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Research Article

Effect of air temperature and relative humidity on the stored Welsh onion (*Allium fistulosum* L.) seeds

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Abstract

In the years 2018-2021, experiments on the effect of air temperature and relative humidity (RH) on the storage of Welsh onion (*Allium fistulosum* L.) seeds were carried out. Three different cultivars and one breeding line received from commercial plant breeding and seed production companies were used. All their seeds at the beginning of storage had high germination over 90%, and moisture content 7-8%. Then, they were stored for 30 months in the controlled conditions at 3 different temparatures: 7-8 °C, 10 °C and 25 °C, each with 2 different air relative humidty (RH): 25% or 45%. During the experiment, their quality was evaluated by germination test according to the International Seed Testing Association (ISTA) protocol. Additionally, after 1 and 2 years of storage at 7-8 °C and 25% air RH, the seeds of the line were evaluated for their incidence of fungi. It was found out that seeds of the tested genotypes kept good germination at 7-8 °C and at 10 °C, i.e. after storing them, the same effect was achieved. Thus, the latter can effectively lower the costs of the seed storage in commercial seed trade of the crop. The electric current consumption for the seeds stored at 7-8 °C in comparison with 10 °C was higher by 11.2%. In seeds of 3 genotypes, after storing them for 30 months, their germination was better at the air RH 45% than at 25%. Generally, low temperature (7-8 °C) favoured growth of *Penicillium* spp. and *Phoma* spp. on Welsh onion seeds during 2 years of storage, while storing at higher temperature (25 °C) resulted in the increase in seed infestation with *Cladosporium* spp. and *Fusarium* spp. The results obtained from this research can be used as a guideline for the storage in climatic and RH controlled chambers of all genotypes belonging to *Allium fistulosum* species, with the aim to maintain its seed germination quality related to the duration of the storage and taking into consideration the operating energy costs.

Keywords: Japanese bunching onion; seed health; seed storage costs; Welsh onion seed storage

Introduction

Welsh onion (*Allium fistulosum* L.) is a vegetable crop popular in the Far East countries. As all other species from the Liliaceae family, its seeds do not have a post harvest dormancy period and easily lose their germination capacity at room temperature, if they are stored for more than 6-12 months (Zhang *et al.*, 2001; Tang *et al.*, 2001; Padula *et al.*, 2022). Poor seed performance after storage has been one of the crucial factors

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that limits Welsh onion commercial production and development. Moreover, results of earlier research proved, that its seeds short lifespan is related to the cells membrane deterioration caused by the oxidation of macromolecules (Dong *et al.*, 2014). Infestation with fungi is additional factor which can significantly reduce quality of onion seeds (Brocklehurst, 1985; Maude, 1996).

Experiments on finding out optimal conditions for longer storage of Welsh onion seeds focused mainly on 2 factors: seed moisture content: from 3.2% to 8.4% and air temperature of the storing room: from -18 °C to 9 °C (Zhang, 2001; Li and Wang, 2005, 2006; Lazarenko *et al.*, 2008; Li *et al.*, 2008; Yanping *et al.*, 2000; Khan *et al.*, 2020). At the same time, much less attention was paid to another important factor when storing the Welsh onion seeds: the relative humidity (RH) of the air during the storage (Padula *et al.*, 2022). Further experiments have also evidenced a connection and high sensitivity between *Allium* spp. seed viability and the level of oxygen in the storing chamber, which in combination with a high percentage of seed moisture, resulted in a rapid loss of seed germination (Hourston *et al.*, 2020).

In the situation of constant seeds overproduction, it is not common for seed companies to keep Welsh onion seeds in stock for longer than 2-2.5 years. The main reason is to avoid high storing costs associated to a difficult stock management (Padula *et al.*, 2022). Kugbei (2000) pointed out a long list of costs related to storing of sowing material. He classified them into 2 categories: permanent ones, not depending on the amount of seeds stored or the period of storage, and variable ones. It was evidenced that out of the latter, most relevant was the expense of electricity (Traill *et al*., 2019). As a result, one of the short-term goals in a seed company management should be lowering it (Hołubowicz, Bralewski, 2004). No information was available in the literature about possible ways of storing Welsh onion seeds at higher than 4°C temperatures with the idea to reach the same goal of their storing at lower cost (Padula *et al.*, 2022).

