International Journal of Food Science +Technology

Original article

Effect of UV-B treatment during the growth process on the postharvest quality of mung bean sprouts (*Vigna radiata*)

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(Received 22 January 2018; Accepted in revised form 4 April 2018)

Summary The production patterns and nutritious qualities of mung bean sprouts are in line with the consumption needs of modern people. This study was conducted to explore the effects of low doses of UV-B irradiation on the 3-day growth period of mung bean sprouts. The results showed that, when mung bean sprouts were irradiated with UV-B (0.738 kJ m^{-2}) for 1 h per day, their total phenolic content increased by 22.80%, and the activity of polyphenol oxidase decreased by 45.45%. Hypocotyl epidermis stomata were closed, reducing water loss and the opportunity for microbes to invade plants, leading to the maintenance of freshness of the sprouts. In conclusion, UV-B irradiation for 1 h per day during the growth of mung bean sprouts can effectively improve their preservation. This method is a low-cost, environmental friendly and pollution-free physical preservation technique, and it has good application prospects.

Keywords Mung bean sprouts, polyphenol oxidase, preservation, stomata, total phenolic content, UV-B radiation.

Introduction

Sprouts are an edible bud seedling grown under darkness or illumination by crop seeds or vegetative organs (Zhang & Wang, 1998; Lin & Lai, 2006; Wongsiri *et al*., 2015). Sprouts possess special characteristics, including soft texture and high vitamin content (Guo *et al*., 2012); however, because of the high water content and presence of a thin cuticle, they are prone to decay. Therefore, the period for which mung beans remain fresh is short-lived (Goyal & Siddiqui, 2014). Although chemical treatment can prevent sprouts from degradation and prolong its shelf life, it is not conducive to maintaining human health and the environment. Therefore, it is particularly important to study and develop preservation methods without the use for chemical reagent treatment. Previous studies have shown that UV-B radiation can induce plants to produce secondary metabolites, such as polyphenols (Luthria *et al*., 2006; Peng & Zhou, 2009; Kaspar *et al*., 2010; Jiang *et al*., 2015; Wang *et al*., 2017), as well as inducing morphological changes within plants to reduce water loss (Peng & Zhou, 2009). Therefore, the idea of using UV-B irradiation on sprouts was put forward. On the one hand, UV-B has been shown to inhibit the growth of microorganisms and prevent spoilage by inducing the production of the secondary metabolites of polyphenols (Lai *et al*., 2016). On the other hand, it can reduce the loss of water and keep the sprouts fresh and tender by inducing morphological changes within the plants (Namli *et al*., 2014). The goal of research in this field is to create a pollution-free, health-friendly physical preservation method. The purpose of this experiment in particular was to evaluate the effect of UV-B irradiation on the quality preservation of mung bean sprouts and to explore the underlying mechanism of UV-B radiation.

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Materials and methods

Experimental design

The mung bean seeds used in this study were washed and soaked in the dark for 4 h at 30 °C. The soaked

doi:10.1111/ijfs.13804

mung beans were then transferred into a sprouter (OUWON/DY801B, Foshan, China) to allow germination to occur. The room temperature was adjusted to 20 °C and was maintained at that temperature throughout the experiment. The darkness conditions were also maintained during germination, and the growth of sprouts was recorded from the time of soaking. The daily UV-B irradiation time (dose) was C: 0 h (0 kJ m^{-2}; control group), T1: 0.5 h (0.369 kJ m^{-2}), T2: 1 h (0.738 kJ m^{-2}), T3: 1.5 h (1.107 kJ m^{-2}), T4: 2 h (1.476 kJ m^{-2}), T5: 2.5 h (1.845 kJ m^{-2}), T6: 3 h (2.214 kJ m^{-2}) and T7: 3.5 h (2.583 kJ m^{-2}), respectively.

The mung bean sprouts were collected after 3 days and immediately placed in a polyethylene box (approximately 250 mL in volume), and stored in a freezer at a temperature of 6 ± 1 °C and relative humidity of approximately 95%. After 120 h of storage time, various indexes (weight loss rate, vitamin C content, total phenolic content, browning index, polyphenol oxidase (PPO) enzyme activity, microbial analysis and observation of epidermal structure) were measured.

