International Journal of Academic and Applied Research (IJAAR)
ISSN: 2000-005X
Vol. 2 Issue 9, September – 2018, Pages: 1-7

Distribution of BCMD and Response of Common Bean Varieties to Bean Common Mosaic Virus in Western Kenya

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Abstract:*Common bean (Phaseolus vulgaris L) is the main legume crop grown by small farm holders in Kenya. Its grains are very vital in human nutrition and source of income for peasant farmers. The yield is approximately 530 kg ha^{-1} and the country's production is estimated at 613,902 tons per year. However, bean production in Kenya has kept on declining due to biotic and abiotic factors. In biotic, viral diseases are major yield reduction factor in bean production among them Bean Common Mosaic Disease (BCMD), caused by Bean common mosaic virus (BCMV) and Bean common mosaic necrosis virus (BCMNV) are most wide spread viruses with disease incidence of up to 100%. The general objective of the study was to determine the distribution of BCMD in three agro ecological zones of western Kenya and the response of common bean varieties to BCMV isolate. A diagnostic survey was done in 6 clusters (Bujumba, Alupe, Madola, Kimaeti, Ndareti and Chebich) of Busia and Bungoma counties in the long and short rain seasons of 2017. Farms for study in each cluster were randomly selected. Bean common mosaic disease (BCMD), incidence and severity were calculated and recorded. Leaf samples from each cluster, were collected for serological analysis. Sixteen bean varieties were planted in a greenhouse and some from each variety inoculated with BCMV isolate. BCMD incidence and severity were calculated, recorded and Leaf samples taken for serological tests. The results showed the occurrence of BCMD in all the clusters with varied incidence; 36.58%, 29.40%, 34.33%, 37.95%, 42.78% and 39.45 respectively as shown above clusters. Sixteen bean cultivars screened for re to BCMD showed that Chinese black kidney and Libya bean cultivars were tolerant to BCMV, but serologically tested positive to BCMV, while Red haricot, KATB1 and Mwitemania were more susceptible to disease with incidence. BCMD occurrence in all agro ecological zones in western Kenya is due infected seeds, availability of aphids or BCMV inoculum in other host plant. Resistant genes should be introgressed in local varieties.*

Keywords: BCMNV, BCMV, beans, Incidence, severity, resistance.

1. INTRODUCTION

The common bean (*Phaseolus vulgaris L*) It's a self-pollinating legume species that can also cross pollinate at a very low rate by insects (Ferreira *et al.*, 2000; Gepts, 2001). Pod formation occurs either after self or cross pollination. Common bean is a short season crop, with a range of varieties maturing from 65 to 110 days (Buruchara *et al.*, 2007). The world leading producer of common bean are Myanmar, India, Brazil and Republic of China producing metric tons; 3,800,000, 3,630,000, 2,936,444, and 1,400,000 respectively (FAOSTAT, 2014). In Africa this crop is grown on more than four million hectares annually. Its grains are consumed by more than 100 million people in rural and urban communities, with an annual per *capita* bean consumption in Eastern Africa (50 - 60 kg) being the highest in the world (ISAR, 2011). Common bean is the main legume crop grown in Kenya for its important value in human nutrition as it contains high protein content (Singh, 2005; Cortes *et al*., 2013). Regular consumption of common bean and other pulses is now promoted by health organizations because it reduces the risk of diseases such as cancer, diabetes or coronary heart diseases (Leterme *et al.*, 2002). It's also the source of income for many rural households (FAO, 2011). The main varieties cultivated in western Kenya include Rosecoco, Canadian wonder, KK8, KATX56, KATX69 and Pinto sugars. Rose coco and Canadian wonder are high yielding varieties but requires heavy rains and high soil fertility (Wronno *et al*., 2001). In Kenya the yield is 530 kg/ha and the country production estimated at 613,902 metric tons (FAO, 2014) that is low compared to a production potential of 1400 – 2000 kg ha^{-1} (Katungi., 2009). Also Kenya's Production is lower compared to Tanzania and Rwanda (885 and 913 kg/ha) respectively (FAOSTAT, 2014). The most common cause of low yields is based on biotic factors. The most important

viruses of common bean in Kenya are *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV) that cause Bean common mosaic disease (BCMD). This disease result into grain yield losses up to 100%. Other viruses infecting common bean that have been reported are CPMMV (Mink and Keswani, 1987; Chang *et al*., 2013), CMV (Davis and Hampton, 1986; Njau *et al*., 2006) and CABMV (Bashir *et al*., 2010).

