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Growth Response and Root Characteristics of Lettuce Grown in Aeroponics, Hydroponics and Substrate Culture

Qiansheng Li[1], Xiaoqiang Li[2], Bin Tang[3], Mengmeng Gu[1,*]

[1] Department of Horticultural Sciences, Texas A&M AgriLife Extension Service, College Station, TX 77843, USA

[2] Shanghai Jieyou Agriculture Sci & Tech Co., Ltd., Shanghai, 201210, China

[3] Spraying Systems (Shanghai) Co., Shanghai, 201611, China

[*] Correspondence: mgu@tamu.edu Tel.: +1-979-845-8567

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Abstract: Aeroponics is a relatively new soilless culture technology, which may produce food in space limited cities or non-arable land with high water use efficiency. The shoot and root growth, root characteristics, mineral contents of two lettuce cultivars were measured in aeroponics, as compared with hydroponics and substrate culture. The results showed that aeroponics remarkably improved the root growth with a significant greater root biomass, root/shoot ratio, and several times higher total root length, root area and root volume. However, the greater root growth did not lead to a better shoot growth compared with hydroponics, due to the limited availability of nutrients and water. It can be concluded that aeroponics systems may be better for high value true root crops production. Further research is necessary to figure out the suitable pressure, droplet size, and misting interval in order to improve the continuously availability of nutrients and water in aeroponics, if it is used to grow crops like lettuce for harvesting above-ground parts.

Keywords: aeroponics; soilless culture; root growth; root/shoot ratio

1 Introduction

Soilless culture, including aeroponics, aquaponics and hydroponics, is considered as one of the innovative agricultural strategies to produce more from less in order to feed 11 billion people by 2100[1]. Soilless culture can be divided into categories with and without a root supporting media, and static aerated or flowing nutrient solution with or without its reuse. Growing plants without soil has a long history, but it was only widely used from 1850 to the mid-1900s in the study of essential elements required by plants. Hydroponics is a relatively new term introduced by W.F. Gericke the 1930s, publicly known as water culture[2]. Aeroponics is a promising technique to grow plants with their root systems exposed constantly to a nutrient mist in a closed chamber[3]. Plants grow well in aeroponics, primarily because of the highly aerobic environment it creates for plants[4]. It is even viable to control root-zone atmosphere when a gas delivery system is combined[5]. Integrated vertical aeroponic farming system with manipulation of root-zone environments such as temperature and CO_2 can be achieved from more efficient use of land area to secure vegetable supply in

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space limited cities[6]. Aeroponics is also an excellent option for space mission life support systems which requires optimum controllable growth parameters[7].

Aeroponics was widely used in plant physiology research[8, 9], but not as commonly used as other hydroponic methods on a commercial scale[8]. However, aeroponics was tried in the growing of numerous vegetable crops such as lettuce, cucumbers, melons, and tomatoes[8], herbs, potatoes and flower crops[9]. Commercial aeroponics was especially adopted in those crops which roots were harvested as the end product. Seed potato production may be the most successful application of aeroponics in commercial scale mostly in China, Korea, South America, and even African countries in recent years [10-13]. Aeroponics is able to produce large numbers of minitubers in one generation and harvest sequentially, thus, eliminating the need for more field multiplications thereby reducing costs and saving time[10]. This technique was also applied to effectively produce minitubers of yam (*Dioscorea rotundata* and *D. alata*) in Nigeria and Ghana[14, 15]. Aeroponics could be an alternative production system for other high-value root and rhizome crops, such as great burdock (*Arctium lappa* L., Asteraceae)[16], Ginger (*Zingiber officinale*)[17], medicinal crops like *Urtica dioica, Anemopsis californica*[18], Saffron (*Crocus sativus*)[19]. Essential oil production of herbs like valerian (*Valeriana officinalis*) growing in aeroponics was also reported[20]. It is also reported as an economic method for rapid root induction and clonal propagation of three endangered and medicinally important plants[21]. Aeroponic was proved had the potential to produce tree saplings (*Acacia mangium*) inoculated with AM fungi[22]. Aeroponics had the advantage of root initiation and subsequent root growth and development in woody (*Ficus*) and herbaceous (*Chrysanthemum*) cuttings due to the well aerated root environment[23].