Determination of weight loss rate

The determination of the weight loss rate was based on the method described by DeEll (DeEll *et al*., 2000), and was calculated according to the following formula: water loss rate = (the quality before preservation − the quality after preservation)/the quality before preservation × 100%.

Determination of vitamin C content

The vitamin C content was quantified using the molybdenum blue colorimetric method, described by Yang *et al*. (2015), with slight modifications. The mung bean sprouts of 0.5 g were weighed precisely, 4 mL oxalate-ethylene diamine tetraacetic acid (EDTA) solutions was added and the sprouts were ground in a cold mortar under dark conditions. The mixture was transferred to tubes and centrifuged at 10 000 ***g*** for 15 min at 4 °C. Supernatant is crude extract. Two milliliter of the supernatant, which is the crude extract, was transferred to a 25 mL flask and mixed with 0.5 mL of 3% (w/v) metaphosphoric acid in 10% (v/v) acetic acid solutions, 1 mL of 5% (v/v) sulphuric acid, and 2 mL of 5% (w/v) ammonium molybdate, diluted with distilled water to volume. The mixture was incubated for 15 min at 30 °C and the absorbance (A) was measured at 760 nm. L-Ascorbic acid (0.4 g L^{-1}) was used as a standard for measurement. Vitamin C value was expressed in terms of mg ascorbic acid equivalents per 100 g in fresh weight (mgAAE/100 g FW). Data were reported as mean ± SD (n = 3).

Determination of total phenolic content

Total phenolic content was determined using the Folin-Ciocalteu method (Singleton *et al*., 1999) with minor modifications. Mung bean sprouts were baked in the oven at 60 °C for 24 hours, then ground into powder with a mortar and passed 60 mesh. Following this, weighed 0.25 g dry powder of mung bean sprouts into centrifuge tubes, added 5 mL 50% ethanol and then placed in a constant temperature water bath shaker (30 °C, 150 r.p.m.) to extract for 1.5 h in a dark place. The absorbance was measured at 766 nm, and 0.4 g L^{-1} gallic acid was used as the standard. The total phenolic content was expressed as the amount of gallic acid equivalents in the dry weight of every 100 g sample. Data were reported as mean ± SD (n = 3).

Determination of browning index

The determination of the browning index was measured according to the method described by Chen *et al*. (2006), with slight modifications. After 5 days of storage, the mung bean sprouts were placed in dark conditions for 5 min. Before the surface water evaporation was complete, cut out the middle part of the hypocotyl, took 2 g, ground it and added distilled water at a ratio of 1:6. Following this, centrifuged the solution at a speed of 6000 ***g*** for 15 min at 4 °C and then diluted the sample (10 times). The absorbance of the supernatant was determined at a wavelength of 420 nm. The browning index (10 × OD_{420} as browning index) was converted in the calculation using distilled water as the control.

Determination of the PPO enzyme activity

Polyphenol oxidase activity was assayed adhering to the method described by Sukhonthara *et al*. (2016), with slight modifications. Weighed 2 g sample, added 5 mL precooled sodium phosphate buffer (pH 7.0, 10.0 g L^{-1} PVP, 5.0 g L^{-1} Triton x-100) to homogenate on the ice. Then it was centrifuged for 30 min under the condition of 4 °C, and the supernatant was crude enzyme extract. PPO activity was determined by measuring the absorbance value at 420 nm. One unit of enzyme activity was defined as the amount of enzyme responsible for a change of 1 absorbance unit at 420 nm h^{-1} at 25 °C and pH 7.0.

Microbiological analysis

The microorganism content was identified according to the method described by Goyal & Siddiqui (2014), with slight modifications. Take 1 g of mung bean sprouts stored for 5 days, and soak it in 99 mL sterile water for

1 h under dark conditions at normal temperature, the original liquid was obtained. Subsequently, the original liquid was diluted ten times using aseptic water, and 0.1 mL was absorbed on the PDA medium and smeared evenly, before being cultivated at 30 °C in darkness. Following culturing, the microbial colonies on the plate were counted and expressed in lg CFU mL^{-1}.

