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Growth Promotion and Increased Potassium Uptake of Tobacco by Potassium-Mobilizing Bacterium *Frateuria aurantia* Grown at Different Potassium Levels in Vertisols

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Potassium-mobilizing bacterial strain Frateuria aurantia *was examined for plant-growth-promoting effects and nutrient uptake on tobacco (*Nicotiana tabacum *L.) grown in vertisols as a field experiment for two crop seasons, 2009–2010 and 2010–2011. Inoculation with bacterial strain* Frateuria aurantia *was found to increase biomass, nutrient content, and leaf quality of flue-cured Virginia (FCV) tobacco. Bacterial strain* F. aurantia *was able to enhance potassium uptake efficiently in tobacco plants when sulfate of potash was added to the soil. In tobacco, the ultimate product is the leaf that is consumed and has commercial value. In tobacco-growing soils treated with soluble potassium and inoculated with strain* F. aurantia*, the potassium content of the leaf was increased by 39%. Bacterial inoculation also resulted in greater nitrogen and phosphorus contents of aboveground plant components. The bacterial isolate was also able to colonize and develop in the rhizosphere soil of tobacco after root inoculation. Solubilization of potassium containing minerals by potassium-mobilizing bacteria in vertisols and their effect on tobacco plant growth, yield, and quality are reported in this study.*

Keywords Bacterial inoculation, *Frateuria aurantia*, potassium, tobacco, vertisols

Introduction

Tobacco is an important commercial crop of India, which is used as foreign exchange, provides raw material to industry, and creates employment opportunities for many. Potassium (K) is the master cation of the plant, and it is the element of quality in tobacco. The cured leaf color, grade, body, texture, elasticity, and fire-holding capacity of the leaf are significantly influenced by K concentration of the leaf. High K content in tobacco leaf is regarded as one of the criteria of quality. Tobacco plant is a luxury consumer of potash and is considered as the indicator plant for K deficiency (Krishnamurthy, Reddy, and Ramakrishnayya 1989).

Ramakrishnayya and Krishnamurthy (1990) reported that K content of the tobacco leaf was strongly and positively correlated with the available K status in sandy loams of northern light soils and constructed the regression equations between the leaf K and

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the soil K for tobacco crop grown in northern light soils of Andhra Pradesh. There is an ample evidence to suggest that the tobacco plant accommodates a high reserve of K in the early stages of plant growth, sufficient enough to sustain the latter phases of development. Ramakrishnayya and Krishnamurthy (1990) studied the K-distribution pattern in irrigated Alfisols of Andhra Pradesh and found that K content of leaf lamina in K-deficient plants increased from bottom to the top. The effect of potash mobilizer on brinjal recorded an increased potash uptake and increased plant biomass in potash-mobilizer-treated plants as compared to the control plants (Ramarethinam and Chandra 2004).

Lin et al. (2002) recorded increases in biomass by 125% K and 150% phosphorus (P) in tomato plant due to inoculation of silicate-dissolving bacteria (*B. mucilaginosus*) over noninoculated plants. Plant-growth-promoting rhizobacteria (PGPR) including phosphate-solubilizing bacteria (PSB) and K-solubilizing bacteria (KSB) as biofertilizers was a sustainable solution to improve plant nutrient and production (Vessey 2003; Lu et al. 2006).

Park et al. (2003) reported that bacterial inoculation could improve P and K availability in the soils by producing organic acid and other chemicals to stimulate growth and mineral uptake of plants. Sheng, Xia, and Chen (2003) studied the effect of inoculation of silicate-solubilizing bacteria (SSB) (*Bacillus edaphicus*) on chili and cotton, which resulted in increased available P and K contents in plant biomass.

Zhang, Tu, and Cheng (2004) reported that the effect of K-mobilizing bacteria on sorghum resulted in increased biomass and contents of P and K in plants than the control. Solubilization of K-bearing minerals by K-solubilizing bacteria as alternative sustainable fertilizers was reported by earlier workers (Han, Supanjani, and Lee 2006; Sheng and He 2006; Raj 2004). The increased K uptake coupled with increased yield in yam and tapioca, while treating the plants with K mobilizer in conjunction with biofertilizers and chemical fertilizers, (Clarson 2004) was also reported. Chandra et al. (2005) reported increased yield by 15 to 20% in yam and tapioca due to the potash solubilizer application and in combination with other biofertilizers such as *Rhizobium, Azospirillum, Azotobacter, Acetobacter*, and P-solubilizing microorganisms (PSM).