Many results clearly showed that aeroponics promotes growth rate through optimizes root aeration because the plant is totally suspended in air, giving the plant stem and root systems access to 100% of the available oxygen in the air[10]. Droplet size and frequency of exposure of the roots to the nutrient solution are the critical factors which may affect the oxygen availability[3]. Large droplets lead to less oxygen available to the root system, while fine droplets produce excessive root hair without

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developing a lateral root system for sustained growth[13]. Three broad categories generally used to classify droplet forming systems and droplet size, regular spray nozzles with droplet size >100μm (spray), compressed gas atomizers with droplet size between 1 to 100μm (fog), ultrasonic systems with droplet size 1 to 35μm (mist)[24]. The most common type is that the nutrient solution is compressed through nozzles by a high pressure pump, forming a fine mist in the growth chambers[10]. Ultrasonic misting system was adopted in sterile aeroponics culture system for in vitro propagation[25].

In this study, air atomizing nozzles (1/4J Series) were employed for the aeroponics system. The air atomizing nozzles require a single air source for the atomizing air and provide independent control of liquid, atomizing air and fan air pressure for fine tuning of flow rate, droplet size, spray distribution and coverage. This air atomizing nozzles were equipped with clean-out needles to eliminate clogging and ensure optimum performance. The objectives of the present study was to compare the shoot and root growth, root characteristics, mineral contents of two lettuce cultivars grown on this aeroponics system to hydroponics (NFT) and substrate culture system.

2 Materials and methods

2.1 Cultivation systems

The experiment was carried out in a 12.8×24 m experimental Venlo type glasshouse, which was equipped with outside and inside shade net, fans and pad, misting system. The aeroponics units were built in an A-frame shape, 1.4m width, 1.4m height, 6m length; both sides were covered with multiple Styrofoam panels at 60° angles. The planting density was 20×20 cm. Six nozzles (AEROJSUMAX-6SS, Spraying Systems (Shanghai) Co.) were placed horizontally at the ends and middle of the A-frame. The nozzles can be operated under the air pressure from 0.7 to 4 bar, with liquid capacity of 7.6 to 63 L/h. AutoJet® Spray System (Spraying Systems (Shanghai) Co.) was installed to monitor and automatically adjust spray pattern, flow rate, droplet size, liquid pressure and atomizing air pressure. Misting lasted 20s with

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30s interval. The droplet size was adjusted to 50μm. Residual nutrition solution was recycled.

The NFT hydroponics system consists of a PVC trough on a slope of 1 per cent. The trough was 10cm width, 5cm depth, 6m length. The cascade troughs were suspended one above the other up to 7 levels. The nutrient solution entered the high end of the slightly sloping top trough, exited at the low end of that trough into the high end of the next one, and so on, to the reservoir from where it was pumped. The planting distance was 20cm.

Substrate culture system was conducted on the square PVC plastic pots, which is 46cm length, 40cm width, 18cm height. Planting density was 20×20cm, 6 plants were planted in each pot. Mix of 50% peat and 50% perlite was used as substrate. The substrate depth was approximately 18 cm. Nutrient solution was supplied with 3 drippers for each pot.

2.2 Planting and experimental arrangement

Lettuce seeds of cultivar 'Nenglv naiyou' and 'Dasusheng' (Institute of vegetable and flower, CAAS, China) were sown in 72 cells polystyrene trays. Each cell was filled by a hydroponic planting basket with sponge as supporting media. From the two true leaves stage, all the plants were head watering with a half strength Hoagland's nutrient solution[26] until seedlings were ready for transplanting. Four weeks after sowing, lettuce seedlings were transplanted to the aeroponics, hydroponics and substrate culture systems at a spacing of 20×20cm. Plants were supplied with full strength Hoagland's nutrient solution. Three A-shape aeroponics facilities, 12 hydroponics troughs, and 24 substrate culture pot were planted for the comparison experiment.

