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Effects of light on phytochemical contents of *Brassica rapa* under controlled environments

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ABSTRACT

Plant growth is predominantly influenced by light, and light intensity is manipulated in indoor farming to allow for mass production. The light-emitting diode (LED) is the most practical artificial light, capable of improving crop quality and making indoor agricultural systems more sustainable. Despite their consistent growth under artificial light, little is known about how light intensities and spectrums affect secondary metabolites in commonly grown *Brassica* species. This study aimed to compare the metabolite profiles of *Brassica rapa* (Chinese cabbage) grown under natural light to those grown under different artificial light intensities and spectrums using gas chromatography-mass spectrometry (GC-MS). Although the biochemical composition of *B. rapa* was comparable under varied light conditions, exposure to magenta and red spectrums produced neophytadiene and myristic acid, respectively, whereas exposure to natural light produced squalene and sulfurous acid. Neophytadiene and myristic acid are antioxidants and flavour enhancers, respectively; whereas squalene and sulphurous acid are involved in odour production and function as disinfectants in plants. As a result, these findings add to our understanding of how light conditions can be controlled to improve the growth and biochemical composition of *B. rapa*, thereby enhancing the palatability of indoor farming.

Keywords: artificial light, *Brassica rapa*, gas chromatography-mass spectrometry (GC-MS), indoor farming, secondary metabolites

INTRODUCTION

According to the United Nations, the world will face significant challenges in meeting the growing demand for food in 2050 (Shamshiri et al., 2018). In addition to environmental degradation and land for urbanization purposes, urban agriculture, such as artificial farming, has emerged. Subterranean crops like potatoes, tapioca, and legumes may be prioritized on the land remaining for agriculture, and these crops can gradually help stabilize the land structure. On the other hand, leafy crops may be produced indoors as an alternative farming approach that uses inputs only as needed for profitable production, reducing resource overlaps (Castle et al., 2016). As a result, artificial farming can produce large quantities of food, especially from plants, to meet rising food demands. Although planting in natural light is still favourable owing to lower production costs, it has numerous drawbacks, including the fact that crops can only be planted horizontally, necessitating more land. Strong light penetration can also cause an increase in water use, while the presence of clouds and weather fluctuation can refract light direction, and cause variability in plant growth.

Artificial light can be used as the primary light source for indoor farming, such as vertical farming, grafted seedling, seed production, and in vitro culture production of medicinal and aromatic plants (Bures et al., 2018). Secondary metabolites, also known as phytochemicals, are products of healthy plants that do not participate in direct plant growth and development compared to primary metabolites. Their absence has a little immediate effect on mortality, but they are important for the long-term survival of producing species (Jain et al., 2019). Their productions were primarily derived from plant defences against herbivory, pathogen attack, and tissue damage, or as a by-product derived from primary metabolites with no significant plant benefits. *Brassica* sp. was selected as a model crop in this study because it has a short cultivating time and is reasonably affordable. Metabolite profiles were examined across different light intensities and spectrums to better understand the effect of light conditions on the accumulation of phytochemicals in *B. rapa*.

MATERIALS AND METHODS

Instruments and Chemicals

The following instruments were used in this study: a Eurosafe timer (Euro, Malaysia), a line quantum sensor (MQ-303, Apogee Instruments, USA), a digital CO_2 meter (HT-2000, Walfront, USA), LED tubes ST8V 16w of 6500k (Osram, Germany), an electronic balance (Mettler Toledo, Malaysia), a freeze dryer (Labconco, USA), a speed vacuum concentrator (Eppendorf Concentrator Plus, Germany), and a GC-MS (Shimadzu GCMS-QP2020 NX, Japan). The main chemicals used in compound extractions were methanol and hexane of HPLC grade (Merck, Germany).

Plant Materials and Growth Conditions

Brassica rapa var. *Chinensis* plants were grown in Kekkilä growth medium (100 g per pot), with seeds obtained from Green World Genetics (GWG) Sdn. Bhd. Seeds were directly sown in pots rather than transferred after germination to avoid plant stress during transfer. After seeding, the growth medium was compressed to prevent seed loss during watering. After two weeks, the plants were watered twice a week with MS medium (Murashige & Skoog, 1962). Plants were grown under natural light (control plants) and artificial light at 75 and 150 $\mu mol\ m^{-2}\ s^{-1}$ light intensities. For *B. rapa* cultivated under different light spectrums, they were grown for four weeks under white artificial light and then exposed to the coloured LED (red, magenta, and blue) for the remaining two weeks. After six weeks, plants were harvested by cutting at the base of the stem. The biomass was then weighed using an electronic balance. The HT-2000 digital CO_2 meter was used to ensure the other environmental variables such as temperature, humidity, and CO_2 were kept constant, and that plant growth was unaffected by these factors.