Potassium is an element essential for plant growth. With the rapid development of world agriculture, available soil K levels have dropped due to crop removal, leaching, runoff, and erosion. Most soils in India are deficient in K. It thus becomes urgent to investigate the bioactivation of soil K reserves so as to alleviate the potash fertilizer shortage (Sugumaran and Janarthanam 2007).

Potassium needs of flue-cured tobacco plant are high and therefore a liberal supply of K fertilizers is essential for rapid growth, maturity, and quality of tobacco. The rate of K absorption is very high during the early stages of plant growth and diminishes rapidly during later phases. Among all the nutrients absorbed from the soil, K is removed in the greatest amounts by the tobacco crop in both the soil zones, indicating the importance of K in the mineral nutrition of flue-cured tobacco (Subhashini 2013). Tobacco is known to be a luxury user of K. Leaf color, texture, combustibility, and hygroscopic properties are enhanced by K fertilizer. Greater leaf K reduces tar and other harmful substances in the smoke. The present study is needed to study the enhancement of K-uptake efficiency in flue-cured tobacco by exploiting K-mobilizing bacteria*, F. aurantia* (FA).

Materials and Methods

Plant and Soil

The traditional black soils (TBS) classified as vertisols contained 36% sand, 14% silt, and 50% clay and have the textural class of clay. The dominant clay mineral is montmorillonite

with less illite. The soils are calcareous with 4.5 to 5.0% free calcium carbonate. The cation exchange capacity (CEC) is 48.26 $cmol_c\ kg^{-1}$. The physicochemical properties of the experimental soil were pH 7.6, electrical conductivity 2.95, organic carbon 0.41%, available P 14 kg ha^{-1}, and available K 633 kg ha^{-1}. Tobacco (cv. Siri) seedlings were obtained from the Division of Crop Improvement, Central Tobacco Research Institute, Rajahmundry, India.

Bacterium

Potassium-mobilizing bacterium *F. aurantia* was obtained from Gokulum Biotech, Pondicherry. The K mobilizer contained a population of 1×10^{10} colony-forming units (CFU) g^{-1} inoculum (wet weight). Mutants of *F. aurantia* strain marked with antibiotic resistance were obtained after plating the parental strain on sucrose-minimal salts agar amended with rifampicin (150 mg L^{-1}). After incubation for 4 days at 28 °C, the rifampicin-resistant strains were selected based on similarities in colony morphology, growth rate, K solubilization, and plant-growth promotion with the parent strain recultured on a medium containing rifampicin to ensure stability of the antibiotic resistance marker.

Increased K Uptake by Plants. A field experiment to study the effects of bacterial strain *F. aurantia* on plant growth and K content of tobacco leaf was conducted for two crop seasons, 2009–2010 and 2010–2011. The experimental design consisted of six treatments: uninoculated control without K fertilizer (T_1K_0); uninoculated plot with 25 kg ha^{-1} of K fertilizer (T_2K_{25}); uninoculated plot with 50 kg ha^{-1} of K fertilizer ($T_3\ K_{50}$); *F. aurantia*–inoculated plot without K fertilizer (T_4K_0); *F. aurantia*–inoculated plot with 25 kg ha^{-1} K fertilizer (T_5K_{25}); and *F. aurantia*–inoculated plot with 50 kg ha^{-1} K fertilizer (T_6K_{50}). Potassium fertilizer was applied in the form of sulfate of potash (SOP); *F. aurantia* was applied at a dosage of 10^3 CFU $plant^{-1}$ (the biofertilizer supplied contained the dosage of *F. aurantia* 10^{10} CFU ml^{-1}). *F. aurantia* was applied after mixing with farmyard manure (FYM) before transplantation of tobacco seedlings.

