosomes and 2:4 lagging chromosomes were frequently observed. Distribution of chromosomes were recorded in about 63 per cent normal and 37 per cent PMCs were abnormal which might be due to delayed separation laggards.

K1155

25kR

Meiosis in PMCs in plants exposed to 25kR gamma-rays showed slightly distributed meiosis. The ring bivalents, rod bivalent and polyvalent were in the order of 1 to 3, 2-4 and 1 to 3 respectively. Univalents and fragments were also vizualized in some of the PMCs at diakinesis (Table 2 & 3). The orientation of chromosomes at the equator was normal at metaphase I (Plate 2—Fig. 14, 15 & 16) in majority of the PMCs. The stickiness was observed in the large number of PMCs. The segregation of chromosomes has been found to normal in majority of cells were seen at both the poles because of delayed separation, irregular distribution of chromosomes and occurrence of laggards was noticed.

30kR

At this dose of gamma-rays sevral meiotic irregularities observed and the frequency of rod bivalent exceed that of ring bivalent. Fragment of chromosomes in free state has also been observed in some of the PMCs (Table 3 & 4) metaphase I (Plate 2—Fig. 17, 18, 19) in majority of the cell was found to be normal in a few cells. However, a few chromosomes were found away from the equator at metaphase I. The stickiness persisted in a good number of cells. In a few PMCs unequal distribution was observed. Presence of lagging chromosomes and delayed separation of chromosomes were commonly seen in abnormal cells. Behaviour of chromosomes fragments happened to be erratic.

40kR

Meiosis in plants raised from dry seeds exposed to 40kR gamma-rays was very much disturbed and the majority of PMCs were found with varying numbers of univalents, bivalents and polyvalents association at diakinesis (Table 3 & 4). The number of ring and rod bivalent were observed in the order of 1 to 2 and 3 to 4 respectively. The frequency of univalents at 40kR dosages was much better than those of lower dosages. The chiasmata frequency per PMCs was 5.6 and chromosome fragment seen in large number of cells. The metaphase I in some of PMCs was much disturbed (Plate 2—Fig. 20, 21 & 22). The behaviour of fragments, univalents and

Table 1 : Chromosomal associations at diakinesis in *Hordeum vulgare* variety Lakhan treated with different dosage of gamma-rays.

Dosages	No. of PMCs scored	Chromosomal associations			Polyvalents mean/range	Chiasmata frequency per PMCs
		Univalents means/range	Bivalents mean/range			
			ring IIs	rod IIs		
25kR	85	0.28	3.0	1.9	1.82	7.9
		(0-1)	(2-4)	(1-3)	(1-3)	
30kR	85	0.35	2.8	1.8	2.05	7.4
		(0-1)	(1-5)	(1-3)	(1-3)	
40kR	100	0.46	2.1	1.5	2.94	5.7
		(0-1)	(0-2)	(2-3)	(2-4)	

Table 2 : Frequency of meiotic irregularities in pollen mother cells of *Hordeum vulgare* variety Lakhan at metaphase I (%) at various dosage of gamma-rays.