Preparation of Plant Extracts

After being dried in a freeze-dryer, the plants were ground into a powder. Approximately 1 g of powdered leaves was extracted in 20 ml methanol and sonicated for 30 min at 30°C at 50 – 60 Hz. A total of 4 ml of the filtered solution was collected and concentrated using a speed vacuum concentrator for 1.5 h before being desiccated for 45 min. Following that, hexane was used to redissolve the concentrated sample since it is more volatile in the GC-MS than methanol. After being redissolved in hexane and syringe filtered using a cellulose acetate membrane filter, about 1 µl of the extracts was injected into the GC-MS for compound analysis.

GC-MS Analysis

The volatile compounds in *B. rapa* were detected using a Shimadzu single quadrupole GC-MS fitted with an RTX-5 capillary GC column (Fisher Scientific, USA) measuring 30 m × 0.25 mm × 0.25 µm. Helium was used as a carrier gas at a pressure of 100 kPa. The ion source temperature was set to 250°C, while the interface temperature was set to 290°C. The mass spectrometer detector was set to full scan mode with a scan range of 35 – 500 m/z. The initial temperature was set to 70°C for 6 min, then increased to 250°C at a rate of 10°C/min for 10 min, then to 300°C at a rate of 10°C/min for 10 min. The isolated compounds were identified and quantified using TIC (Total Ion Count). The mass spectra of the identified compounds were compared to the NIST17 Library (Version 2.3).

Statistical Analysis

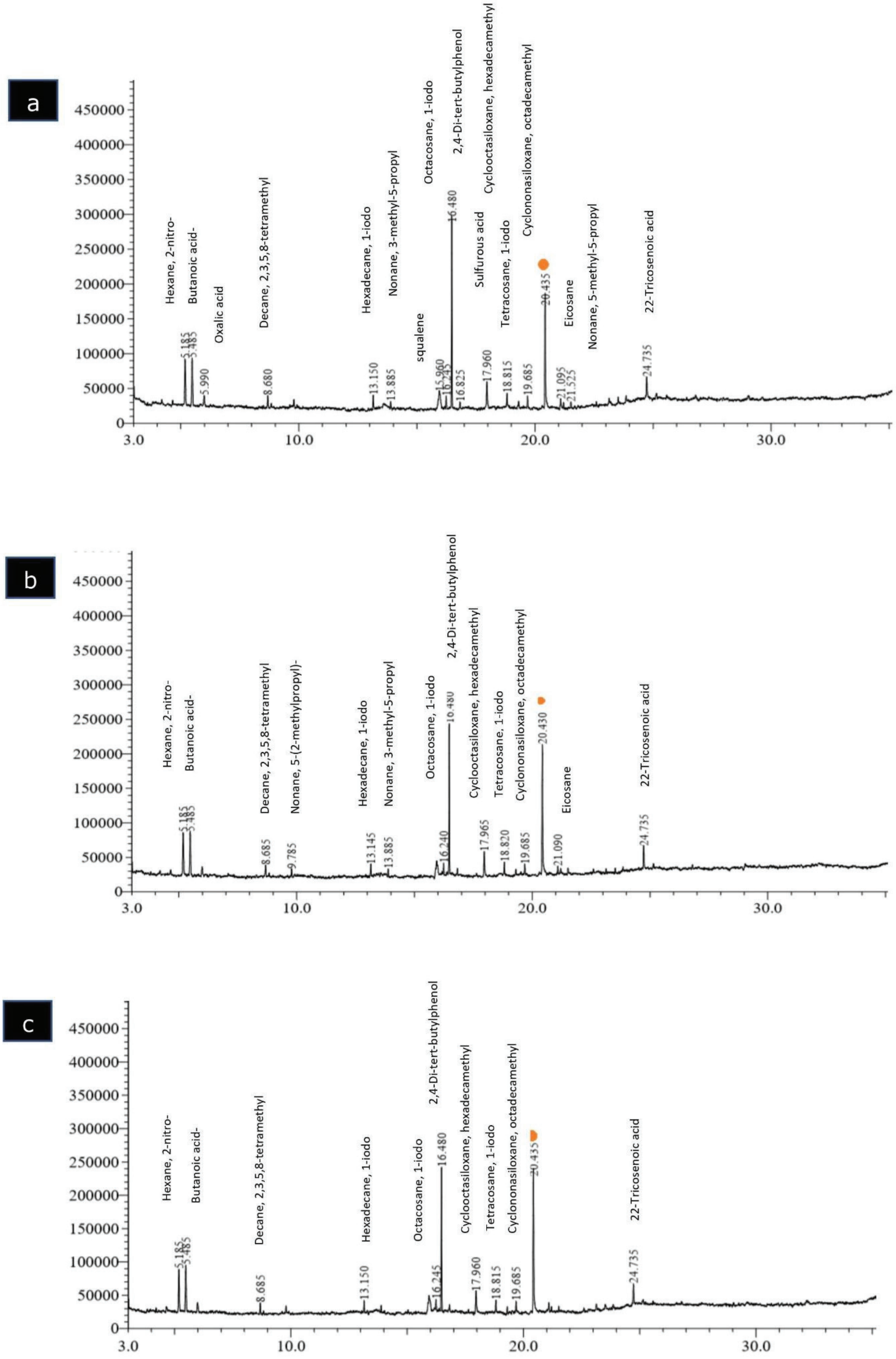
All experiments were carried out in triplicate, and the data were statistically analyzed using R statistical software version 3.3.0 (R Core Team, 2016).