Plants were grown up to 120 days on residual moisture in vertisols. After 55 days of transplantation, observations on gas exchange parameters including photosynthetic rate, transpiration rate, and stomatal conductance were measured using a portable photosynthetic system (LICOR-6400 Portable Photosynthesis System, LiCOR BioSciences, Lincoln, Neb., USA), and chlorophyll content index was measured using chlorophyll content meter model CCM-200 (Optisciences, Inc., Hudson, N.J., USA). Biometric observations on plant height, number of leaves, and stem girth were recorded at the grand growth period of the crop. Matured leaves were harvested and cured. Yield data such as cured leaf and bright leaf were recorded and grade index was calculated. Cured leaf samples collected were analyzed for quality parameters such as nicotine, reducing sugars, and chlorides (Harvey, Starh, and Smith 1969), and nitrogen (N) and P were determined by the vanidomolybdate method and K by flame photometry (Jackson 1973). Soil analysis was carried out after the completion of the crop for estimation of percentage of organic carbon (C), available P, and available K. Organic C content of the soil (taken as an index of N availability) was estimated by the chromic acid digestion method of Walkley and Black (1934). Available P was determined by sodium bicarbonate extraction (Olsen et al. 1954). Neutral normal ammonium acetate extraction technique (Black 1965) was adopted in quantifying the available K status of the soils.

Survival of Antibiotic-Resistant Bacterium after Inoculation. This investigation was conducted with an antibiotic-resistant mutant of the *F. aurantia.* Plants were harvested after ripening of the tobacco leaves. After harvesting, adhering soil was removed from the crop roots. For determination of soil colonization, 1 g soil removed from the roots was shaken with 10 mL sterile water and 1% fungicidin solution for 30 min. To determine the rhizosphere colonization, 1 g washed roots were macerated and shaken with 10 mL sterile water (Egamberdiyeva and Höflich 2003). The resulting suspensions were evaluated for colony-forming units (CFU) according to the dilution-plate method on sucrose-minimal salts agar (Sheng and Huang 2002) with addition of 150 mg rifampicin. By adding fungicidin and rifampicin, the native fungal and bacterial flora were mostly excluded from the plates. After incubation for 7 days at 28 °C, the reisolated rifampicin-resistant strains were identified against the parent strains.

Statistical Analysis

All the data were tested by analysis of variance (ANOVA). Treatment means were compared to the control using the least significant different test ($LSD_{0.05}$) (Panse and Sukhatme, 1985).

Results

Observations were recorded on initial soil analysis, plant height, green leaf, cured leaf, bright leaf yield, grade index, uptake of N and K by the plants, and FA count in the rhizosphere. Yield data revealed significant increase in the production and yield due to the bacterium *F. aurantia.*

Survival and Establishment of Antibiotic-Resistant Bacterium in Tobacco Rhizosphere

A rifampicin-resistant mutant of *F. aurantia* was tested for its ability to colonize tobacco roots and soil. The inoculated bacterium was able to establish a population on the roots and in the rhizosphere soil of tobacco up to 10 weeks after transplantation. However, its survival was much better on K_{50} + FA tobacco of *F. aurantia* in the rhizosphere of flue-cured Virginia (FCV) tobacco, which revealed that the population of FA increased with the increase in the levels of K fertilizer showing the maximum colonization of 344 CFU g^{-1} (Figure 1).

Soil Properties

Inoculation of *F. aurantia* resulted in a significant increase in soil organic-matter content when compared to uninoculated control (Table 1). Similar findings were reported in sudan grass (Basak and Biswas 2009). However, the results indicated that the increase was not directly induced by the activity of soil microorganisms. The treatments with K fertilizer as sulphate of potash (SOP) exhibited a larger population size of *F. aurantia* in the rhizosphere. The significant correlation ($LSD < 0.05$) between soil organic-matter content and plant biomass (Tables 1 and 2) suggested that the organic-matter content in the rhizosphere was mainly influenced by plant growth, metabolism, and physiological activities. The results are in agreement with those of Mikhailouskaya and Tcherhysh (2005).

