Evaluation and management of fungal-infected carrot seeds

Xue Zhang¹, Ruiting Wang¹, Hailong Ning¹,², Wenxia Li¹,², Yunlong Bai³ & Yonggang Li¹,*

Carrot (Daucus carota L.), which is one of the 10 most important vegetable crops worldwide, is an edible root vegetable desired for its taste as well as its medicinal uses. However, a fungus isolated from carrot seeds was observed to substantially decrease the germination rate. The isolate was identified as Alternaria alternata based on morphological and molecular characteristics as well as a phylogenetic tree. The maximum seed infection rate of selected carrot cultivars was approximately 60%, with the main infection site just underneath the seed shell. Additionally, the germination rate of infected seeds decreased by 28.7%. However, the seed infection rate varied among the examined carrot cultivars. Regarding the effects of chemical fungicides, the optimal treatment involved immersing seeds in amistar top suspension concentrate (SC) (effective concentration of 0.65 g/L) for 6 h, which effectively killed the fungi inside the carrot seeds. The results of this study provide a theoretical basis for the development of efficient methods for preventing the infection of carrot seeds by specific fungi and increasing the germination rate and vigour index.

Carrot (Daucus carota L.), which is cultivated worldwide, is one of the most important root vegetables in the family Apiaceae¹. The importance of carrots in fulfilling the nutritional needs of communities has long been known². In addition to being an edible root vegetable with a desirable taste, carrots have a medicinal use. The largest carrot producer worldwide is China, wherein the vegetable is cultivated mainly in the northern, northeastern, central, and southwestern parts of the country to generate 43% of the global carrot yield³.

Current studies on carrots mainly focus on cultivation, breeding, tissue culture, nutrient content, increasing yield, and regulating carotenoid synthesis⁴,⁵. There has been comparatively less research regarding fungal infections of seeds. We recently revealed that some carrot varieties have a low germination rate and a weak seedling growth potential. Preliminary analyses have suggested that these characteristics may be related to the infection of seeds by specific fungi. Seeds form the basis of crop production and are vital for plant associations with microorganisms, which may be damaging to the seeds or the seedlings that germinate from infected seeds⁶. Of the 16% annual crop loss due to plant diseases, at least 10% is caused by seed-borne diseases⁷.

During seed production, storage, and transport, the seeds are exposed to many kinds of microorganisms, ultimately resulting in fungal infections that may adversely affect seeds by decreasing germination and vigour, shortening the storage period, and inducing physiological changes⁸,⁹. Seeds infected by fungi may survive for 5 years if they are air-dried and stored at 4 °C. However, seed quality directly determines the quality of agricultural products⁴. Additionally, seed-borne pathogens can be the primary source of infection and disease transmission¹⁰.

Fungi infecting seeds can attach to the seed surface or penetrate the seeds. Consequently, the effectiveness of chemical seed treatments may vary because deep-seated infections may be unaffected¹¹. The application of chemical pesticides is a fundamental agronomic practice regarding crop protection¹². However, their excessive use may decrease the sensitivity of the target pathogens to these chemicals. Therefore, developing novel fungicides with low toxicity and establishing appropriate application protocols for managing fungal infections of seeds are crucial. For example, Sudisha et al. (2006) revealed that a carbendazim wettable powder, captan 50 WP, and dithane M-45 can inhibit melon stem blight due to infected seeds¹³. Wang et al. observed that treatments with 75% chlorothalonil, 50% thiram, or 80% carbendazim are significantly inhibitory to the pathogens infecting seeds, and that 75% chlorothalonil and 80% carbendazim can promote seed germination¹⁴. Thus, research involving the screening and use of chemical fungicides may result in effective methods for controlling fungal infections of seeds.
The aim of the current study was to isolate the fungi associated with carrot seeds, evaluate the efficacy of chemical fungicides against the detected fungi, and optimize the application of fungicides to maintain a relatively high germination rate and vigour index.

**Materials and methods**

**Isolation and identification of fungi infecting carrot seeds.** The tested carrot seeds (cultivars Kaixinmin (Kaix- min and Berlin) were purchased from a commercial market in Shuangyangshan, Heilongjiang province, China. The seeds (30 seeds/cultivar) were surface-sterilized with 0.5% NaOCl for 5 min, rinsed three times with sterilized distilled water, placed on potato dextrose agar (PDA), and incubated at 26 °C. Single spores were obtained from the fungal cultures for morphological and molecular analyses as previously described16. The fungi were identified based on morphological characteristics as described in published methods7,18,19. To further identify the fungi, genomic DNA was extracted from the isolates with the Fungal Genomic DNA Kit (Tiangen, Beijing, China); and Berlin (Bejo Zaden B.V., Harenkarspel BEJO, Harenkarspel, The Netherlands).