SEM observation of hypocotyl epidermal structure of mung bean sprouts

The observation using scanning electron microscopy (SEM) was conducted according to the method described by Wang *et al.* (2015). A double-sided blade was used to cut the middle section of the hypocotyl of the treated mung bean sprouts. The cut length was approximately 5 nm, and it was cleaned three times using sodium phosphate buffer solution with 1–2 min in between each clean. The treated mung beans were then fixed with 2.5% glutaraldehyde for 24 h at 4 °C. Subsequently, ethanol gradient dehydration (30%, 50%, 70%, 80%, 90%, 95%, 100%, 100%) was conducted, and each level was dehydrated for 15 or 20 min. Following this, different concentrations of tert-butyl alcohol (70%, 80%, 90%, 100%, 100%) were added for 15–20 min for each level. After that, they were dried for 1–2 h using an Alpha 1–2 LDplus freeze dryer. The treated mung beans then underwent SBC-12 type ion sputtering, before being observed using a scanning electron microscope (TM3030Plus, Hitachi High-Technologies Corporation, Hitachinaka-shi, Ibaraki-ken, Japan) set to 15 kV.

Statistical analysis

Statistical analyses were conducted using SPSS software 14.0 (SPSS Inc., Chicago, IL, USA), and the results were analysed by ANOVA. Differences between the means were calculated using Tukey's multiple comparison test. Pearson correlation analysis was used to determine whether there was any correlation among variables. Significant differences were determined at $P < 0.05$, and all data were expressed as mean ± standard deviation and each treatment was set up for three replications ($n = 3$).

Results and analysis

Effect of UV-B irradiation on the water loss rate of mung bean sprouts

It was shown in Table 1 that the water loss rate of the mung bean sprout in control group was 5.44%. In the T2 group, the water loss rate was 2.72%, which reflects a decrease of 49.92% compared to that of the control group. UV-B irradiation in the T2 group could significantly improve the water conservation and preservation effects of mung bean sprouts. However, excessive UV-B irradiation would lead to an increase in water loss rate. In the T7 group, the rate of water loss was 8.00%, which was an increase of 47.12% compared to that of the control group.

Effect of UV-B irradiation on the browning index of mung bean sprouts

The browning index of mung bean sprouts in the control group was 3.9 (Fig. 2). A low dose of UV-B irradiation resulted in a decrease in browning index, meaning that the browning of mung bean sprouts was inhibited with respect to the control group. In the T2 group, the browning index of mung bean sprouts was 2.8, which was 29.60% lower than that of the control group. However, excessive irradiation of UV-B was known to cause cell damage and induce further browning of mung bean sprouts. In the T7 group, the browning index of mung bean sprouts was 4.52, which showed an increase of 14.13% compared to that of the control group.

Effect of UV-B irradiation on the PPO enzyme activity in mung bean sprouts

In the control group, the activity of PPO in mung bean sprouts was 5.3 $h^{-1} \cdot g^{-1}$ FW (Fig. 3). UV-B irradiation led to a significant change ($P < 0.05$) in PPO activity in the control group. Under these experimental conditions, the change in PPO activity showed a trend of decreasing firstly and then increasing; the lowest recorded value being 2.9 U h^{-1} g^{-1} FW in the T2 group. This reflects a 45.5% decrease compared to the results recorded at the control group.

Table 1 The effect of UV-B radiation in mung bean sprouts

Treatment	Driage (%)	Vitamin C content (mg ascorbic acid equivalents/ 100 g FW)	Total phenolic content (mg GAE/100 g DW)
C	5.44c	11.83c	546.51c
T1	4.09d	13.83b	604.98b
T2	2.72e	15.33a	687.17a
T3	4.18d	15.23a	571.76c
T4	5.38c	12.10c	439.48d
T5	6.58b	9.57d	380.65e
T6	7.15ab	7.47e	366.66e
T7	8.00a	6.10f	271.58f

GAE: gallic acid equivalent.
Different letters within the same column indicate significant difference at $P < 0.05$. Values are mean ± SD ($n = 3$).

Effect of UV-B irradiation on total number of colonies in mung bean sprouts

The changes to the total number of colonies in mung bean sprouts following UV-B preservation treatment are presented in Table 1. The total number of colonies in mung bean sprouts was 5.014 ± 0.025 lg CFU mL^{-1} (Fig. 4) in the control group. UV-B irradiation caused a significant change ($P < 0.05$) in the total number of colonies at all recorded times, when compared to the control group. The total number of colonies was lowest at 4.472 ± 0.080 lg CFU mL^{-1}, recorded at the T2 group, reflecting a decrease of 10.80% compared to that recorded at the control group.