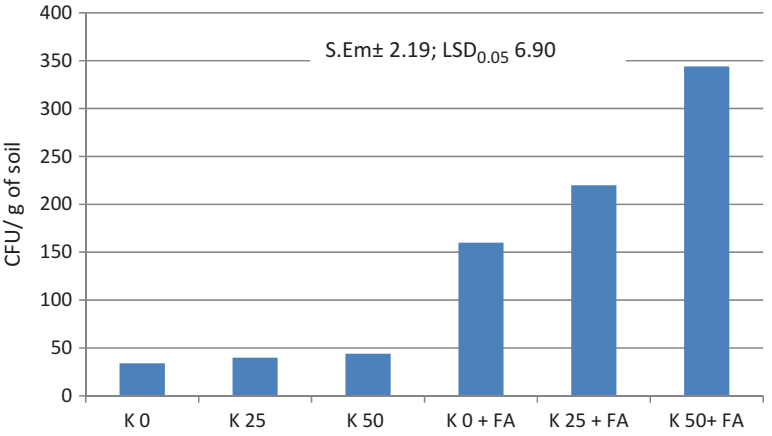

Figure 1. Establishment of *F. aurantia* (FA) in the rhizosphere of TBS tobacco.

Table 1
Response of inoculation of *F. aurantia* on the nutrient status of soil in vertisols

No.	Treatment	OC (%)	P (kg/ha)	K (kg/ha)
1	K_0	0.52	39	778
2	K_{25}	0.53	41	870
3	K_{50}	0.59	49	955
4	K_0 + FA	0.48	49	867
5	K_{25} + FA	0.61	55	1146
6	K_{50}+FA	0.67	42	1112
	SEM±	0.06	0.11	89.88
	$LSD_{0.05}$	*NS*	*NS*	*NS*
	CV (%)	20.56	51.74	18.82

Note. Initial soil values: pH, 7.6; EC, 2.95; OC, 0.41%; P, 14 kg/ha; K, 633 kg/ha.

Plant Biomass Accumulation

Response of inoculation of *F. aurantia* on the growth parameters of FCV tobacco in TBS indicates the beneficial effects on plant height, number of leaves, fresh and dry weights of the stem, and root and stem girth over the control (Table 2).

Observations on plant growth were recorded 90 days after transplantation. The control plants showed very poor growth, which may be attributed to nutrient deficiency, for example, the lack of available K in the unfertilized soil (Table 2). Bioinoculation effect on plant growth was much more pronounced due to the application of sulfate of potash at different levels and its combination with *F. aurantia*. The maximum cured leaf yield of 2768 kg ha^{-1} was recorded in the treatment K_{50} + FA, whereas the K_{25} + FA treatment achieved the yield of 2653 kg ha^{-1}. The effect of FA on yield characters was recorded (Figure 2). Cured leaf yield and grade index increased with K_{50} + FA followed by K_{25} + FA. *F. aurantia* alone was better than the uninoculated control. Significant differences were not observed among the treatments at graded levels of K with inoculated and corresponding uninoculated treatments. The results are in agreement with those of Sheng and He (2006).

Table 2
Effects of *F. aurantia* on growth parameters of tobacco

No.	Treatment	Plant height (cm)	No. of leaves /plant	Stem girth (cm)	Stem weight (g)		Root weight (g)	
					Fresh	Dry	Fresh	Dry
1	K_0	131.75	26	7.37	349.37	111.50	85.50	34.00
2	K_{25}	140.25	27.25	7.56	520.50	146.00	108.12	43.00
3	K_{50}	143.75	27.75	7.94	727.25	185.50	131.50	51.75
4	K_0 + FA	140.87	27	7.81	609.87	171.00	120.87	47.00
5	K_{25} + FA	142.62	28	8.12	611.75	172.50	136.37	54.50
6	K_{50}+FA	149.37	27.5	8.35	738.50	208.50	145.62	57.00
	SEM±	5.55	0.73	0.31	72.08	15.57	14.93	6.25
	$LSD_{0.05}$	*NS*	*NS*	*NS*	*217.24*	*46.93*	*NS*	*NS*
	CV (%)	7.85	5.33	7.94	24.32	18.78	24.62	26.11