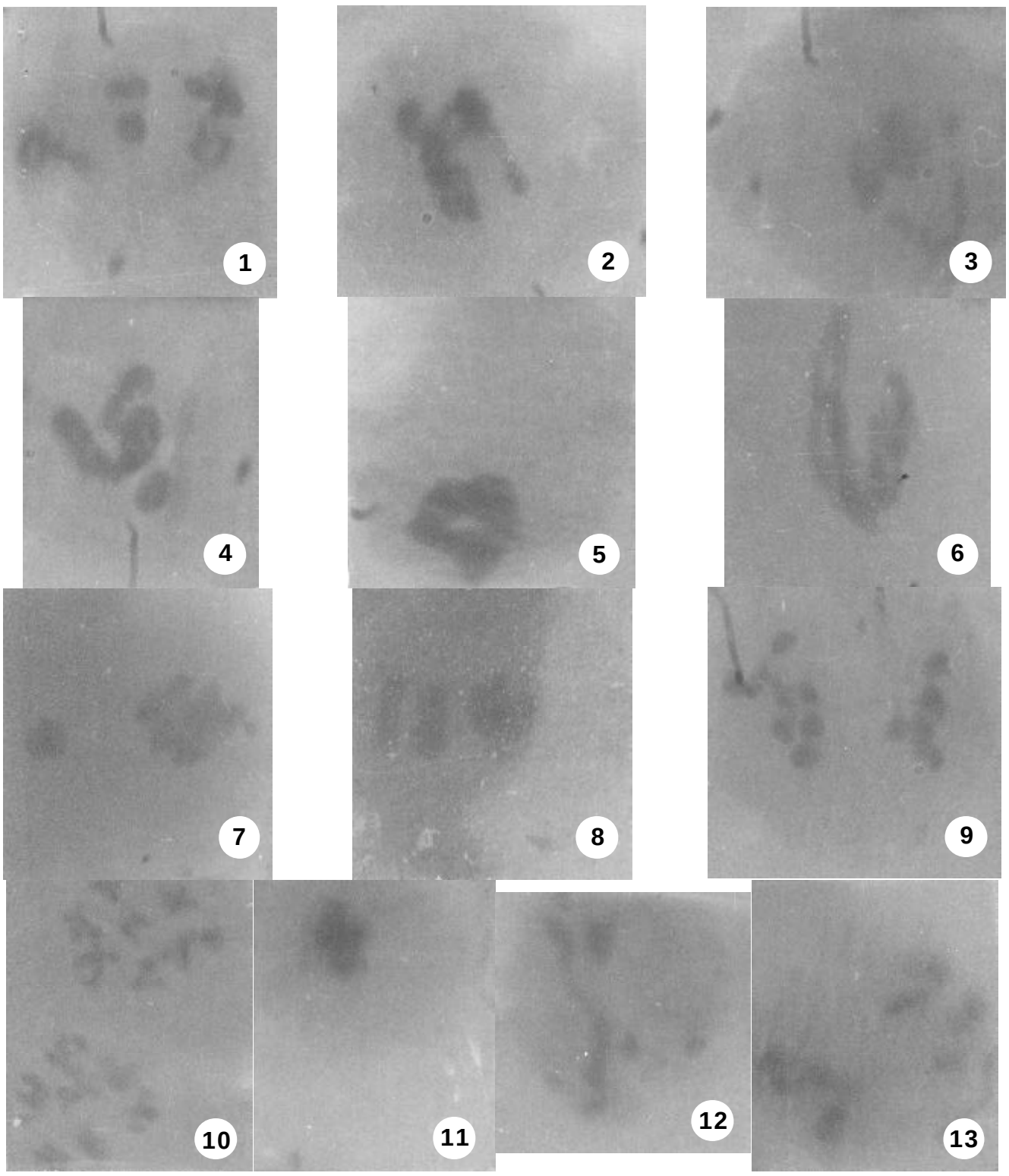
Dosages	No. of PMCs scored	Percentage of PMCs with meiotic irregularities at metaphase I			Percentage of abnormalities
		Stickiness	Fragments	Multivalent	
25kR	85	3	—	12	60
30kR	85	4	—	12	70
40kR	100	5	10	13	75

Table 1 : Chromosomal associations at diakinesis in *Hordeum vulgare* variety K1155 treated with different dosage of gamma-rays.

Dosages	No. of PMCs scored	Chromosomal associations			Polyvalents mean/range	Chiasmata frequency per PMCs
		Univalents means/range	Bivalents mean/range			
			ring IIs	rod IIs		
25kR	85	0.26	2.8	2.1	1.84	7.7
		(0-1)	(1-3)	(2-4)	(1-3)	
30kR	85	0.29	2.5	1.8	2.41	6.8
		(0-1)	(2-3)	(2-3)	(2-3)	
40kR	100	0.31	2.0	1.6	3.09	5.6
		(0-1)	(1-2)	(3-4)	(1-2)	