The fungi from genera *Alternaria*, *Aspergillus*, *Botrytis*, *Cladosporium*, *Epicoccum*, *Fusarium* and *Rhizopus* are commonly associated with *Allium* spp. seeds (Tylkowska and Dorna 1996, 2001). Increase in seed infestation with fungi, especially with saprotrophic *Aspergillus* and *Penicillium* species, is one of the factors responsible for seed deterioration during storage (Maude, 1996). Unfortunately, low temperatures, optimal for seed storage, usually also favours viability of seedborne fungi (Hewett, 1987).

The aims of this research were: 1/ to identify the optimal air temperature and RH for the storage of Welsh onion seeds, 2/ to identify the seeds effective storing conditions in the experiment with the lowest electric current consumption, and 3/ to evaluate the effect of storage at temperature 7-8 °C, and at 25 °C on seed infestation with fungi.

Materials and Methods

Plant material selection

Selecting plant material for the experiments was based on 2 factors: its breeding background and place of reproduction. The first parameter included different genotypes identified on the seed market as cultivars or breeding lines. They were received from plant breeding and seed production companies. The second one was based on seed production location. All the seeds used in the experiments were collected from locations considered in the seed world as optimal for Welsh onion seed multiplication (Hołubowicz, 2016). Table 1 shows their main characteristics.

Table 1. Characteristic of Welsh onion plant material used in the experiments

Commercial use	Breeding status	Seed lot no.	Production place
Negi group	F_1* cultivar	270322	Chile
Negi group	Inbred line	17TSITGH03	Italy
Salad onion	OP cultivar	1240694	South Africa
Salad onion	F_1 cultivar	170403214	South Africa

*(F1- hybrid, OP- open pollinated)

Description of the selected genotypes

The genotype 270322 is a hybrid (F_1) cultivar developed by a Japanese seed company. Its seeds were produced in Chile. It belongs to the Negi commercial group. Its main use is to eat fresh or cooked long white pseudo stem, similar to leek (*Allium porrum* L.), and long from 35 to 45 cm. This effect is achieved through a blanching, i.e., covering the bottom parts of the stem with the soil (Padula *et al*., 2022). Its seeds are used for producing transplants to be planted in the field in spring for autumn harvest.

The genotype 17TSITGH03 belongs to the same commercial group as the genotype above. It is a very successful inbred male sterile (MS) line, developed by a Japanese plant breeding and seed production company. It has been used as a female parental line in a commercial hybrid (F_1) seed production (Padula *et al*., 2022). The seeds were produced in a greenhouse located in Italy, with the use of its proper complementary line and in full insect isolation.

The genotype 1240694 is an open pollinated (OP) cultivar developed by one of the European leading plant breeding and seed production companies. Seeds of this genotype were produced in South Africa. As the cultivar's main use is for fresh salad, it has short and sweet pseudo stems long 9-11 cm, and it is suitable for spring and summer high density field production. It has a short growing cycle of only 70 to 75 days.

The genotype 170403214 is a hybrid (F_1) cultivar developed by one of the best South Korean plant breeding and seed production companies. It is used to produce young seedlings for freshly eaten salads. It is commonly used in commercial production in South Korea, Europe and in the US. As the genotype above, its seeds were produced in South Africa.

Seed source

Once the seeds of all the genotypes reached the maturity, they were all harvested by hand and then forced under artificial drying to decrease their moisture level to the range of 7-8%. All the seeds delivered by the companies had high quality: germination over 90% and moisture content 7-8%. The results of the preliminary germination test, done before starting the experiment, did confirm the quality information provided by each of the seed companies.

Seed storing conditions

Seeds of the genotypes were placed into natural textile sacks, similar to ones used today in standard seed storing conditions. Then, they were kept in 6 high-tech Italian designed and built laboratory climatic chambers of the type FDM CB-CS series. Their range of controlled conditions covers from -25 °C to + 70 °C and an air RH - from 10 to 98%. Their electric characteristic (according to the Italian producer) includes the "+++" ikon, i.e., the third (highest) energy saving category. The chamber has been recommended for providing strictly controlled climatic conditions for storing any plant and human live tissues material. The seeds were stored for 30 months under controlled both air temperature and RH. The seed storage parameters kept for each chamber were as follows: 25 °C with 25% air RH (chamber 1) and 45% air RH (chamber 2); 10 °C with 25% air RH (chamber 3) and 45% air RH (chamber 4); and below 10 °C with 25% RH (chamber 5) and 45% air RH (chamber 6).