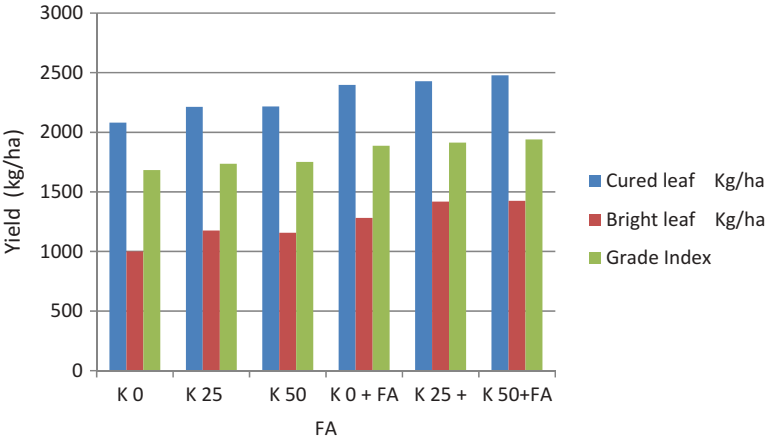

Figure 2. Response of inoculation of *F. aurantia* on the yield of FCV tobacco in vertisols.

With regards to the increase in plant biomass, *F. aurantia* seemed to be more effective in enhancing K-uptake efficiency of the plant. However, the effect of the *F. aurantia* with or without SOP on plant yield was significantly different. It was noted that plants grown with the application of bioinoculants produced more dry matter than plants grown in the uninoculated control (Sheng 2005; Han and Lee 2005). These results suggest that *F. aurantia* inoculation could contribute to the nutrient availability in vertisols. The low biomass of plants grown on the control treatments could be attributed to the absence of *F. aurantia*, which may be essential to increase nutrient bioavailablility and uptake in the rhizospheric soil. Stimulation of different crops by rhizobacterial inoculation was demonstrated by other studies both in laboratory and field trials (Khan, Ahemad, and Oves 2009).

Yield data on the effect of K-mobilizing bacteria in TBS indicated the beneficial role of *F. aurantia* in increasing the yield of tobacco at three different levels of K fertilization (0, 25, and 50 kg of K along with FA). Yield data (Figure 2) showed that the treatment *F. aurantia* in combination with 50 kg of K resulted in an increased yield of 10% green leaf, 12% cured leaf, and 16% bright leaf and 10% grade index over the control. The results are in accordance with those of Fang and Yan (2006) and Hu, Chen, and Guo (2006).

Gas Exchange Parameters and Chlorophyll Content Index

Significantly greater photosynthetic rates were observed with K_{50} + FA followed by the plants inoculated with K_{25} + FA and K_0 + FA (Table 3). Lower rates of photosynthesis, transpiration, and chlorophyll content index were found in uninoculated plants.

Nutrient Acquisition

The treatment K_{50} + *F. aurantia* seemed to be the most effective treatment combination to improve plant nutrient uptake (Table 4). Potassium concentration in plants under different treatments ranged from 3.92 (control) to 5.45%. The inoculation with *F. aurantia* had a more stimulating effect on the assimilation of K than SOP at different levels as fertilizer K. However, FA performed well in stimulating N and P uptake also, when combined with SOP at different levels. The pattern of P and K uptake by plants under different treatments

Table 3
Response of inoculation of *F. aurantia* on the physiological observations of FCV tobacco in vertisols

No.	Treatment	Photosynthetic rate (μ mol m^{-2} s^{-1})	Stomatal conductance (mol m^{-2} s^{-1})	Transpiration rate (mol m^{-2} s^{-1})	Chlorophyll content index (m mol m^{-2} s^{-1})
1	K_0	16.90	0.41	6.26	35.50
2	K_{25}	19.43	0.50	6.39	40.40
3	K_{50}	20.47	0.61	7.24	48.10
4	K_0 + FA	25.80	0.42	6.38	43.00
5	K_{25} + FA	25.60	0.70	8.48	49.90
6	K_{50} + FA	28.90	0.82	9.50	53.83
	SEM±	0.26	0.01	0.07	1.83
	$LSD_{0.05}$	*0.81*	*0.03*	*0.21*	*5.75*
	CV (%)	1.96	2.64	0.30	7.57

Table 4
Response of inoculation of *F. aurantia* on the nutrient content of leaf in vertisols

No.	Treatment	N (%)	P (%)	K (%)
1	K_0	1.95	0.09	3.92
2	K_{25}	2.00	0.10	4.44
3	K_{50}	2.25	0.11	4.56
4	K_0 + FA	2.10	0.10	4.44
5	K_{25} + FA	2.25	0.12	4.62
6	K_{50} + FA	2.27	0.13	5.45
	SEM±	0.11	0.28	0.28
	$LSD_{0.05}$	*NS*	*NS*	*0.85*
	CV (%)	10.08	21.05	12.16

was similar to N assimilation. The lowest P and K uptake was detected in plants grown in uninoculated and unfertilized plots. Either single treatment with FA inoculation resulted in an increase in P and K uptake to different degrees when compared to the control. The maximum P and K assimilation were obtained with the treatment K_{50} + FA (Sheng, He, and Huang 2002).