2.3 Harvesting and measurement

2.3.1 Biomass and root/shoot ratio

Nine plants from each cultivation system were harvested and washed with tap water. Substrates in the roots of plants from substrate cultivation treatment were gently washed off. Fresh weight of shoots and roots were weighed immediately after removing the free surface moisture with soft paper towel. Shoots and roots samples

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were then oven dried at 85 °C for 48 h, and weighed for dry weight on a scale accurate to 0.0001g. Root/shoot ratio was calculated as dry weight of roots/dry weight of top of plant).

2.3.2 Root characteristics

The washed roots were immersed and spread out in a 40×25×10cm square blue plastic container which was filled with tap water in 3 cm depth. The entire root system was photographed from above with a digital camera (Nikon D90, Nikon Corporation, Japan) and saved as jpeg format (Fig. 1 A). The photographs were re-cropped, scaled (Fig. 1 B), and processed with GiA Roots software to obtain a thresholded image (Fig. 1 C) for measuring the characteristics of all roots. The measured root characteristics included average root diameter (width), root length (network length), root area (network surface area), root volume (network volume), maximum number of roots, median number of roots, and network perimeter[27]. Five plants were measured from each treatment.

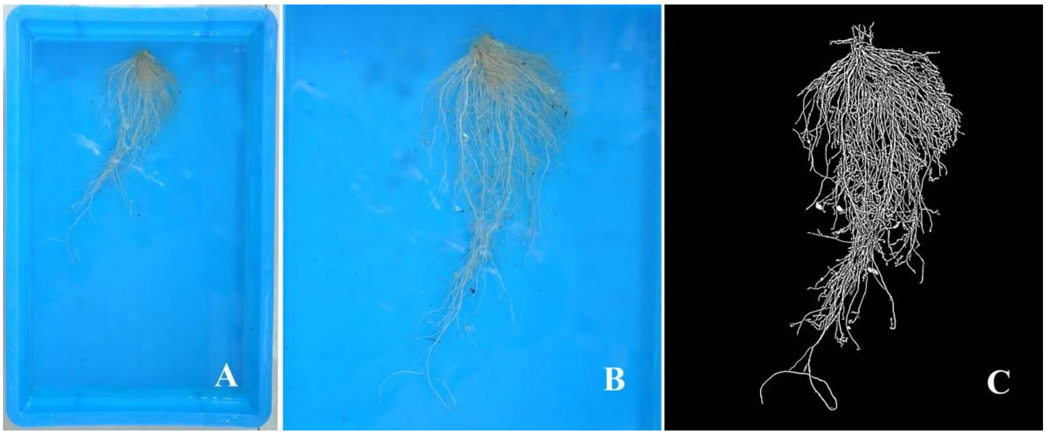

Fig. 1. The entire root was immersed and spread out in a 40×25×10cm square plastic container which filled with tap water in 3 cm depth (A), the image was re-cropped after scaled (B), and changed to thresholded image (C) with GiA Roots for root characteristics measurement.

2.3.3 Plant leaf Nitrogen, phosphorus and potassium content.

Dried above ground parts of lettuce from different treatments were milled and passing through a 1 mm screen. The grounded dry material (~0.2000g) was wet

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digested by H_2SO_4-H_2O_2 solution. Nitrogen was determined by the Kjeldahl method[28]. Phosphorus was determined by ascorbic acid molybdenum blue method method[29]. Potassium was determined by flame emission spectrophotometry[30]. Three replicates of each different treatment were determined.

2.4 Statistical analysis.

All results were subjected to analysis of variance using SPSS Statistics 19.0. Where significant differences occurred, means were separated using Duncan's multiple range tests at P = 0.05.The results are expressed as means±SE.

3 Results

3.1 Plant growth and biomass

The cultivation systems significantly influenced the growth of both lettuce cultivars. Lettuce grown in hydroponics had larger shoot (head), while the aeroponic lettuce had more roots instead of shoots; plants from substrate cultivation had smallest size (Fig. 2).