Seed germination tests

After 10 and 30 months of storage in the chambers, 2 tests of the seed germination were done. They followed the rules for germination test of the International Seed Testing Association (ISTA) (Anonymous, 2012). The seeds were previously pre-chilled at 5 °C for 4 days. Then, 4 replicates of 50 seeds (each placed in one 9 cm Petri dish) were placed on 6 layers of blotting paper and tested at 20 °C with 16 hours light and 8 hours dark phases. The first and the final counts were done after 6 and 12 days, respectively.

Storage costs calculation

It included only one variable: the used electric current in KW per 1 hour, when one of the used in the experiment climatic chamber was set for 30 months at 6-7 °C (below 10 °C) and the other one - at 10 °C. The received energy use data was received directly from the chamber's Italian producer. The use of the electricity (in KWh) of the first chamber with below 10 °C (6-7 °C) was taken as 100%.

Mycological analysis of the seeds

It started after 1 year and was repeated after 2 years of storage of seeds at temperature 7-8 °C at 25% RH and at 25 °C. It was done only for one genotype: 17TSITGH03. The seeds of this line were produced in the greenhouse with complete insect isolation and optimal conditions for their high health status. For mycological analysis, 400 seeds (4 replications of 100 seeds) were placed on the potato dextrose agar (PDA) medium in 9 cm diameter Petri dishes, 10 seeds per dish, and then incubated at 20 °C for 10 days under 12 hours alternating cycle of NUV light and darkness. Streptomycine sulfate at 100 ppm concentration was added to the media to prevent growth of bacteria (Tylkowska and Dorna, 1996). Determination of fungi was based on the appearance of their colonies and sporulation (Watanabe, 2002; Mathur and Kongsdal, 2003). Additionally, the percentage of the seeds free from fungi was determined.

Statistical data evaluation

For the received data, the variance was calculated. The significant differences were calculated based on the Duncan's test. Means followed by the same letters were not significantly different for $\alpha = 0.05$.

Results and Discussion

Seeds of the tested genotypes responded differently to storing them at the same conditions for 10 and 30 months.

For the seeds of the lot 270322, after 10 months of their storage, no differences in their seed germination capacity (second count) were recorded (Table 2). The same test repeated after 30 months of storage showed some difference in the same parameter: the germination capacity decreased to 80%, when the seeds were stored at 25 °C and at 25% air RH. The main observation here was that that the germination capacities of the seeds of this genotype after storing them for 30 months at 7-8 °C and 10 °C did not differ. Moreover, when comparing the germination capacities of this genotype after 30 months of storage, there were differences between 25% and 45% air RH: for 7-8 °C and 25 °C. The recorded germination capacities were better at 45% than at 25% RH (Table 2).

Table 2. Effect of various storing conditions of Welsh onion seeds lot 270322 on their germination after 10 and 30 months

Seed lot 270322 storage conditions	After 10 months				After 30 months			
	1st count	STAT	2nd count	STAT	1st count	STAT	2nd count	STAT
7-8 °C, 25% RH	92.00%	b*	94.00%	a	79.00%	b	86.00%	b
7-8 °C, 45% RH	95.00%	b	96.00%	a	75.50%	ab	92.00%	c
10 °C, 25% RH	82.00%	a	94.00%	a	70.00%	a	91.00%	bc
10 °C, 45% RH	93.00%	b	94.00%	a	75.50%	ab	92.00%	c
25 °C, 25% RH	94.00%	b	95.00%	a	78.00%	b	80.00%	a
25 °C, 45% RH	94.00%	b	96.00%	a	76.00%	ab	90.00%	bc

*Means of a given count followed by the same letters are not significantly different according to the Duncan's test for α=0.05

For the seed lot17ISITGH03, the results of their germination capacities for the storage after 10 months were different only in one case: their germination was higher (91%) when the seeds were stored at 25 °C at 25% air RH than the seeds stored at 7-8 °C and 45% air RH (Table 3). The same test done after 30 months of storage showed again only one difference: the germination of the seeds stored at 7-8 °C at 25% air RH was worse than when they were stored at 25 °C at 25% air RH. All other values had no significant differences (Table 3).