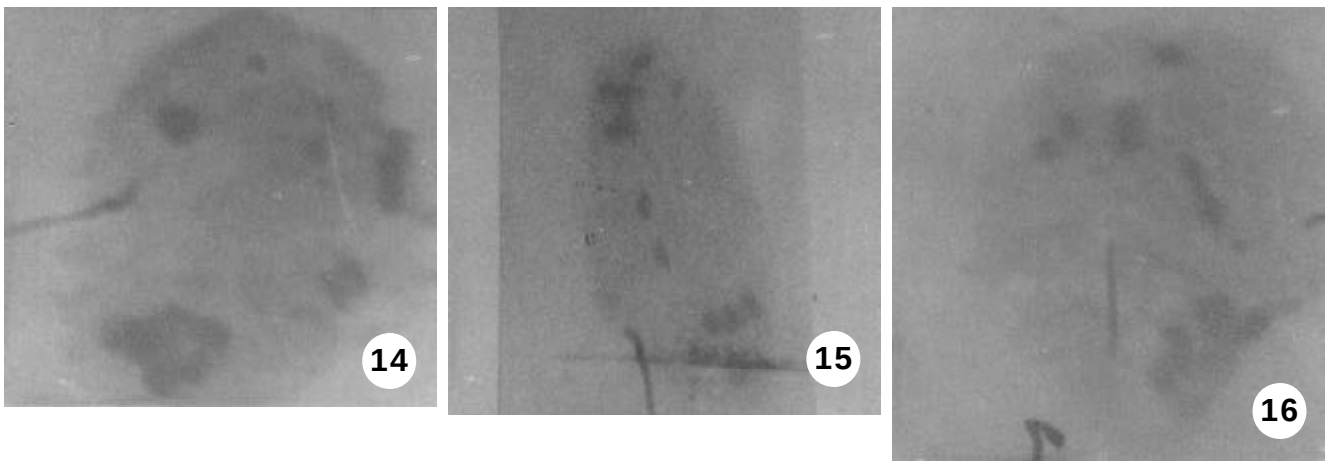
Table 2 : Frequency of meiotic irregularities in pollen mother cells of *Hordeum vulgare* variety K1155 at metaphase I (%) at various dosage of gamma-rays.

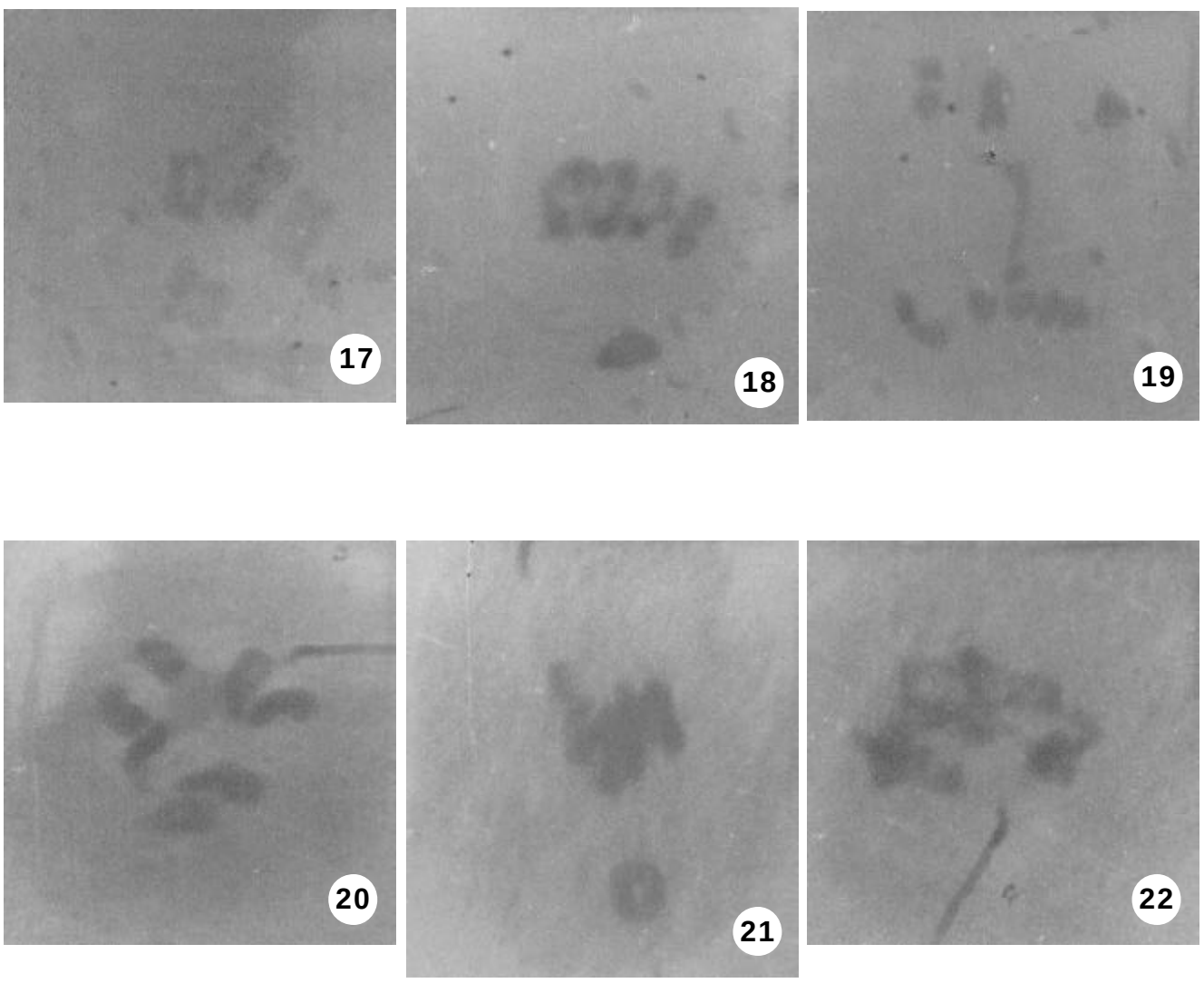
Dosages	No. of PMCs scored	Percentage of PMCs with meiotic irregularities at metaphase I			Percentage of abnormalities
		Stickiness	Fragments	Multivalent	
25kR	85	6	3	12	87
30kR	85	6	2	13	89
40kR	100	7	1	13	90