**Screening of chemical fungicides.** Carrot seeds (cv. Hanhong) were collected to analyse the impact of fungal infections on germination. amistar top SC (325 g/L) was diluted to 0.65 g/L (effective concentration), after which carrot seeds were immersed in the diluted fungicide solution for 8 h to kill all of the fungi within the seeds. Control seeds were treated with sterile water. All seeds were then air-dried and analysed. The seed infection and germination rates were determined by culturing on PDA. Specifically, the seeds were sterilized, after which 30 seeds per treatment were placed on PDA medium and incubated at 25 °C for 5 days. The appearance of the resulting fungal colonies was recorded. This analysis was completed with three replicates for a total of 90 seeds, which were used to calculate the seed infection and germination rates with the following equations:

\[
\text{Seed infection rate} (%) = \frac{\text{Number of seeds infected by fungi}}{\text{Total number of seeds}} \times 100;
\]

\[
\text{Seed germination rate} (%) = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100
\]

**Carrot seed parts infected by fungi.** Carrot seeds (cultivar Hanhong) were collected to analyse the impact of fungal infections on germination. amistar top SC (325 g/L) was diluted to 0.65 g/L (effective concentration), after which carrot seeds were immersed in the diluted fungicide solution for 8 h to kill all of the fungi within the seeds. Control seeds were treated with sterile water. All seeds were then air-dried and analysed. The seed infection and germination rates were determined by culturing on PDA. Specifically, the seeds were sterilized, after which 30 seeds per treatment were placed on PDA medium and incubated at 25 °C for 5 days. The appearance of the resulting fungal colonies was recorded. This analysis was completed with three replicates for a total of 90 seeds, which were used to calculate the seed infection and germination rates with the following equations:

\[
\text{Seed infection rate} (%) = \frac{\text{Number of seeds infected by fungi}}{\text{Total number of seeds}} \times 100;
\]

\[
\text{Seed germination rate} (%) = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100
\]

**Determination of the seed infection rate of 10 carrot cultivars.** The following 10 carrot cultivars were analysed: Hanhong (Beijing Baikutian Seedling Co., Ltd., China); Zhimeihong, Qihongyun, Hongyu 6, Kaixinmin, Kaixin666, Kaixin668, Kaixin838, and Kaixin1 (Qingdao Yuanshengtai Seed Industry Co., Ltd., China); and Berlin (Bejo Zaden B.V., Harenkarspel BEJO, Harenkarspel, The Netherlands).

The tested carrot seeds were surface-sterilized. For each carrot cultivar, 30 seeds per cultivar were placed on PDA medium, with three replicates for a total of 90 seeds per cultivar. The seeds were incubated at 26 °C for 5 days, after which the seed infection rate was calculated with the formula provided above and compared among the 10 cultivars.

**Screening of chemical fungicides.** Carrot seeds (cultivar Hanhong) were treated with the following fungicides: azoxystrobin (250 g/L SC) (Shanghai Future Industry Co., Ltd., Shanghai, China); bosalid (50% WG) and sercadis plus (12% SC) [BASF Plant Protection (Jiangsu) Co., Ltd., Rudong, China]; difenoconazole (10% WG), ipronil (25 g/L DS), amistar top SC (325 g/L SC), and procymidone (18.7% SC) [Syngenta (Nantong) Crop Protection Co., Ltd., Nantong, China]; carbeniladim (80% WP) (Shandong Haier Sanli Biochemical Co., Ltd., Zhucheng, China); and captan (80% WG) (Adama Makhteshim Ltd., Beersheba, Israel).