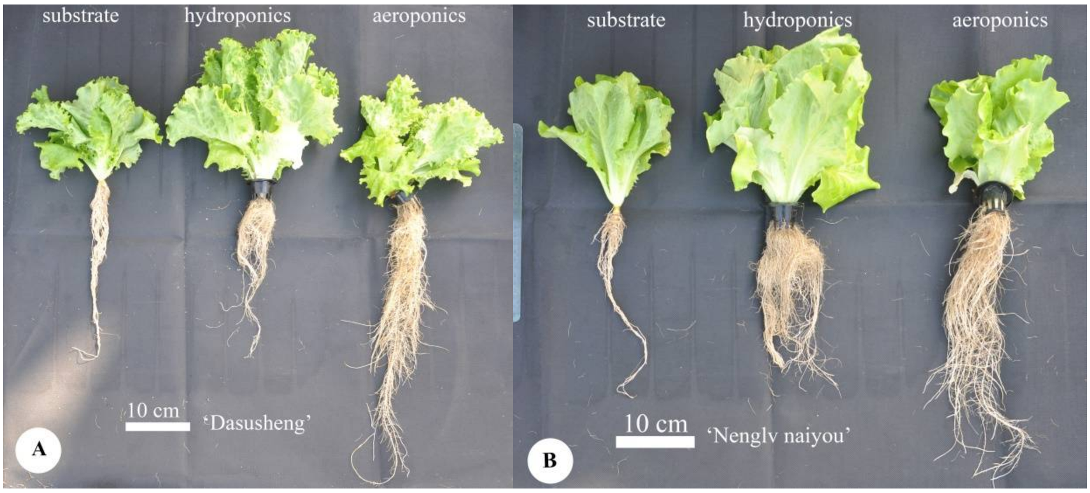

Figure 2. Whole plants of lettuce cultivar 'Dasusheng' and 'Nenglv naiyou' 45 days after transplanting in aeroponics, hydroponics, and substrate cultivation systems.

In both cultivars, the shoot fresh and dry weights of hydroponics lettuce were approximately twice that of aeroponics and substrate cultivated lettuce (Table 1). Root fresh weights of aeroponics and hydroponics lettuce were significantly higher than

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that of substrate cultivated lettuce. And the root dry weights of both cultivars in aeroponics were significant higher than that of hydroponics and substrate cultivation. The most remarkable difference between 3 growing methods was the root/shoot ratio. In both cultivars, the root/shoot ratio of aeroponics lettuce was significant higher than that of the other two growing methods (Table 1).

Table 1 Shoot, root fresh weight (FW), dry weight (DW) and shoot to root ratio of two lettuce cultivars grown on three cultivation systems.

	Shoot FW (g)	Root FW (g)	Shoot DW (g)	Root DW (g)	Root/shoot ratio
		Lactuca sativa 'Dasusheng'			
aeroponics	37.8±2.67b	8.67±1.20a	2.40±0.17b	0.80±0.15a	0.32±0.04a
hydroponics	88.8±9.47a	8.78±1.24a	4.86±0.54a	0.59±0.06b	0.12±0.01c
substrate	49.2±2.34b	6.92±0.43b	3.23±0.13b	0.69±0.03b	0.22±0.02b
		Lactuca sativa 'Nenglv Naiyou'			
aeroponics	50.9±2.60b	10.3±0.46a	2.58±0.11b	0.77±0.07a	0.30±0.03a
hydroponics	96.1±4.23a	11.5±1.07a	4.80±0.16a	0.54±0.03b	0.11±0.01b
substrate	39.4±1.72c	3.9±0.35b	2.03±0.06c	0.26±0.03c	0.13±0.01b

Note: Values are mean±SE (n=9). In the same cultivar, values followed by the same letter are not significantly different ($p \leqslant 0.05$).

3.2 Root characteristics

Further analysis of the root characteristics by GiA Roots software revealed more detail of the influence of growing methods on the root growth. Corresponding to the significant higher root biomass of aeroponics lettuce (Table 1), root length, root area, root volume, and network perimeter of aeroponics lettuce (both cultivars) were significantly greater than that of hydroponics and substrate cultivated lettuce (Table 2). Especially in cultivar 'Dasusheng', the root length, root area, root volume, and perimeter were several times that of the hydroponics and substrate cultivation. However, the average root diameter had no significant difference in cultivar 'Dasusheng'. Average root diameter of hydroponics lettuce 'Nenglv naiyou' was significantly greater than that of substrate cultivated lettuce. Maximum and median numbers of roots of aeroponics lettuce 'Dasusheng' were 2-3 times higher than that of hydroponics and substrate cultivation; but such a great difference were not found in the cultivar 'Nenglv naiyou'.