Table 3. Effect of various storing conditions of Welsh onion seeds lot 17TSITGH03 on their germination after 10 and 30 months

Seed lot 17TSITGH03 storage conditions	After 10 months				After 30 months			
	1st count	STAT	2nd count	STAT	1st count	STAT	2nd count	STAT
7-8 °C, 25% RH	88.00%	bc*	90.00%	ab	81.00%	ab	83.00%	a
7-8 °C, 45% RH	83.00%	a	85.00%	a	83.50%	ab	87.00%	ab
10 °C, 25% RH	88.00%	bc	89.00%	ab	77.50%	a	88.00%	ab
10 °C, 45% RH	89.00%	bc	89.00%	ab	87.00%	b	89.00%	ab
25 °C, 25% RH	91.00%	c	91.00%	b	84.00%	ab	90.50%	b
25 °C, 45% RH	86.00%	ab	89.00%	ab	83.00%	ab	87.00%	ab

*Means of a given count followed by the same letters are not significantly different according to the Duncan's test for α=0.05

For the seeds of the lot 1240694, after 10 months of their storage, only one difference in the germination capacities was recorded. When the seeds were stored at 25 °C, and at both 25% and 45% air RH, their germination capacities were worse than when the seeds were stored at 7-8 °C and at 45% air RH (Table 4). When the same test was repeated after 30 months of storage, again only one difference was recorded, the seeds stored at 25 °C and at 25% air RH, performed worse germinated capacity than at any other conditions provided in this experiment (Table 4).

Table 4. Effect of various storing conditions of Welsh onion seeds lot 1240694 on their germination after 10 and 30 months

Seed lot 1240694 storage conditions	After 10 months				After 30 months			
	1st count	STAT	2nd count	STAT	1st count	STAT	2nd count	STAT
7-8 °C, 25% RH	95.00%	a*	96.00%	ab	85.00%	c	87.00%	b
7-8 °C, 45% RH	94.00%	a	97.00%	b	85.50%	c	89.00%	b
10 °C, 25% RH	95.00%	a	96.00%	ab	76.50%	b	90.00%	b
10 °C, 45% RH	93.00%	a	94.00%	ab	77.50%	b	90.00%	b
25 °C, 25% RH	91.00%	a	92.00%	a	71.00%	a	74.00%	a
25 °C, 45% RH	91.00%	a	92.00%	a	79.00%	b	87.00%	b

*Means of a given count followed by the same letters are not significantly different according to the Duncan's test for α=0.05

For the seeds of the lot 170403214, after 10 months of their storage, only one difference in their seed germination capacity was recorded (Table 5). The seeds stored at 25 °C at 25% RH had worse germination capacity than the ones stored at the same air RH but at 7-8 °C and 10 °C. The same test repeated after 30 months of storage showed more differences in the values of this parameter. The seeds stored at 7-8 °C, at 45% air RH and at 10 °C at both 25% and 45% air RH germinated better than all other seeds. Moreover, the seeds stored at 7-8 °C and at 25 °C had better germination capacities when stored at the air RH 45% than at the 25% RH (Table 5).

Table 5. Effect of various storing conditions of Welsh onion seeds lot 170403214 on their germination after 10 and 30 months

Seed lot 170403214 storage conditions	After 10 months				After 30 months			
	1st count	STAT	2nd count	STAT	1st count	STAT	2nd count	STAT
7-8 °C, 25% RH	62.00%	b*	73.50%	b	34.50%	b	39.00%	a
7-8 °C, 45% RH	63.50%	b	69.50%	ab	62.00%	d	64.00%	c
10 °C, 25% RH	63.00%	b	75.50%	b	53.00%	c	58.00%	c
10 °C, 45% RH	65.00%	b	76.00%	b	59.00%	d	60.00%	c
25 °C, 25% RH	54.00%	a	64.00%	a	27.00%	a	35.00%	a
25 °C, 45% RH	64.00%	b	75.00%	b	48.00%	c	51.00%	b

*Means of a given count followed by the same letters are not significantly different according to the Duncan's test for α=0.05

The carried-out experiments showed that in terms of the seed quality, the 4 lots used in the experiment seeds should be divided into 2 groups: the first 3 batches (270322, 17TSITGH03, 1240694) with high germination capacities after the storage, and the last one (170403214) which clearly showed the lowest germination quality after 30 months of storage compared to the 3 previous ones. This observation only confirmed earlier findings about serious problems with keeping high quality of Welsh onion seeds on the commercial market (Lazarenko *et al.*, 2008; Li *et al.*, 2008; Yanping *et al*., 2000; Khan *et al.*, 2020; Padula *et al.*, 2022).