The chemical fungicides were diluted for an effective concentration of 0.65 g/L. Carrot seeds (180 seeds/fungicide) were immersed in the diluted solutions or sterile water (control) for 6 h. After air-drying the seeds, 90 of them were added to PDA medium to assess the effects of the fungicides on the seed infection rate. This analysis was completed with three replicates for a total of 90 seeds. The remaining 90 seeds were added to pots containing sterilized soil, with 10 seeds per pot and nine pots per treatment. The seeds were covered with 1 cm of soil. After a 15-day incubation in the greenhouse, the germination rate, seedling height, and fresh weight were determined. The seed infection rate was calculated with the formula provided above and compared among the 10 cultivars.

\[
\text{Control effect} (%) = \frac{(\text{seed infection rate of the control group} - \text{seed infection rate of the treatment group})}{\text{seed infection rate of the control group}} \times 100
\]
Optimizing fungicide applications on carrot seeds. Fungicide applications were optimized, including the amistar top SC concentration and treatment time. Specifically, carrot seeds (90 seeds/treatment) were treated with amistar top SC (effective concentrations of 0.33, 0.41, or 0.65 g/L) or sterile water (control) for 6 h, after which 30 seeds per treatment were surface-sterilized and added to PDA medium (10 seeds/plate) and incubated at 26 °C. The remaining 90 seeds were added to a culture bowl (10 seeds/bowl). The seeds were incubated for 20 days in a greenhouse with a 28 °C (day): 22 °C (night) cycle. The germination rate, plant height, and fresh weight of the carrot seedlings were determined.

Sterilized carrot seeds (90 seeds/treatment) were immersed in amistar top SC solution (effective concentration of 0.65 g/L) or sterile water (control) for 2, 4, 6, or 8 h. The seed infection rate was calculated with the formula provided above. Additionally, the effect of the treatment on seedling growth was assessed.

Statistical analysis. All experiments were conducted twice under similar conditions. Data underwent an analysis of variance with the SPSS Statistics 19.0 program (IBM/SPSS, Chicago, IL, USA). The significance of any differences in the mean values for treatments was determined with Duncan's multiple range test ($P < 0.05$).

Results
Isolation and identification of fungi infecting carrot seeds. Ten fungi were isolated from cultivars Kaixin666 and Berlin and then subcultured by transferring hyphal tips. Single-microconidium isolates were generated from the fungal cultures as previously described16 and the morphological characteristics of the isolates were analysed. Colonies on PDA medium consisted of dark grey mycelium (Fig. 1). Conidia produced after a 4-day incubation at 26 °C in darkness were pale brown to dark brown, straight or flexuous, obclavate to obpyriform or ellipsoid, and had a short conical beak at the tip or were beakless. The spores had a smooth or verrucose surface and were 3.6–11.5 μm × 6.3–35.2 μm, with 0–4 transverse septa and 0–1 longitudinal septa. Ten fungal isolates had identical morphological characteristics and were identified as Alternaria alternata19.

Genomic DNA was extracted from the single conidial cultures of two representative isolates, Cbailin and Akaixin, and the internal transcribed spacer (ITS) regions and translation elongation factor 1-alpha (TEF-1α) genes and RNA polymerase II second largest subunit gene (RPB2) were amplified by PCR with primers ITS1 and ITS420, EF1-728F/EF1-986R21 and RPB2–5F2/IRPB2–7cR22. A BLAST analysis revealed the amplified sequences of Cbailin and Akaixin were 100% identical to sequences from A. alternata isolates Alt-C81 (MN044802.1) and 12A (MK248606.1) for ITS and isolates Aa1 (MK733276.1) and PaF-3 (MN692926.1) for TEF-1α, isolates 15–239 (LC132700.1) and 14–358 (LC132699.1) for RPB2, respectively.

A combined tree based on the ITS, TEF-1α and RPB2 sequences indicated that Cbailin and Akaixin were A. alternata (Fig. 2). The sequences of the Cbailin and Akaixin amplicons were deposited in the GenBank database (accession numbers MN337233 and MK332248 for ITS, and MT178330 and MT178329 for TEF-1α, and MT593329 and MT593330 for RPB2, respectively). To the best of our knowledge, this is the first report of the isolation of A. alternata from carrot seeds.

Effects of fungal infections on carrot seed germination. The carrot seeds treated to kill all fungi germinated better than the control carrot seeds (Table 1). Specifically, the mean germination rate of seeds lacking A. alternata was 28.7% higher than that of control seeds infected by fungi.

Detection of carrot seed parts infected by fungi. An analysis of the carrot seed parts infected by fungi revealed the seed infection rates of the whole seed and the cut seed were both 53.3% (Fig. 3). The lack of significant difference ($P < 0.05$) between the seed infection rates suggested that the fungi were just underneath the seed shell (i.e., internally seed-borne and extra-embryonic).