Effect of UV-B irradiation on the content of vitamin C

It is shown in Table 1 that the vitamin C content in mung bean sprouts was 11.83 mg/100 g FW, recorded at the control group. UV-B irradiation led to a significant change ($P < 0.05$) in vitamin C content at all recorded times, when compared to the control group. Under these experimental conditions, the accumulation of vitamin C showed an initial rise, before declining. The highest value was 15.33 mg/100 g FW, recorded at the T2 group, reflecting an increase of 29.58% compared to that recorded at the control group.

Effect of UV-B irradiation on total polyphenol content in mung bean sprouts

The total polyphenol content in mung bean sprouts was 546.51 mg/100 g DW, recorded at the control group. UV-B irradiation led to a significant change ($P < 0.05$) in the total polyphenol content at recorded times, when compared to the control group (Table 1). Under these experimental conditions, the accumulation of polyphenols over the course of irradiation showed an initial rise, before declining. The highest value was 687.17 mg/100 g DW, recorded at the T2 group reflecting an increase of 25.74% compared to that in the control group.

Effect of UV-B irradiation on stomata of the mung bean hypocotyl epidermis

The main edible part of mung bean sprouts is the hypocotyl axis. UV-B irradiation induces stomatal depression and closure on the hypocotyl epidermis. As shown in Fig. 1a, the hypocotyl surface of the control group is uneven, and the degree of water loss and shrinkage of the epidermal cells is quite serious, and the stomata were open. The surface of the mung bean sprouts' hypocotyl was smooth in the T2 group, the shrinkage of epidermal cells was improved, and stomata were closed (Fig. 1b).

Discussion and conclusion

UV-B radiation, a type of oxidative stress, affects plants in many ways, including their morphological structure, physiology, biochemistry and genetic characteristics, as well as the growth cycle. In general, it is considered that UV-B is largely detrimental to plant growth by reducing biomass, inhibiting photosynthetic processes and damaging organelles. However, UV-B stress can increase the content of secondary metabolites and prolong the shelf life of fruits and vegetables. In this study, it was found that UV-B could prolong

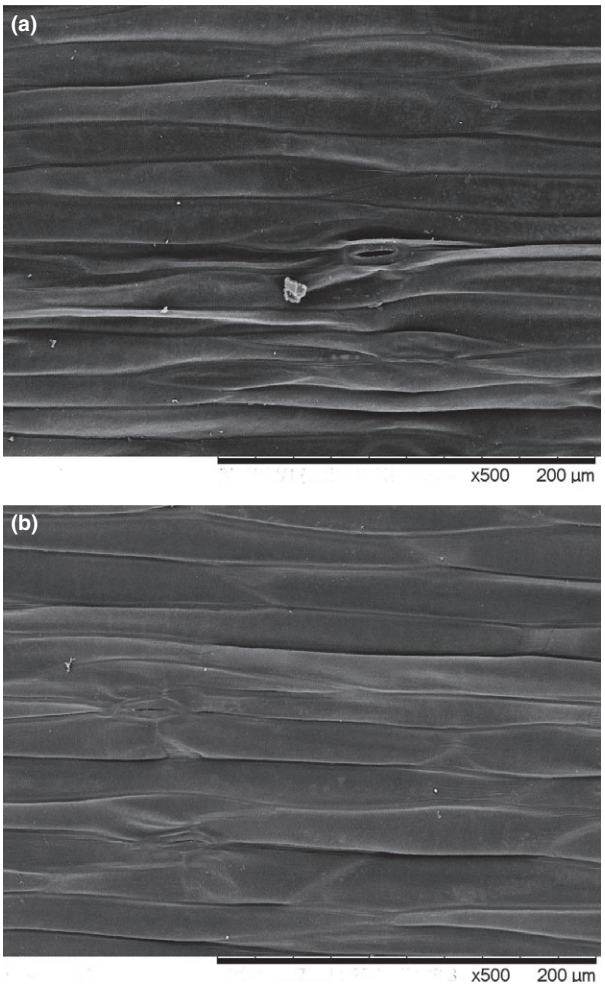

Figure 1 Effect of UV-B pretreatment on morphological changes in mung bean sprouts observed with SEM. (a) In the control group, the mung bean sprouts' hypocotyl surface is uneven and stomata were open. (b) In the T2 group, the hypocotyl surface was smooth and stomata were closed.