The received results of the germination capacities for almost all of the samples showed that the seed germination capacities after storing them for 30 months at 7-8 °C and at 10 °C did not differ. For the genotype 170403214, at 25% air RH, the 30-month seed storage at 10°C even showed better results than at 7-8 °C. Thus, in a private seed company trading on the day-to-day basis Welsh onion seeds, there is no reason to store them up to 2.5 years at the commonly recommended temperatures range from 0 °C to 4 °C (Doijiode, 2001; Black *et al*., 2006). The seed germination capacities of the 3 genotypes, after storing them for 30 months, were higher when seeds were stored at the air RH 45% than at the air RH 25%. However, there were also in the results of our tests, cases with no effect of this factor on the seed germination of the seeds. Our finding is in agreement with the earlier information that the optimal air RH when storing seeds of onion-like crops is 35% (Doijiode, 2001). Due to high market demand and price of the Welsh onion seeds (Padula and Hołubowicz, 2022; Padula *et al*., 2022), this problem, due to generated costs of storage, needs first further research, then followed by extension advice for plant breeders and seed traders.

Seed storage costs savings

The carried-out calculations of the costs of the Welsh onion seeds in these experiments at 10 °C instead of 7-8 °C evidenced that the chamber used 11.2% less energy (Table 6). Although, there have been various stable and variable elements of the costs of string sowing material, the easiest way to prove savings caused by temperature of the air is to compare the cost of the used electric current. We were not able to find results of such calculations done for storing seeds of other crops.

Table 6. The comparison (%) of the electric current consumption (KWh) when storing Welsh onion seeds in the climatic chambers of the type FDM CB-CS series at 2 different temperatures: 7-8 °C and 10 °C

Temperature for storage	Used energy (KWh)	%
Below 10 °C	0.675	100.0
10 °C	0.600	88.8

Fungus test

Numerous saprotrophic and pathogenic fungi were identified on the stored Welsh onion seeds, however some of them infested relatively low percentage of seeds (Table 7). Prolongation of storage, regardless of temperature, resulted in a significant increase in seed infestation with *Alternaria alternata*, *Aurobasidium pullulans*, *Cladosporium* spp. and *Penicillium* spp. Moreover, 2 years of storage at 25 °C increased seed infestation with *Fusarium* spp. and *Trichothecium roseum*, while after storage at 7-8 °C higher percentage of seeds infested with *Phoma* sp. was observed. Two years of storage, especially at 25 °C, decreased significantly the percentage of seeds infested with *Rhizopus* sp. in comparison with seeds stored for 1 year.

Table 7. Welsh onion genotype 17TSITGH03 seed infestation with fungi after 1 and 2 years of storage at temperature below 10 °C and the air RH 25%, and at room (25 °C) conditions