![Figure 1. Morphological figures of Alternaria alternata strain Cbailin on PDA. (a) Colony; (b) Conidiophore.](https://www.nature.com/scientificreports/)
Alternaria alternata Akaixin^T (MT593330) (RPB2)
Alternaria alternata Cbailin^T (MT593329) (RPB2)
Alternaria alternata 14-358^T (LC132699.1)
Alternaria caroïticae CBS 109381^T (KC584386.1)
Alternaria botryospora CBS 478.90^T (KC584461.1)
Alternaria tumida CBS 539.83^T (KC584466.1)
Alternaria planifonda CBS 537.83^T (KC584463.1)
Alternaria aspera CBS 115269^T (KC584474.1)
Alternaria avenicola CBS 121459^T (KC584380.1)
Alternaria dennisii CBS 476.90^T (KC584454.1)
Alternaria telluistris CBS 538.83^T (KC584465.1)

Alternaria alternata Akaixin^T (MT178329) (TEF)
Alternaria alternata WCS1-5^T (MK791316.1)
Alternaria alternata Cbailin^T (MT178330) (TEF)

Figure 2. Phylogenetic tree based on the internal transcribed spacer sequence (ITS), translation elongation factor 1-α gene (TEF), and RNA polymerase II second largest subunit gene (RPB2) for identifying Alternaria alternata isolates Cbailin and Akaixin.

Table 1. Effects of fungal infections on the germination of carrot seeds. Values followed by different letters were significantly different according to Duncan’s multiple range tests (P < 0.05), as follow tables. a The treated time was 8 h, as follow tables. b Values in the column indicate mean ± standard error (SE) of the carrot seed carrying rate, as follow tables.

| No | Treated time | Fungal inhibition rate (%) | Germination rate (%) | Reduction rate of germination (%) |
|----|--------------|-----------------------------|----------------------|----------------------------------|
| 1  | 8 h          | 0.0 ± 0.0a                  | 80.0 ± 11.5b         | –                                 |
|    | CK           | 47.2 ± 8.0b                 | 56.7 ± 8.8a          | 29.1                              |
| 2  | 8 h          | 1.3 ± 0.9a                  | 79.8 ± 3.1b          | –                                 |
|    | CK           | 50.1 ± 1.7b                 | 59.2 ± 4.1a          | 25.8                              |
| 3  | 8 h          | 1.0 ± 0.6a                  | 83.1 ± 2.9b          | –                                 |
|    | CK           | 50.8 ± 3.4b                 | 57.2 ± 5.5a          | 31.2                              |
Calculation of the seed infection rate of 10 carrot cultivars. Significant differences in the seed infection rates were revealed among the 10 analysed carrot cultivars (Table 2). Cultivars Hanhong and Kaixinmingtu were the most heavily infected, with seed infection rates of 60.0% and 23.3%, respectively. Second, cultivars Kaixin 1 and Bailin were very few with seed infection rates of 6.7% and 3.3%. Finally, other varieties were free of fungi.

Seed treatments with various fungicides. The treatments with nine chemical fungicides resulted in considerable variability in the seed infection rate, germination, and seedling growth (Table 3). For example, amistar top SC killed all of the fungi within carrot seeds (100% control of fungal infection), whereas captan, procymidine, and azoxystrobin were less effective (40% control of fungal infection). Moreover, boscalid, difenoconazole, carbendazim, benzofuramid, and fipronil were relatively ineffective for controlling fungal infections.

Table 2. Calculation of carrot seed infection rates of various carrot cultivars.

| Varieties       | Carrying rate  |
|-----------------|----------------|
| Kaixinmintu     | 23.3 ± 6.1b    |
| Zhimeihong      | 0.0 ± 0.0a     |
| Bailin          | 3.3 ± 3.3a     |
| Quhongyu        | 0.0 ± 0.0a     |
| Kaixin 1        | 6.7 ± 4.2a     |
| Hanhong         | 60.0 ± 5.2c    |

Table 3. Effects of seed treatments with nine chemical fungicides on carrot seed infection rates, germination, and seedling growth.