Plate–1

Fig. 1. PMC at diakinesis with one fragment. Fig. 2. PMC at diakinesis with bivalents in chains. Fig. 3. PMC with bivalents in two chains at diakinesis Fig. 4. PMC with 2 chains of bivalents and one isolated bivalent. Fig. 5. A closed ring of seven bivalents seen at diakinesis. Fig. 6. An open ring of 7 bivalents in a PMCs at diakinesis. Fig. 7. PMC at metaphase I showing one bivalent away from the equator. Fig. 8. Bivalents seen at equator during metaphase I in 4 groups. Fig. 9. 6:8 distribution of chromosomes at the poles and one chromosome in centre. Fig. 10. PMC showing delayed separation of chromosomes of a lagging bivalents at anaphase I. Fig. 11. All bivalents seen at equator during metaphase I. Fig. 12. Delayed separation of chromosomes at anaphase I and scattered univalents. Fig. 13. Anaphase I with equal distribution of chromsomes in PMC.





Plate–2

Fig. 14. PMC with disturbed metaphase I. Fig. 15. Delayed separation of two homologues of one bivalent at anaphase I. Fig. 16. Anaphase I with delayed separation of chromosomes. Fig. 17. Irregular orientation of bivalents at metaphase I (3 x 211 + one II). Fig. 18. PMC at metaphase I showing one bivalent away from the equator. Fig. 19. Delayed separation of chromosomes at anaphase I. Fig. 20. Seven bivalents seen at diakinesis. Fig. 21. PMC at metaphase I showing one bivalent away from the equator. Fig. 22. PMC at metaphase I showing irregular orientation of bivalents.

sometimes bivalents has been found to be erratic at metaphase I. Anaphase I a quite good number of PMCs 1 to 3 lagging chromosomes were recorded. The chromosomal distribution was found to be normal 7.7 on 67% PMCs.

Reduction in chiasmata frequency may be attributed either to mutation in those particular genes which regulated chiasmata formation or to the presence of univalents in many cells at diakinesis and metaphase I. Such observation are in close confirmity with the observation of Prasad & Tripathi (1986) and Vazquez and Monge (1987) with gamma radiated plants. The higher dosages of gamma radiation induced chromosomal association. Other than bivalents which includes chains of 6 or 7 chromosomes.

Non-orientation of chromosomes at metaphase I and Anaphase irregularities, delayed separation like presence of laggards ad unequal distribution of chromosomes at the poles seen in a large number of PMCs might be due to the advance effect of gamma radiation on the physiology and genetic systems of *H. vulgare*.

Aforesaid study confirms that 30kR of gamma-rays found to be efficient dose. John (1999) has reported that efficiency of gamma-rays was high at lower dose of radiation in hybrids of cowpea.

ACKNOWLEDGEMENT

Thanks are due to Dr. V.K. Srivastava, Principal, D.A.V. College, Kanpur, for providing necessary facilities in the Department of Botany and also for his constant encouragements.

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