Fungus	Storage conditions	Seed infestation with fungi (%) after storage				Mean	
		1 year		2 years			
Alternaria alternata	7-8°C	0.25	a	7.00	c	3.63	A
	25°C	2.50	b	7.00	c	4.75	B
	Mean	1.38	A	7.00	B		
Aspergillus spp.	7-8°C	1.75	ab	1.00	ab	1.38	A
	25°C	3.75	a	0.25	a	2.00	A
	Mean	2.75	B	0.63	A		
Aureobasidium pullulans	7-8°C	1.00	a	25.25	c	13.13	A
	25°C	6.75	a	7.75	b	7.25	A
	Mean	3.88	A	16.50	B		
Botrytis cinerea	7-8°C	0	a	0.25	a	0.13	A
	25°C	0	a	0.50	a	0.25	A
	Mean	0	A	0.38	A		
Cladosporium spp.	7-8°C	16.75	a	27.0	b	21.88	A
	25°C	14.50	a	38.0	c	26.25	A
	Mean	15.63	A	32.5	B		
Epicoccum nigrum	7-8°C	0	a	0.25	a	0.13	A
	25°C	0	a	0.50	a	0.25	A
	Mean	0	A	0.38	A		
Fusarium spp.	7-8°C	2.75	a	2.00	a	2.38	A
	25°C	2.25	a	40.75	b	21.50	B
	Mean	2.50	A	21.38	B		
Mucor sp.	7-8°C	2.50	ab	0	a	1.25	A
	25°C	7.50	b	0	a	3.75	A
	Mean	5.00	B	0	A		
Penicillium spp.	7-8°C	7.50	a	42.5	c	25.00	B
	25°C	6.00	a	18.75	b	12.38	A
	Mean	6.75	A	30.63	B		
Phoma sp.	7-8°C	0	a	4.00	b	2.00	B
	25°C	0	a	0.75	a	0.38	A
	Mean	0	A		B		
Rhizopus stolonifer	7-8°C	40.00	ab	1.25	ab	20.63	A
	25°C	36.25	b	0	a	18.13	A
	Mean	38.13	B	0.63	A		
Stemphylium botryosum	7-8°C	1.50	a	0.75	a	1.13	A
	25°C	0.25	a	1.50	a	0.88	A
	Mean	0.88	A	1.13	A		
Trichothecium roseum	7-8°C	0	a	0	a	0	A
	25°C	0.25	a	2.00	b	1.13	B
	Mean	0.13	A	1.00	A		

*Means followed by the same letters are not significantly different for a given fungus

Dorna *et al*. (2013) suspected that the reduction of the expansion of one of the microorganisms made possible the growth of the other one. The increase in seed infestation with storage fungi, especially *Penicillium* spp., may resulted in seed deterioration (Maude, 1996). These fungi can tolerate quite wide range of temperatures (Pitt, 1999). Nearly all species from genus *Penicillium* are capable of growing below 5 °C, and some even at 0 °C. Therefore, it is difficult to prevent spreading of these fungi during storage by temperature control. Moreover, it was found that some species from this genus could be highly pathogenic to the *Allium* spp. (Valdez *et al*., 2009). On the other hand, at 7-8 °C, the growth of *Fusarium* spp. on the stored seeds was limited. This, in turn, may positively affect growth of the plants in the field, as some of these fungi are seed-transmitted and pathogenic to them (Köycü and Özer, 1997). According to discussed results, despite the used air temperature, seed infestation with both pathogenic and saprotrophic fungi may increase significantly during long term storage. Therefore, it is important to control health of the seeds being prepared for storage to avoid losses of seed viability, and to prevent transmission of seedborne pathogens.

Conclusions

The results obtained from this research can be used as a guideline for the storage in climatic and RH controlled chambers of all genotypes belonging to Allium fistulosum species, with the aim to maintain its seed germination quality related to the duration of the storage and taking into consideration the operating energy costs.

Seeds of the tested genotypes kept good germination at 7-8 °C and at 10 °C. By storing them at 7-8 °C and 10 °C, the same effect was achieved. In seeds of 3 genotypes (270322, 1240694, 170403214), after storing them for 30 months, their germination was better at the air RH 45% than at 25%.

The calculated electricity costs for 30-month seed storage at 10 °C was 11.2% lower than at 7-8 °C. From the company's point of view, considering the electricity cost, seed storage at 10 °C is more recommended than at 7-8 °C.

Generally, the temperature 7-8 °C favoured growth of *Penicillium* spp. and *Phoma* sp. on Welsh onion seeds during 2 years of storage, while in seeds stored at 25 °C it increased seed infestation with *Cladosporium* spp. and *Fusarium* spp.

Authors' Contributions

Conceptualization: G.P. and R.H.; Data curation: G.P., X.X.Z. and D.S.; Formal analysis: X.X.Z. and D.S.; Funding acquisition: G.P.; Investigation: G.P.; Methodology: G.P.; Project administration: R.H.; Resources: G.P.; Software: G.P. X.X.Z. and D.S.; Supervision: R.H.; Validation: G.P.; Visualization: G.P.; Writing - original draft: G.P.; Writing - review and editing: G.P., X.X.Z., D.S. and R.H. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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