2. MATERIAL AND METHODS

Occurrence and distribution of BCMD in western Kenya

Extensive diagnostic survey was conducted in the long rain seasons of 2017 in two major bean growing counties (Bungoma and Busia) of western Kenya. This survey covered three agro ecological zones: low midland zone 1 (LM1), low midland zone 2 (LM2) and upper midland zone 1 (UM1). It was carried on during long rain seasons of 2017. It covered 108 farms in three agro ecological zones in Busia County (LM1, LM2); (Madola, Bujumba and Alupe) and Bungoma county (LM2, UM1); (Chebich, Ndareti and Kimaeti) of western Kenya. One to two sampling units measuring 10m x 10m were randomly selected on each farm depending on farm size. Data obtained (BCMD incidence and severity) were recorded. Leaf Samples were taken from farms for serological Analysis. A GPS device (Magellan Triton "Windows CE Core 5.0" X11-15302) was used to measure the coordinates and altitude of the location.

Disease incidence and severity determination

Viral symptoms were record to determine the disease incidence and severity in each farm. Information on the type of variety grown and sources of seeds was also recorded. Sampling points within a farm was randomly selected. On each point incidence and severity was determined in an area of $10m^2$. A maximum of two sampling points was sampled depending on farm size.

Disease incidence was calculated as the percentage of plants showing BCMD symptoms to the total number of plants observed in the field. The average incidence and severity of the sampling points per farm was use as the actual plot disease incidence and severity. The degree of disease (BCMD) incidences was assessed and analyzed according to (Nono-Womdim, 1996) as the proportion of diseased plants in an area.

The presence and absence of viral disease on common bean varieties planted was scored using a rating scale basing on (Nono-Womdim, 1996) where low incidence=1-20%, moderate incidence= 21-49% and high incidence=50-100%. Disease symptoms severity was scored on a scale of 0-3 according (0du *et* a*l*, 2004) of which;

0 =No disease symptoms on plant,
1= Mild foliar disease symptoms,
2= Moderate foliar disease symptoms,
3= Severe distortion malformation of leaves or stem and stunting.

The viral symptoms that were scored are, leaf mosaic mottling, vein banding and vein clearing chlorotic leaves, leaf curl and crinkling, small leaves with inter-veinal yellowing, stunted growth, or a combination of these. The leaf samples were collected, put in a cool box and taken to the lab for analysis by DAS-ELISA and TAS-ELISA for BCMNV and BCMV respectively.

Enzymes- Linked Immunosorbent Assay (ELISA)

The detection of BCMV and BCMNV viruses by serological techniques was done using polyclonal antibodies (IgG) for coating and monoclonal antibodies (MAb) for detection in ELISA. The following buffers was used;

Coating buffer, pH 9.6 (per litre)
1.59 g Sodium carbonate (Na_2CO_3).
2.93 g Sodium bicarbonate ($NaHCO_3$)
0.20 g Sodium oxide (Na_2O)
Will be dissolved in 900 ml H_2O

PBS (pH 7.4) phosphate buffer Saline
8.00 g Sodium chloride (NaCl)
0.20 g monobasic potassium phosphate (KH_2PO_4).
1.15 g Dibasic Sodium phosphate Na_2HPO_4)
0.20 g potassium chloride (KCl)
0.20 g Sodium oxide (Na_2O)
Was dissolved in 900ml H_2O pH and adjusted from 7.4 to 11 with NaOH

PBS- Tween (PBST)
PBS+0.5 ml Tween 20 per litre
Sample extraction buffer (pH 7.4).
PBST+2% pvp (pvp- is polyvinyl pyrrolidone).
Conjugate buffer
PBST+2% pvp+0.2% egg albumin
Substrate buffer
97 ml diethanolamine
600 ml H_2O
0.20 g Sodium oxide (Na_2O)
Adjusted to pH 9.8 with HCl and make up to 1 litre with H_2O.