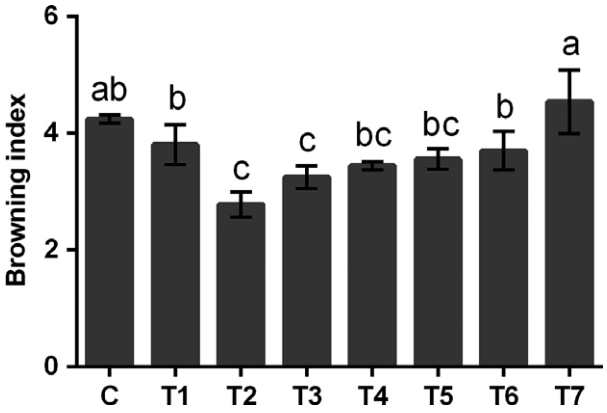

Figure 2 Effect of different dose of UV-B stress on the browning index of mung bean sprouts. C indicates that the daily UV-B irradiation time is 0 h; T1-T7 indicates that the daily UV-B irradiation time is 0.5, 1, 1.5, 2, 2.5, 3 and 3.5 h, respectively. Each value represents the mean of three replicates, and error bars indicate standard deviations (±SD). Different letters denote significant differences ($P < 0.05$).

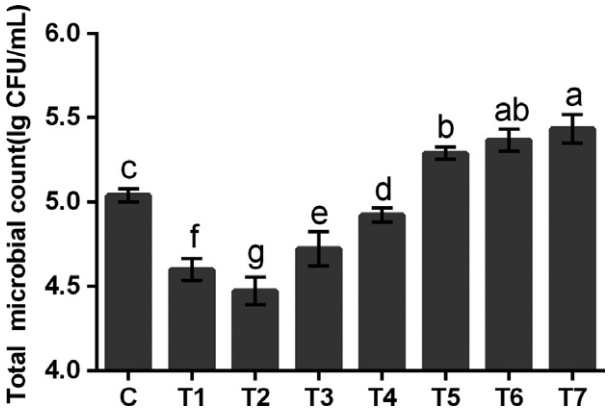

Figure 4 Effect of different dose of UV-B stress on the total microbial count of mung bean sprouts. C indicates that the daily UV-B irradiation time is 0 h; T1-T7 indicates that the daily UV-B irradiation time is 0.5 1, 1.5, 2, 2.5, 3 and 3.5 h, respectively. Each value represents the mean of three replicates, and error bars indicate standard deviations (±SD). Different letters denote significant differences ($P < 0.05$).

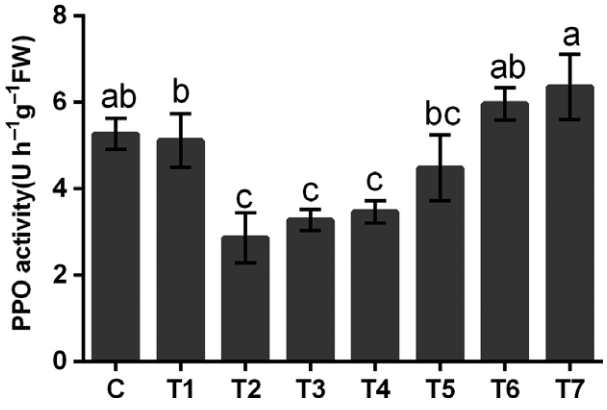

Figure 3 Changes of the PPO activity in mung bean sprouts under different dose of UV-B stress. C indicates that the daily UV-B irradiation time is 0 h; T1-T7 indicates that the daily UV-B irradiation time is 0.5, 1, 1.5, 2, 2.5, 3 and 3.5 h, respectively. Each value represents the mean of three replicates, and error bars indicate standard deviations (±SD). Different letters denote significant differences ($P < 0.05$).

the shelf life of mung bean sprouts by delaying the decrease of secondary metabolites, inhibiting the increase of microbes and delaying the quality degradation.