| Fungicides | Active ingredient | Carrying rate (%) | Control effect (%) | Plant height (cm) | Germination rate (%) | Fresh weight (g) |
|------------|-------------------|-------------------|--------------------|-------------------|----------------------|------------------|
| Azoxystrobin | 250 g/L           | 30.0 ± 6.8b       | 50.0               | 7.1 ± 0.5ab       | 76.7 ± 12.0ab        | 0.021 ± 0.003a   |
| Boscalid   | 50%               | 40.0 ± 7.3bc      | 33.3               | 6.1 ± 0.7a        | 63.3 ± 8.8ab         | 0.025 ± 0.003ab  |
| Difenconazole | 10%              | 43.3 ± 6.1bc      | 27.8               | 6.1 ± 0.4a        | 60.0 ± 10.0a         | 0.028 ± 0.002bc  |
| Carbendazim | 80%               | 60.0 ± 7.3c       | 0.0                | 8.0 ± 0.2b        | 93.3 ± 6.7b          | 0.041 ± 0.003d   |
| Procymidine | 18.7%             | 33.3 ± 8.4d       | 44.4               | 6.8 ± 0.3a        | 60.0 ± 10.0a         | 0.029 ± 0.001bc  |
| Sercadis plus | 12%              | 40.0 ± 8.9bc      | 33.3               | 6.3 ± 0.3a        | 73.3 ± 8.8ab         | 0.030 ± 0.004bc  |
| Captan     | 80%               | 33.3 ± 8.0b       | 44.4               | 7.1 ± 0.3ab       | 70.0 ± 10.0a         | 0.023 ± 0.001ab  |
| Fipronil   | 25G/L             | 56.7 ± 6.1c       | 5.6                | 6.8 ± 0.1a        | 90.0 ± 0.0ab         | 0.032 ± 0.001c   |
| Amistar top SC | 325 g/L         | 0.0 ± 0.0a        | 100.0              | 6.7 ± 0.4a        | 80.0 ± 5.8ab         | 0.030 ± 0.002bc  |
| CK         | –                 | 60.0 ± 5.2c       | –                  | 6.4 ± 0.3a        | 73.3 ± 8.8ab         | 0.029 ± 0.002bc  |

Figure 3. Determination of the carrot seed parts infected by fungi. (a) Whole seeds. (b) Cut seeds.
Seed treatments with varying amistar top SC concentrations. The three tested amistar top SC concentrations were inhibitory to the fungal infection of carrot seeds (Table 4). The 0.65 g/L treatment was the most effective (99.2% control of fungal infection). Additionally, seed germination and seedling growth were not inhibited.

Effects of varying amistar top SC seed treatment times. The four tested amistar top SC treatment times inhibited the infection of seeds by fungi, with obvious differences among the analysed times (Table 5). Specifically, the 6-h and 8-h treatment times were the most effective, with no significant difference between these two time-points. However, plant height was significantly lower after the 8-h treatment. The 6-h seed treatment with amistar top SC was the most appropriate based on the overall performance.

Discussion
Carrots are an important vegetable grown worldwide and represent a source of carotenoids in the human diet. However, the emergence rate of some carrot cultivars was relatively low. The decreased germination rate was due to an infection of the seeds by A. alternata. The genus Alternaria Nees includes imperfect fungal species that are cosmopolitan and economically important. In the current study, the germination rate of seeds not infected with A. alternata was 28.7% higher than that of the control seeds infected with fungi. Thus, screening for suitable fungicides and optimizing their applications are important.

Seeds are critical for viable crop production. Pathogen-free seeds are essential for generating healthy plant populations and a good harvest. In many crops, fungal infections are responsible for low-quality seeds. Additionally, the presence of seed-borne pathogenic fungi in beans results in decreased germination, emergence, growth, and yield. This is consistent with our finding that the highest carrot seed infection rate was approximately 60%. Moreover, seed infection rates differed among the tested carrot cultivars. Selecting carrot cultivars uninfected by fungi represents a good agronomic practice for minimizing the chances of fungal infections.

Microorganisms associated with seeds may be pathogens, weak parasites, or saprophytes. They may be present within or on the surface of seeds and may infect seeds via exposures to contaminated sclerotia, galls, fungal bodies, infected plant parts, and soil particles. Fungal pathogens may be externally or internally seed-borne, extra- or intra-embryonic, or associated with seeds as contaminants. To clarify which carrot seed parts are infected by fungi, the seed infection rates were calculated for surface-sterilized whole and cut seeds. Our results suggest the primary fungal infection site of carrot seeds is just underneath the seed shell (internally seed-borne and extra-embryonic). However, a more precise localization of the fungi is necessary.