RESULTS AND DISCUSSION

According to the plant growth results under varied light conditions, artificial light produced more consistent biomass than natural light (control). Natural light-grown plants yielded average biomass of 40.31 ± 10.00 g whereas artificial light-grown plants produced a lower but more constant output of 29.76 ± 5.72 g under 150 µmol m^{-2} s^{-1} light intensity and 5.13 ± 1.15 g under 75 µmol m^{-2} s^{-1} light intensity. In terms of light spectrums, blue light produced the highest biomass yields (14.21 ± 3.70 g), followed by magenta (12.59 ± 2.87 g) and red (10.65 ± 2.92 g) lights.

Light intensity and spectrum quality are two of the most critical environmental factors for crop physiology and biochemistry. Even minor changes in light intensity can induce significant changes in leaf morphology and structure, and low light conditions can limit photosynthetic rate, transpiration, and stomatal conductance (Feng et al., 2019). Besides, within the photosynthetically active radiation spectrum of 400 to 700 nm, red and blue lights influence photosynthesis and plant morphogenesis by influencing chlorophyll content, photosynthetic enzyme activity, stomatal opening, and carbohydrate distribution in plants (Dou et al., 2017), but little research has been conducted to investigate the effects of magenta light (a combination of violet and red light).

In this study, the presence of volatile organic compounds (VOCs) in plants treated with various light treatments was determined using GC-MS. Figure 1 depicts the chromatogram of *B. rapa* cultivated under natural and artificial lighting. A total of sixteen compounds were detected in *B. rapa* growing in natural light, thirteen in artificial light at 75 µmol m^{-2} s^{-1}, ten in artificial light at 150 µmol m^{-2} s^{-1}, twelve in the red spectrum, eleven in the magenta spectrum, and thirteen in the blue spectrum. The retention time of compounds, and the light treatments that trigger the production of the compounds, are summarized in Table 1.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Hexane, 2-nitro-