Triple Antibody Sandwich ELISA (TAS ELISA)

TAS ELISA was done as described by Were *et al*., (2013) Without modifications microlitre plates (96 wells) was coated with BCMV IgG diluted 1:1000 (v/v) in a coating buffer and incubated for 2 h at 37 ^{0}C. Blocking was done by adding 2% skimmed milk in PBST (200 µ1/well) and incubated for 30 min at 37 ^{0}C. Sap extracts sample was added and incubated at 4^{0}C. Extracted from each variety of a healthy plant (beans) and those infected with BCMV were used as negative and positive controls, respectively. MAbs

raised against BCMV was used in detecting antibodies at dilution of 1:100 (v/v) in conjugate buffer were used for detection. 100µ1 of each supernatant dilution was loaded onto microtitre plates and incubate for 2 h at 37^{0}C. After washing the plates, an alkaline phosphate labeled phosphate as (99Rabbit- anti- mouse) diluted 1:1000 (v/v) in conjugate buffer added and the plate incubated for 45 min at 37 ^{0}C. The substrate, P Nitrophonyl phosphate diluted 1mg/ml in substrate buffer was added and incubate for 2 h at 37 ^{0}C.

Double Antibody sandwich ELISA (DAS ELISA)

Double antibody sandwich ELISA was done with no modification as per Clark and Adams (1977). For detection of BCMNV in bean varieties, microtiters plates were coated with BCMNV IgG diluted 1:1000 (v/v) in coating buffer and incubated for 4 hours at 37 ^{0}C. Sample extracts were added and incubate at 4 ^{0}C. Extracts from healthy bean varieties and those of infected with known BCMNV was used as negative and positive controls, respectively. IgG- alkaline phosphate conjugates diluted 1:1000 (v/v) in conjugate buffer was added and incubated for 2 h at 37 ^{0}C substrate

Screening Common bean varieties for response to BCMV.

Popularly grown bean varieties were screened for resistance to BCMV from western Kenya: KATX56, KK8, GLP2- Rosecoco, Chelalang variety, KATB1 bean cultivar, Chinese black kidney beans, GLP24- Canadian wonder, Kenya sugar Bean (E7), M22- Rosecoco (simlaw), GLP585-Red haricot, Mwitemania bean cultivar, K131 bean variety, Libya bean cultivar, K132 bean cultivar, Saitoti bean cultivar. Selected bean cultivars were grown in 500 ml pots filled with sterile soil medium composed of loam soil, manure and sand in the ratio of 2:1:1 in a greenhouse. Two seeds per cultivars were planted in each pot and 10 pots were used for each cultivar. After germination, the seedlings were thinned to one seedling per pot. The set-up was replicated thrice. Isolates of BCMD from the survey were macerated into tiny pieces and grounded using a pestle and mortar in freshly prepared ice-cold 0.01M potassium phosphate buffer (K2HPO4 +KH2PO4), pH 7.0, containing 0.2% sodium sulphite and 0.01M mercapto-ethanol (1:6 [w/v] tissue: buffer). At the 3 leaf stage, ten bean plants were inoculated by gently rubbing the inoculum on leaves dusted with carborundum. The other ten plants were not inoculated and used as healthy controls. After inoculation, the plants were gently sprayed with water to remove excess carborundum. The test plants were observed for symptom development 3 days after inoculation and thereafter on weekly basis for 5 weeks. Data collected included: number of symptomatic development plants per variety (disease incidence) and disease severity (using 0-3 scale). Leaf sample was collected at 5 weeks and tested for the presence viruses BCMV using TAS-ELISA. Plants that tested positive for BCMV were regarded as susceptible. This was used to grade the resistance levels of different varieties to BCMV.

3. RESULTS AND DISCUSSION

Occurrence of BCMD in western Kenya.

The study covered an Altitudinal and coordinates from 1164 meters above sea level (masl) in LM1 (N00.48997; E 034.12290) to 1751 masl in UM1 (N00.80968; E034.60663). The typical symptom of both BCMV and BCMNV were light green and yellow leaf colour, dark green mosaic pattern on leaves, puckering of leaves, distortion and rolling of leaves, mottling of leaves. Other symptoms included mottling of leaves, dwarfing of bean plant, curling and malformation of leave. Viral symptoms were observed in all agro ecological regions surveyed with varied incidence. Ndareti (UM1) had the highest disease mean incidence of (42.78 %), with maximum mean of (90 %) and no disease symptom was noted in some farms. Chebich (UM1) was second with mean incidence of (39.45 %). Alupe (LM1) had the least BCMD mean incidence of (29.4 %). (Table 1). The ANOVA was used to obtain least significant difference (L.S.D) to separate the means at p=0.05 which indicated a positive correlation (r= 0.745; p<0.0001) between incidence and severity of BCMD.