Seeds infected by fungi influence the germination, overall health, and final crop stand under field conditions. Seed-borne as well as seed-associated fungal infections can be effectively inhibited if the seeds are treated with fungicides before sowing. The application of chemical fungicides can completely control fungal infections, but it can be costly and harmful to human health and the environment. To prevent the undesirable effects of fungicides on carrots, we systematically screened for effective fungicides and examined the effects of their application. The optimal seed treatment involved a 6-h immersion in amistar top SC (effective concentration of 0.65 g/L), which killed all of the fungi infecting the seeds, with no deleterious effects on the seeds or seedlings.

The results of this study provide a theoretical basis for the development of effective methods for controlling the fungal infections of carrot seeds, thereby increasing the germination rate and vigour index.

| Effective concentration (g/L) | Fungal carrying rate (%) Control effect (%) Germination rate (%) Plant height (cm) Fresh weight (g) |
|-----------------------------|---------------------------------|---------------------------------|-----------------------------|-------------------------------|-----------------------------|
| 0.33                        | 38.6 ± 1.9c                     | 33.7                            | 59.1 ± 2.6a                 | 8.4 ± 0.1a                    | 0.2 ± 0.0ab                 |
| 0.41                        | 11.2 ± 0.8b                     | 81.2                            | 72.3 ± 2.1b                 | 8.6 ± 0.1a                    | 0.2 ± 0.0ab                 |
| 0.65                        | 0.5 ± 0.5a                      | 99.2                            | 73.2 ± 2.6b                 | 8.4 ± 0.1a                    | 0.2 ± 0.0b                  |
| CK                          | 59.7 ± 1.5d                     | –                               | 57.9 ± 2.7a                 | 8.2 ± 0.0a                    | 0.1 ± 0.0a                  |

Table 4. Effects of various amistar top SC concentrations on carrot seed infection rates, germination, and seedling growth.

| Treated time | Inhibition rate (%) Control effect (%) Germination rate (%) Plant height (cm) Fresh weight (g) |
|--------------|---------------------------------|---------------------------------|-----------------------------|-------------------------------|-----------------------------|
| 2 h          | 61.1 ± 8.2ab                    | 17.6                            | 70.0 ± 5.8a                 | 8.5 ± 0.1c                    | 0.03 ± 0.00a                |
| 4 h          | 72.2 ± 5.5b                     | 41.1                            | 63.3 ± 8.8a                 | 8.4 ± 0.1c                    | 0.03 ± 0.00a                |
| 6 h          | 94.4 ± 3.5c                     | 88.2                            | 59.7 ± 8.8a                 | 8.2 ± 0.0b                    | 0.03 ± 0.01a                |
| 8 h          | 100.0 ± 0.0c                    | 100.0                           | 80.0 ± 11.5a                | 6.9 ± 0.1a                    | 0.02 ± 0.00a                |
| CK           | 52.8 ± 8.0a                     | 56.7 ± 8.8a                     | 7.9 ± 0.1b                  | 0.03 ± 0.00a                  |

Table 5. Efficacy of various amistar top SC seed treatment times.
Received: 14 January 2020; Accepted: 17 June 2020
Published online: 02 July 2020