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Table 2 Root characteristics of lettuce grown in aeroponics, hydroponics, and substrate culture systems.

	Average Root Diameter (mm)	Root Length (cm)	Root Area (cm^2)	Root Volume (cm^3)	Maximum Number of Roots	Median Number of Roots	Network Perimeter (cm)
			Lactuca sativa 'Dasusheng'				
aeroponics	0.501±0.017a	3043±231a	479±42a	7.24±0.77a	75.0±5.8a	39.0±4.1a	6019±473a
hydroponics	0.551±0.025a	581±113b	100±20b	1.64±0.35b	24.6±2.9b	13.8±1.6b	1164±221b
substrate	0.501±0.007a	724±126b	114±21b	1.67±0.35b	32.6±5.3b	17.4±2.2b	1437±245b
			Lactuca sativa 'Nenglv Naiyou'				
aeroponics	0.511±0.0023ab	2634±260a	424±46a	6.63±0.84a	63.0±7.4a	34.4±3.1a	5197±557a
hydroponics	0.554±0.0009a	1688±239b	296±46b	4.91±0.85ab	54.4±3.0a	19.0±4.8b	3379±473b
substrate	0.487±0.0006b	1378±58b	211±10b	2.99±0.15b	51.2±1.6a	37.0±2.6a	2755±113b

Note: Values are mean±SE (n=5). In the same cultivar, values followed by the same letter are not significantly different ($p \leqslant 0.05$).

3.3 Leaf N, P, K contents

In both cultivars, leaf N content of hydroponic lettuce was significantly higher than that of aeroponics and substrate cultivated lettuce; but there was no difference between aeroponics and substrate cultivation (Fig. 3 A). The leaf P content of hydroponics lettuce was significantly higher than that of the aeroponics and substrate cultivated lettuce (Fig. 3 B). Potassium content in both aeroponics and hydroponics lettuce was significantly higher than that of substrate cultivated lettuce, but there was no difference between the aeroponics and hydroponics lettuce (Fig. 4 C).

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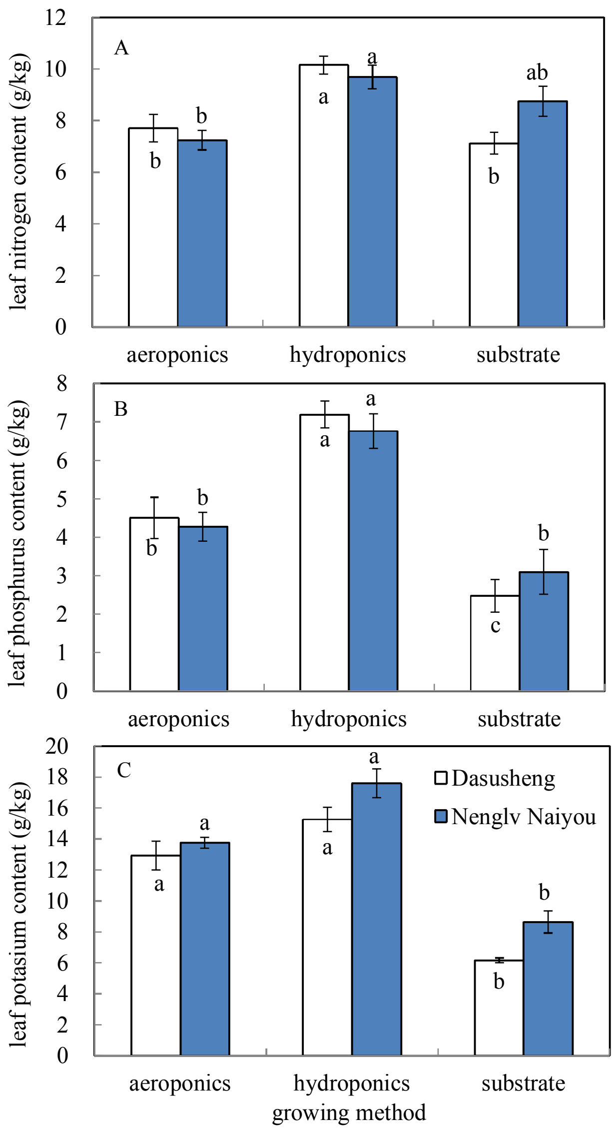

Figure 3. Leaf nitrogen (N), phosphorus (P), and potassium (K) contents of two lettuce cultivars grown on aeroponics, hydroponics, and substrate cultivation systems. Values are mean±SE (n=3). In the same cultivar, values followed by the same letter are not significantly different ($p \leqslant 0.05$).