Butanoic acid-
Oxalic acid
Decane, 2,3,5,8-tetramethyl
Hexadecane, 1-iodo
Nonane, 3-methyl-5-propyl
squalene
Octacosane, 1-iodo
2,4-Di-tert-butylphenol
Cyclooctasiloxane, hexadecamethyl
Sulfurous acid
Tetracosane, 1-iodo
Cyclononasiloxane, octadecamethyl
Eicosane
Nonane, 5-methyl-5-propyl
22-Tricosenoic acid
5.185
5.485
5.990
8.680
13.150
13.885
15.960
16.245
16.480
16.825
17.960
18.815
19.685
20.435
21.095
21.525
24.735
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250000
200000
150000
100000
50000
0
3.0
10.0
20.0
30.0
Hexane, 2-nitro-
Butanoic acid-
Decane, 2,3,5,8-tetramethyl
Nonane, 5-(2-methylpropyl)-
Hexadecane, 1-iodo
Nonane, 3-methyl-5-propyl
Octacosane, 1-iodo
2,4-Di-tert-butylphenol
Cyclooctasiloxane, hexadecamethyl
Tetracosane, 1-iodo
Cyclononasiloxane, octadecamethyl
Eicosane
22-Tricosenoic acid
5.185
5.485
8.685
9.785
13.145
13.885
16.240
16.480
17.965
18.820
19.685
20.430
21.090
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Hexane, 2-nitro-
Butanoic acid-
Decane, 2,3,5,8-tetramethyl
Hexadecane, 1-iodo
Octacosane, 1-iodo
2,4-Di-tert-butylphenol
Cyclooctasiloxane, hexadecamethyl
Tetracosane, 1-iodo
Cyclononasiloxane, octadecamethyl
22-Tricosenoic acid
5.185
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18.815
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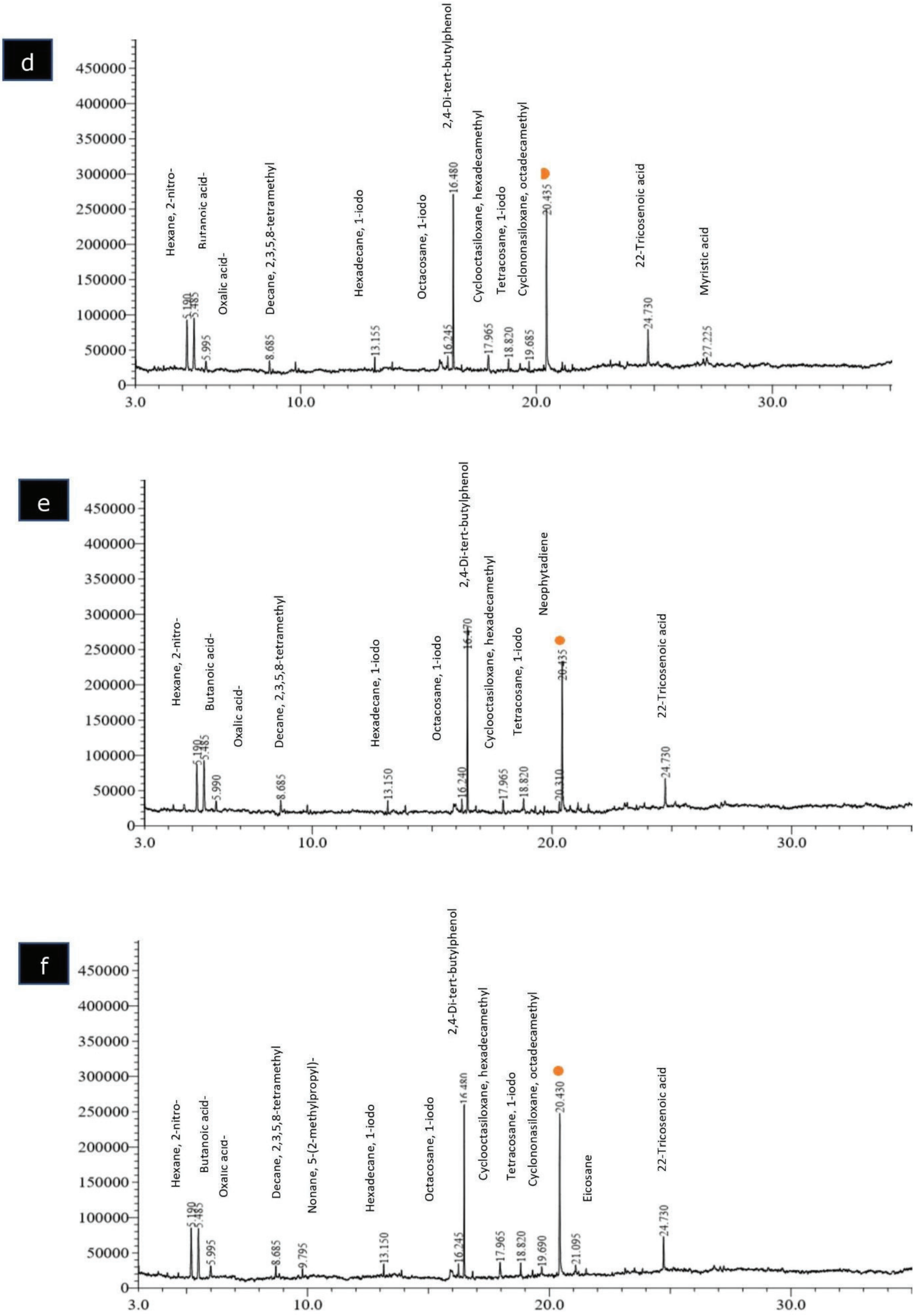

Figure 1 Chromatogram of *B. rapa* grown in (a) natural Light; (b) artificial light at 75 µmol m^{-2} s^{-1}; (c) artificial light at 150 µmol m^{-2} s^{-1}; (d) red spectrum; (e) magenta spectrum; and (f) blue spectrum. The orange dot is a standard that was inserted into the sample to ensure the system was calibrated.