Table 1: BCMD incidences in 6 cluster areas surveyed in western Kenya.

County	Cluster	AEZs	Mean	Max	Std error of mean
Bungoma	Chebich	UM1	39.45	90	2.522
Bungoma	Ndareti	UM1	42.78	90	2.723
Bungoma	Kimaeti	LM2	37.95	100	2.322
Busia	Alupe	LM1	29.4	75	1.875
Busia	Madola	LM2	34.33	72	2.112
Busia	Bujumba	LM1	36.583	72	2.211

BCMD severity in Bean growing areas in Busia and Bungoma Counties

Severity mean showed a variation from one cluster to the other; Ndareti (UM1) had the highest severity mean of

(1.670) with maximum severity of (3) followed by Chebich (UM1) with severity mean of (1.450) with a maximum of (3). Alupe (LM1) had the lowest severity mean of (1.100) with maximum severity of (2). (Graph 1)

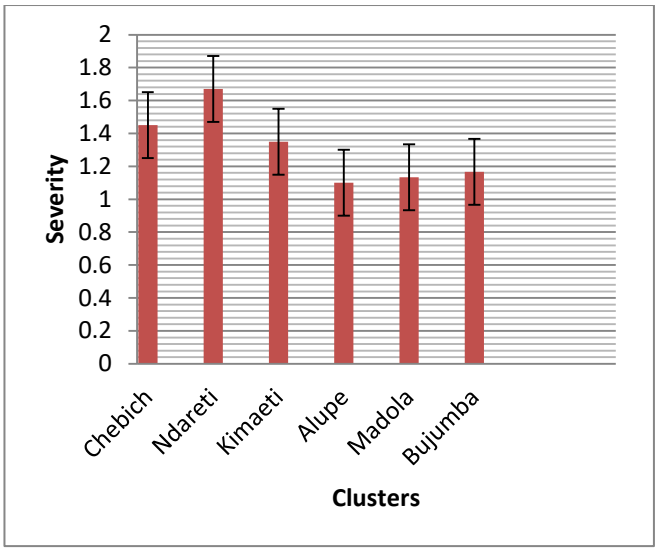

Figure 1: graph showing BCMD severity in 6 cluster areas of western Kenya

Serological tests for BCMV and BCMNV.

Serological tests showed that samples from Ndareti (UM1) had the highest incidence for BCMV (25 %), followed by Kimaeti and Bujumba (LM2, LM1) with (12%) tested positive for BCMV. Samples from Chebich (UM1), Alupe (LM1) and Madola (LM2) had the lowest percentage (10 %) of samples that tested positive for BCMV. None of the samples tested positive for BCMNV. (Table 2).

Table 2: Visual and ELISA incidence of BCMV and BCMNV in western Kenya

County	Cluster	AEZs	Mean Incidence	BCMV-ELISA (%)
Bungoma	Chebich	UM1	39.450	10.00
Bungoma	Ndareti	UM1	42.780	25.00
Bungoma	Kimaeti	LM2	37.950	12.00
Busia	Alupe	LM1	29.400	10.00
Busia	Madola	LM2	34.330	10.00
Busia	Bujumba	LM1	36.748	12.00

Seed Quality Tests.

P.vulgarise germplasm for each cultivar randomly picked for seed healthy test for BCMV. The selected seeds prepared for BCMV test according to international rules for seed health (ISTA) (ISTA, 2014). The seeds wiped with cotton wool and soaked into 70% Ethanol, then rinsed with distilled water. Transferred to Petri dish water soaked paper towels and sprout.

Screened common bean varieties on resistance levels to BCMD.