References
1. Que, F. et al. Advances in research on the carrot, an important root vegetable in the Apiaceae family. Hortic. Res. Engl. 6, 69–82 (2019).
2. Sumekar, Y. Weed diversity in carrot (Daucus carota L.) crop in Majalengka Regency, West Java Province. Indonesia. Res. Crops 20, 109–115 (2019).
3. Fu, Y. L., Liu, T. Z., Fan, J. Y., Zhao, X. & Niu, R. S. Present Situation and development trend of carrot breeding in China. Hebei Agr. Sci. 14, 100–101 (2010).
4. Michael, T. P. & VanBuren, R. Progress, challenges and the future of crop genomes. Curr. Opin. Plant Biol. 24, 71–81 (2015).
5. Luby, C. H., Maeda, H. A. & Goldman, I. L. Genetic and phenological variation of tocochromanol (vitamin E) content in wild (Daucus carota L. var. carota) and domesticated carrot (D. carota L. var. sativa). Hortic. Res. Engl. 1, 14015 (2014).
6. Biswas, S. et al. Isolation of seed-borne and seed associated fungi of lablab purpureus (l) Sweet and their biological control. J. Microbiol. Biotech. Food Sci. 5, 136–141 (2015).
7. Baka, Z. A. M. Biological control of the predominant seed-borne fungi of tomato by using plant extracts. J. Phytopath. Pest Manage. 1, 10–22 (2014).
8. Duan, C. X., Wang, X. M., Zhu, Z. D. & Wu, X. F. Testing of seedborne fungi in wheat germplasm conserved in the National Crop Genebank of China. Agric. Sci. China 6, 682–687 (2007).
9. Maude, R. B. Seed-borne diseases and their control: principles and practice (CAB International, Wallingford, UK, 1997).
10. Ilieva, V., Mitrev, S., Karov, I., Markova, N., & Todorovska, E. Seed quality and its importance in agricultural production and safety of agricultural products. In International Conference "Quality and Competence 2013", pp. 1–11 (2013).
11. Agarval, V. K. & Sinclair, J. B. Principles of seed pathology (CRC Press Inc, Boca Raton FL, 1987).
12. Dube, E., Sibiya, J. & Fanadzo, M. Early planting and hand sorting effectively controls seed-borne fungi in farm-retained bean seed: research article. S. Afr. J. Sci. 110, 73–80 (2014).
13. Miyake, T., Tateishi, H., Sakuma, Y. & Saino, T. A novel soil-type biopesticide KNB422-soil against rice seedling diseases. J. Pestic. Sci. 37, 129–134 (2012).
14. Sudisha, C. I., Niranjana, S. R., Unmesh, S., Prakash, H. S. & Shetty, H. S. Transmission of seed-borne infection of muskmelon by Didymella bryoniae and effect of seed treatments on disease incidence and fruit yield. Biol. Control 37, 196–205 (2006).
15. Wang, H., Li, K. M., Wang, L. L., Fan, J. X. & Qiereyazidan, J. Y. Inhibitory effect of 6 kinds of fungicides on alfalfa seed carrying Didymella bryoniae and effect of seed treatments on disease incidence and fruit yield. J. Pestic. Sci. 37, 129–134 (2012).
16. Leslie, J. F. & Summerell, B. A. The Fusarium Laboratory Manual (Blackwell Publishing, Victoria, Australia, 2006).
17. Ellis, M. Dematiaceous hyphomycetes 601–608 (Commonwealth Mycol. Institute. Kew, Surrey, England, 1971).
18. Aleoxopolous, J. & Mims, W. Introductory Mycology (Third Edi. John Wiley and Sons. Inc, USA, 1979).
19. Li, Y. G. et al. Occurrence of leaf spot of early lilac caused by Alternaria alternata in Heilongjiang Province in China. Plant Dis. 101, 1048 (2017).
20. Yin, J. et al. Aggressiveness and diversity of Phytophthora capsici on vegetable crops in Georgia. Ann. Appl. Biol. 160, 191–200 (2012).
21. Carbone, I. & Kohn, J. M. A method for designing primer sets for species detection in filamentous ascomycetes. Mycologia 91, 553–556 (1999).
22. Woudenberg, J. H. C., Groenewald, J. Z., Binder, M. & Crous, P. W. Alternaria redefined. Stud. Mycol. 75, 171–212 (2013).
23. Macura, R., Michalczyk, M., Fiutak, G. & Maciejasz, E. Effect of freeze-drying and air-drying on the content of carotenoids and anthocyanins in stored purple carrot. Acta Sci. Pol. Technol. Aliment. 18, 135–142 (2019).
24. Neergaard, P. et al. Histopathology of seed borne infections in common bean (Phaseolus vulgaris cv. INTA Rojo) in Nicaragua. PLoS ONE 11, e0168662 (2016).
25. Singh, D. & Mathur S. B. Location of fungal hyphae in seeds. in Histopathology of seed borne infections (Singh D, & Mathur SB, Eds.). Boca Raton, FL, USA: CRC Press, pp. 101–168 (2004).

Acknowledgements
This study was financially supported by Shuangyashan Dong Hao Agricultural Science and Technology Development Co., Ltd; the National Natural Science Foundation of China (31971760) and Heilongjiang Collaborative Innovation and Extension System of Modern Agricultural Industry Technology of Forage and Feed.

Author contributions
X.Z., R.W., H.N. and Y.L. designed the experiment. X.Z., R.W., W.L. and Y.B. conducted the experiment and wrote the article. X.Z., R.W., and Y.L. helped in statistical analysis. H.N. and Y.G.L. revised the article. All authors approved the final article after reading.

Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to Y.L.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