Leaf Quality. The tobacco leaf is an economic product and sensitive to applied chemical fertilizers such as nitrogen. Nicotine, reducing sugars, and chlorides determine the quality of FCV tobacco leaf. Nicotine is an alkaloid that is synthesized in tobacco roots and is regulated more by N supply than any other nutrient (Collins and Hawks 1993). *F. aurantia* inoculation proved to be the best treatment in terms of quality, recording greatest percentage of reducing sugars and the lowest of chlorides. Regarding percentage of nicotine, the control (K_0) alone showed increased level of nicotine (Table 5).

Table 5
Response of inoculation of *F. aurantia* on the quality of FCV tobacco in vertisols

No.	Treatment	Nicotine (%)	Reducing sugars (%)	Chlorides (%)
1	K_0	2.60	13.03	1.93
2	K_{25}	2.50	13.21	1.95
3	K_{50}	2.47	13.17	1.75
4	K_0 + FA	2.46	13.72	1.90
5	K_{25} + FA	2.41	13.62	1.94
6	K_{50} + FA	2.29	14.14	1.85
	SEM±	0.22	0.89	0.16
	$LSD_{0.05}$	*NS*	*NS*	*NS*
	CV (%)	17.78	13.38	17.06

Discussion

This region has extensive FCV tobacco cultivation. In uninoculated soils, the K content in leaf lamina was greatly increased if soluble K was added, but this increment was greater when the soil was inoculated with the strain *F. aurantia,* supporting the contention of earlier workers (Supanjani et al. 2006). Although in soils inoculated with strain *F. aurantia* the K content in leaf is lower than in soil amended with soluble K (K_{50}), the plants have greater N content and greater shoot and root dry weight (Egamberdiyeva and Hoflich, 2003). Lin et al. (2002) also demonstrated that bacterial inoculation resulted in growth promotion and greater K contents of plant components. Bacterial inoculation also increased N and P contents in plants. Increased nutrient uptake by plants inoculated with plant-growth-promoting bacteria has been attributed to the production of plant-growth regulators at the root interface, which stimulated root development and resulted in better absorption of water and nutrients from the soil (Kloepper et al. 1991). Auxin was detected in the bacterial suspension (Sheng and Huang 2001). This release (auxin) was reflected in the greater intake of N and P due to the release of auxin by *B. edaphicus*; as successful plant-growth-promoting inoculants, bacteria must be able to rapidly colonize the root system during the growing season (Defreitas and Germida 1992). Present results also showed that *F. aurantia* was able to colonize the rhizosphere soil and roots of FCV tobacco. According to Subhashini and Padmaja (2010) competitive and effective bacterial strains must be screened and isolated from the pool of indigenous soil bacteria, which supposedly are adapted to the particular conditions of the site. The results showed that the survival of strain *F. aurantia* (from the rhizosphere soil of tobacco) was much better in rhizosphere soil of K_{50} + FA than in that of K_0 + FA. These results demonstrate that applied soluble K influences root colonization activity of introduced strains.

Conclusion

From the results it is concluded that effective plant-growth-promoting bacterium–plant systems must be tested and established in different agroclimatic zones growing tobacco with consideration of the specific ecological site conditions of practical applications (soil type, plant type). However, the extent of stimulation of plants by the tested bacterial strains and the persistence of plant-growth-promoting activity under actual field conditions

remains unclear. Thus, experiments concerning stimulation of tobacco must be followed by investigations under field conditions in different agroclimatic zones growing tobacco. The K-releasing bacteria used here may be exploited in the amelioration of K-deficient soils to enhance the K uptake efficiency of the plant in K-sufficient soils, and further research could lead to an alternative means of K nutrition improvement for use in agriculture.

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