Sixteen differential bean varieties screened for host resistance to BCMV inoculum and evaluated. The study revealed a variation in disease incidence and severity in different varieties

Figure 2a: Hypersensitive reaction observed on inoculated leaves of KATB1 bean cultivar in greenhouse. (Resulting into leaf mosaic and yellowing of leaves due to inoculation with BCMV). B: Chinese black kidney bean variety was more tolerant to inoculation with BCMV.

Screened Bean cultivars for resistance to BCMD incidences

Study indicated that the Chinese black kidney and Libya bean varieties were the more tolerant to BCMV with mean incidence of (0.00%). most of bean varieties screened had lower BCMD incidence (1-20%); (GLP2 Rose coco, GLP24-canadian wonder, KATX56, Kenya sugar bean (E7), Rose coco (M22), K131, K132, GLP1004). KATB1, GLP 585-Red haricot and Mwitemania cultivars were the most susceptible to BCMD infections with mean incidences of (75%), (75%) and (60%) respectively, with minimum incidence of (8%) and maximum incidence of (100%).

Table 4: screened bean varieties for resistance levels to bcmd incidences in western kenya.

Bean variety	N	Mean	Max	Min
Chelalang Variety	18	27	38	8
KK8	16	22	44	11

GLP2.Rosecoco	18	15	40	00
KATB1	16	75	100	33
Chinese black kidney	20	0	00	00
GLP 24. Canadian Wonder	16	14	29	00
KAT56	18	8	22	00
Kenya sugar bean (E7)	16	14	31	8
Rose coco (M22)	20	10	25	00
GLP 585. Red haricot	20	75	100	50
Mwitamania	18	60	100	8
K131	16	8	16	00
Libya beans	20	0	00	00
K132	18	17	25	8
Saitoti beans	20	30	44	11
GLP1004	20	19	50	00

Screened common Bean varieties on resistance levels on BCMD severity

This Study reveals that the Chinese black kidney and Libya bean variety were the most tolerant common bean cultivar with mean of severity (0.000). Most of bean varieties screened had the same resistance level on BCMD severity. Table 10 showed that (KK8, GLP2 Rose coco, GLP24-canadian wonder, KATX56, K131, K132, Saitoti bean, K1004) had a mean severity of (1.000) with minimum (0.000) and maximum mean of (1.000). Chelalang Variety, Red hericort and Mwitamania had BCMD severity mean (2.000) with minimum severity (1.000) and maximum of (2.000). KATB1 had the highest severity mean (3.000), with minimum severity (2.000) and maximum of (3.000). (Table 3).

Table 3: Screened bean varieties on resistance levels on BCMD severity in western Kenya.

Bean variety	N	Mean	Max	Min
Chelalang Variety	18	2.000	2.000	1.000
KK8	16	1.000	1.000	0.000
GLP2. Rosecoco	18	1.000	2.000	0.000
KATB1	16	3.000	3.000	2.000
Chinese black kidney	20	0.000	1.000	0.000
GLP 24. Canadian Wonder	16	1.000	2.000	0.000
KAT56	18	1.000	1.000	0.000
Kenya sugar bean (E7)	16	1.000	2.000	1.000
Rosecoco M22	20	1.000	2.000	0.000
GLP 585. Red hericort	20	2.000	3.000	1.000
Mwitamania	18	2.000	2.000	1.000
K131	16	1.000	1.000	0.000
Libya beans	20	0.000	1.000	0.000
K132	18	1.000	1.000	0.000
Saitoti beans	20	1.000	1.000	1.000
GLP1004	20	1.000	1.000	0.000

BCMV SYMPTOMATIC AND ELISA INCIDENCES ON SCREENED BEAN VARIETIES

Serological tests on sixteen screened P.vulgarise differential genotype samples show that all the cultivars reacted positive for BCMV (TAS ELISA) with varied incidences.KATB1, GLP 585-Red haricot and Mwitemania had high ELISA incidences percentage of (39%), (32%) and (27%) respectively. Chinese black kidney and Libya varieties had the lowest ELISA percentage of (4%). (Table 5).