Table 1 List of compounds present in the respective light treatments

No.	RT ± 0.05 min	Compound	Light treatment
1.	5.185	Hexane, 2-nitro	All
2.	5.485	Butanoic acid	All
3.	5.990	Oxalic acid	Natural light, red, magenta and blue spectrum
4.	8.685	Decane, 2,3,5,8-tetramethyl-	All
5.	9.785	Nonane, 5-(2-methylpropyl)-	75 μmol $m^{-2} s^{-1}$ and Blue Spectrum
6.	13.145	Hexadecane, 1-iodo	All
7.	13.885	Nonane, 3-methyl-5-propyl	Natural Light and 75 μmol $m^{-2} s^{-1}$
8.	15.960	Squalene	Natural light
9.	16.240	Octacosane, 1-iodo	All
10.	16.480	2,4-Di-tert-butylphenol	All
11.	16.825	Sulfurous acid	Natural light
12.	17.960	Cyclooctasiloxane, hexadecamethyl	All
13.	18.820	Tetracosane, 1-iodo	All
14.	19.685	Cyclononasiloxane, octadecamethyl	All except magenta spectrum
15.	20.310	Neophytadiene	Magenta spectrum
16.	21.095	Eicosane	Natural light, 75 μmol $m^{-2} s^{-1}$ and blue spectrum
17.	21.525	Nonane, 5-methyl-5-propyl	Natural light
18.	24.735	22-Tricosenoic acid	All
19.	27.225	Myristic acid	Red spectrum

Aside from crop physiology and biochemistry, light has a significant impact on the biosynthesis and accumulation of various secondary plant metabolites that are important for crop quality, such as anthocyanins, carotenoids, and flavonols, which are not essential for life but play a significant role in plant fitness and are found in most fruits and vegetables (Dou et al., 2017). The biosynthesis and accumulation of those secondary metabolites are primarily triggered by light, as plant wavelength-specific photoreceptors are linked to signalling pathways and lead to gene expression changes when activated by photons, subsequently interact with other signal transduction elements, and are responsible for triggering various processes, including the biosynthesis of secondary metabolites (Thoma et al., 2020). The most common compound found in *B. rapa* under all light conditions was 2,4-di-tert-butylphenol, a member of the phenol family. This compound is essential in plants because of its antifungal and antioxidant properties (Varsha et al., 2015). Butanoic acid and 22-tricosenoic acid, both derived from fatty acids, were also detected and are crucial in providing plants with baseline immunity (Kachroo & Kachroo, 2009). Other alkane compounds identified in all samples were dodecane, hexadecane, octacosane, and cyclooctasiloxane.

Plant secondary metabolites have various roles, including cell pigmentation to attract pollinators and seed dispersers and protection against abiotic and biotic stressors, and they are produced in response to environmental stimuli or as defence mechanisms against invading pathogens (Isah, 2019). Due to their antioxidant activity, the metabolites have a variety of health benefits in humans, and many of them include antimicrobial, anti-inflammatory, and anti-allergic properties, allowing them to prevent various diseases (Thoma et al., 2020). Neophytadiene, a terpene-derived compound, was only produced when *B. rapa* was grown in the magenta spectrum. It is predominantly involved in antioxidant activities in plants. Myristic acid (tetradecanoic acid), was only found in plants cultivated in the red spectrum, and the compound is primarily used as a flavour enhancer (Goff & Klee, 2006). This finding implies that growing plants in the red spectrum may improve the flavour of the *Brassica* family. Squalene was also detected, but only in *B. rapa* grown under natural light. Terpenes are aromatic volatiles that gives plants their fragrance and flavour (Goff & Klee, 2006) and serve as a protection against herbivores and as a response to biotic and abiotic conditions to protect photosynthetic tissues (Tholl, 2015). The presence of squalene in naturally grown *B. rapa* may indicate that the plants have developed a barrier to defend themselves from penetration of high light intensity. In addition to squalene, sulfurous acid was detected exclusively in naturally grown *B. rapa*, and the compound is known for its disinfectant properties (Ramya et al., 2015).

CONCLUSION

According to this study, changing the intensity and spectrum of light affects the production of various compounds in *B. rapa*. Compounds such as 2,4-di-tert-butylphenol, butanoic acid, 22-tricosenoic acid, decane, hexadecane, octacosane, and cyclooctasiloxane were detected under all light conditions. However, neophytadiene and myristic acid were identified only in the magenta and red spectrums. Squalene and sulfurous acid, on the other hand, were found exclusively in naturally grown but not artificially grown *B.rapa* plants. This study revealed that cultivation under artificial light produces similar compounds to cultivation under natural light, except for squalene and sulfurous acid production. In addition, GC-MS is a quick and straightforward analytical technique for detecting compounds in plants, and depending on the compound generated, it may also aid in the discovery of metabolism or biological activity in plants.

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