4 Discussions

The most impressive result of this study was the remarkable improvement of root growth of lettuce in aeroponics system. In both cultivars, the root dry weight was significantly higher than that of the hydroponics and substrate cultivation; and the root/shoot ratio of aeroponics lettuce was 2-3 times that of the other two. The root characteristics, such as greater total root length, root area, and root volume were further proved that aeroponics was beneficial to root growth. However, the greater root system of aeroponics did not lead to more shoot biomass (yield) growth than hydroponics. Instead, the shoot biomass of aeroponics lettuce was significant less than

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that of hydroponics. It may due to the sufficient nutrients and water supply when the root system was submerged in nutrient solution all the time. This may also explain that the leaf N, P, K contents were higher in the hydroponics lettuce (Fig. 3).

In aeroponics, the nutrient solution was only sprayed in fine droplets by interval, which may limit the shoot growth and improve the root growth. In valerian (*Valeriana officinalis* L.) cultivation trials, it was also found that both leaf area and biomass production in the aeroponics systems were lower than in the floating raft hydroponics system and growing media systems; and concluded that it may be caused by the higher proliferation of roots inside the frame reducing the performance of nozzles [20]. The root number of Saffron (*Crocus sativus* L.) plants was also significantly greater in aeroponics than that in hydroponics and soil culture, but no significant difference of shoot growth[19]. The larger distance between misting sprayers and roots restricted root access to the water micro droplets, resulting in decreasing nutrients availability and absorbance. In this case, plants are enforced to compensate by increasing root surface area and weight[31]. Thus, the droplet size and the misting interval will have great effects on the plant growth in aeroponics.

The advantage of root growth in aeroponics due to highly aerated root environment. Aeroponics significantly improved the adventitious roots formation in rapid root induction and clonal propagation of three endangered and medicinally important plants than the soil grown stem cuttings[21]. Aeroponics showed higher yield and better size distribution in potato minituber production, but the growth can be influenced by such factors as the variety, the availability of nutrients, the stretching of the cycle and the culture density [11]. Higher root vitality of plants was observed in aeroponics and aerohydroponics than that of deep water culture[32]. If the roots of plants in aeroponics can always achieve the nutrient and water readily, it will support the better growth of above-ground part.

However, the major disadvantage of aeroponics was the possibility of irreversible damages or complete loss, since there is no substrate at all (neither solid nor water) that would enable the plants to survive in the event of a technical failure or power cut [10, 12]. During our experiment, there was one day mechanical breakdown of

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aeroponics system, which could also affect the later growth.

5. Conclusion

It can be concluded that aeroponics was beneficial to the root growth, supporting with the remarkable results of higher root/shoot ratio, root length, root area, and root volume in aeroponics from this study. So aeroponic systems may be superior for producing high value true root crops, particularly for medicinal plants, as Hayden suggested[16, 18]. When grow root crops in aeroponics, clean products may be harvested sequentially. To grow crops like lettuce for harvesting above-ground parts, further research is necessary to figure out the suitable pressure, droplet size, and misting interval in order to improve the continuously availability of nutrients and water in aeroponics, thus the above-ground parts of plants can also grow better and quicker.

Author Contributions

Conceptualization, Qiansheng Li; Data curation, Qiansheng Li; Formal analysis, Qiansheng Li; Investigation, Xiaoqiang Li; Methodology, Qiansheng Li; Resources, Bing Tang; Supervision, MENGMENG Gu; Writing – original draft, Qiansheng Li; Writing – review & editing, MENGMENG Gu.

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Conflicts of Interest

The authors declare no conflict of interest.

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