TABLE 5: BCMV SYMPTOMATIC AND ELISA INCIDENCES ON SCREENED BEAN VARIETIES

Bean variety	N	Mean	Max	Min	ELISA (%). Incidence
Chelalang Variety	18	27	38	8	18
KK8	16	22	44	11	14
GLP2.Rosecoco	18	15	40	00	9
KATB1	16	75	100	33	39
Chines black kidney	20	0	00	00	4
GLP 24. Canadian Wonder	16	14	29	00	10
KAT56	18	8	22	00	6
Kenya sugar bean (E7)	16	14	31	8	8
Rose coco (M22)	20	10	25	00	5
GLP 585.Red haricot	20	75	100	50	32
Mwitamania	18	60	100	8	27
K131	16	8	16	00	5
Libya beans	20	0	00	00	4
K132	18	17	25	8	8
Saitoti beans	20	30	44	11	18
GLP1004	20	19	50	00	10

4. DISCUSSION

The survey revealed that BCMD exist in all agro ecological zones of bean growing regions in western Kenya. The symptoms observed in the farms were mainly leaf mosaic, leaf mottling and distortion, yellowing of leaves, leaf curling and malformation. These are the major BCMD symptoms that have been reported. (Mangeni *et al.,* 2014). Serological tests using TAS-ELISA confirmed the presence of BCMV in all clusters surveyed. DAS ELISA showed absence of BCMNV. This could be due to the fact that bean infected with this virus (BCMNV) were killed by black root syndrome therefore they did not survive to the next generation (Grogan and Walker, 1948). Therefore, the

germplasm used were either free from BCMNV strains or had low level of inoculum that could not be expressed serologically. Upper midland zone 1 (UM1) had the highest disease incidence (41.23 %). This was probably due to high altitude which enhanced the incidence of BCMV on beans (Myers *et al*., 2000) or the prevailing conditions like low temperature favored (Kumar *et al.*, 1997) the activities of vectors (aphids) in transmission of BCMV into host plant. Disease incidence in Alupe (LM1) was low with mean incidence of (29.04%), this may be due to low altitude (Myers *et al.*, 2000) or availability of other host plants which aphids preferred than common beans, resulting into reduced disease pressure. BCMD incidence correlated positively with severity ($r=0.745$; $p<0.0001$). Also early infection of farms thus high viral incidence and rapid built up of viral titres which leads to increased severity of the disease. This also explains the positive correlation between viral disease incidence and severity that was observed in Bean farms where severity increased with increase in disease incidence. The difference in incidence and severity in all agro ecological zones surveyed could imply that the viral strain (s) in Kenya are different due to differences in geographical and climatic conditions or the virus detected serologically could just be closely related to BCMV. Most of bean cultivars screened were found to be susceptible to BCMV, although a few cultivars (Chinese black kidney and Libya), were tolerant to BCMV but serologically tested positive for BCMV. There were variations in titre levels between the varieties screened shows that viral establishment varied from variety to variety, a finding that confirms reports of (Morales and Castano, 1987; Bos, 1971). This gives an indication that some of the varieties may be having mechanisms of reducing virulence or resisting viral multiplication which slow down viral establishment. But with time the system becomes overwhelmed and the viral disease is expressed. The genetic interaction between BCMV inoculum and bean genotypes have relationship which make them to have a variation in incidence. That genotype with genes that are overwhelmed by corresponding pathogenicity genes possess by the virus strain(s) became more susceptible to the virus, thus more disease incidence.

5. CONCLUSIONS

This study has revealed that bean common mosaic disease is widespread in all bean growing regions of western Kenya across all the AEZs surveyed (LM1, LM2 and UM1) although its incidence varies in all agro ecological zones. Different bean genotypes have different levels of tolerance to BCMV inoculum. This reveals that there are no resistance cultivars in Kenya which farmers can use to withstand BCMD infection, or if they are available then farmers are not aware of these varieties.

6. RECOMMENDATION

This study recommends farmer to use certified seeds that are free from BCMV and BCMNV inoculum strains and avoid using those from markets or farmer saved due to contamination with disease inoculum strains. Although there were variations in viral titre among the screened varieties, such varieties with slow viral establishment are not recommended to farmers, but can be studied further to establish the factors leading to slow virus multiplication that can help in breeding for resistance cultivars.

7. ACKNOWLEDGEMENT

We appreciate the McKnight foundation for funding this work as well as the Masinde Muliro University of Science and Technology (MMUST) and (International Centre for Tropical Agriculture-CIAT-Uganda) for providing facilities for this study. Secondly we acknowledge the service provided by Mr Warren Arinaitwe in compiling data

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