Water is an important factor affecting the tenderness, freshness and taste of fruits and vegetables. Mung bean sprouts have high water content, but they are not replenished after harvest. It is easy to evaporate and lose water during storage and transportation, causing wilting. In this experiment, an increase in UV-B dose caused the water loss rate to firstly decrease, before increasing towards the end of the experiment (Table 1). This indicates that an appropriate dose of UV-B pretreatment can prolong the storage period by reducing the water loss rate of mung bean sprouts.

In response to UV-B stress, plants produce some secondary metabolites. Many of these secondary metabolites are beneficial to the human body, such as ascorbic acid, phenols and flavonoids. Previous studies have shown that appropriate UV-B stress can improve the nutritional quality of mung bean sprouts and promote the accumulation of antioxidants. In this experiment, as the UV-B dose was increased, the levels of ascorbic acid and phenols demonstrated a similar trend, that is, the levels first increased and then decreased. The highest and lowest values were observed in the T2 and T7 group, respectively (Table 1). This may be because low dose UV-B pretreatment activated the *in vitro* defense response of mung bean sprouts through hormesis to delay the decrease in antioxidant content. Conversely, following high-dose UV-B pretreatment, a more significant, irreversible damage occurred, thus, inhibiting the synthesis of antioxidants.

Browning is considered to be an important factor that limits the quality of fresh fruits and vegetables during storage (Sikora & Swieca, 2018). Previous studies have shown that browning of fruits and vegetables is mainly caused by the formation of quinones by enzymatic oxidation of endogenous phenolic compounds in fruits and vegetables through the enzyme PPO (Xiao & Shangguan, 2007). In this experiment, the browning index and PPO enzyme activity showed

a similar trend (from a decline to an increase), with both their lowest values recorded at the T2 group (Figs 2 and 3). Browning includes enzymatic browning and nonenzymatic browning. Nonenzymatic browning also includes Maillard reaction, ascorbic acid browning and the like. Although enzymatic browning is the predominant cause in most cases, nonenzymatic browning can also have some effect on the outcome in some cases. Anyway, the experimental results showed that UV-B pretreatment can reduce the browning index of mung bean sprouts during storage by inhibiting the activity of PPO, thus, prolonging the preservation period.

Microbial activity is an important cause of decay and deterioration of fruits and vegetables during their storage period (Swieca & Gawlik-Dziki, 2015; Lutza *et al*., 2017). Reducing the number of surface microorganisms of fruits and vegetables can prolong the time, for which they are considered fresh. In this experiment, the total number of colonies was at the lowest in the T2 group (Fig. 4). This indicates that UV-B treatment of mung bean sprouts can prolong the storage life by inhibiting the growth of microbes on the surface. UV-B treatment works by inhibiting and killing microbes, and also by inducing an increase to total polyphenol content. This is significant as polyphenols are thought to inhibit microbial activity (Baydar *et al*., 2006; Rodriguez-Vaquero *et al*., 2007). Therefore, UV-B treatment could inhibit the growth of microbes on the surface of mung bean sprouts, prevent the corruption of mung bean sprouts, and prolong their freshness period. However, with the enhancement of UV-B radiation in T3 to T7 groups (Fig. 4), the microbial content increased again. This is because high levels of UV-B radiation can not only inhibit the growth and reproduction of microorganism, but also damage plant, and consequently reduced the ability of plant to resist microorganisms.

Changes in the epidermal microstructure of fruits and vegetables during the storage period will affect their storage, transportability, shelf life, ability to resist microbial intrusion and even consumer preferences. The results of scanning electron microscopy showed that, in the UV-B treatment group, the hypocotyl epidermis of mung bean sprouts was smooth and compact, and the stomata were closed. It is worth noting that the stoma acts as the microbial entry point into the plant (Henrik *et al*., 2014). Therefore, stomatal closure not only reduces the evaporation of water but also reduces the chance of microbes invading plants, thereby preventing bean sprout rot caused by microbial invasion.

In conclusion, UV-B irradiation for 1 h (0.739 kJ m^{-2}) per day during the growth process of mung bean sprouts can effectively improve their preservation effect. It is a low-cost and environment-friendly physical preservation technique, which has potential implications for the future.

Acknowledgments

This work was financially supported by the National Science Foundation of China (No. 31270225 and 31370219), Henan Province Production and Research Cooperation Project (182107000028) and Key Research Project Foundation of the Higher Education Institutions (No. 16A180045).

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