Isolation, identification and herbicidal potential of Fusarium avenaceum HY-041 from the Qing-Tibetan Plateau

Haixia Zhu (zhuhaixia0101@163.com)
Qinghai University
huan Li
Qinghai Universsity
Yongqiang Ma
Qinghai University

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Abstract

In order to screen a biocontrol strain with broad-spectrum and high herbicidal activity, potential herbicidal strain HY-041 isolated from the leaves of naturally diseased Cephalanoplos setosum was tested. Based on the morphological characteristics and gene sequence analysis, the fungus was identified as *Fusarium avenaceum*. Herbicidal activity to 9 target weeds were tested, the results showed that the pathogenicity of strain HY-041 to different weeds in *vitro* was as follows: *Polygonum lapathifolium* > *Malva verticillata* > *Avena fatua* > *Chenopodium album* > *Polygonum aviculare* > *Elsholtzia densa* > *Amaranthus retroflexus*> *Thlaspi arvense* > *Galium aparine*, the pot experiment showed that the fermentation filtrate was highly pathogenic to *C. album*, *E. densa* and *A. fatua* by spray inoculation. The safety evaluation results showed that the strain was moderately susceptible to *Brassica napus* L., slightly infected *Triticum aestivum* L. and *Hordeum vulgare*, relatively safe to *Pisum sativum*L. and *Vicia faba* L.. The scanning electron microscope observation showed that strain HY-041 hyphae destroyed the epidermis tissue, invaded *A. fatua* tissue from the stoma and parasites, and propagated in the tissue to produce spores. The results showed that strain HY-041 had the potential to develop microbial biocontrol herbicide.

Introduction

Weed control is one of the main problems faced by agricultural ecosystems. Affected by weeds, six countries including the United States, the United Kingdom, Australia, South Africa, India and Brazil, have annual economic losses of up to 132.4 billion US dollars. The area of land harmed by weeds throughout the year in China exceeds 73 million hm2, and the economic loss directly caused by weeds is up to more than 90 billion yuan (Green 2014). At present, chemical control methods are widely used to control weeds in farmland and lawn because of their large product supply, labor saving and fast operation speed (Izelmar et al., 2018). However, the long-term and extensive use of chemical agents has also brought many ecological and environmental problems to farmland, such as the deterioration of natural resources, the decline in the quality of agricultural products, weed resistance, soil pollution, water quality degradation, and harm to non-target organisms (Mejri et al., 2013; Pawei et al., 2015; Yasuor et al., 2010). With the development of social civilization and the improvement of public health awareness, it is very urgent to develop new microbial herbicides with broad spectrum, high efficiency and low toxicity, especially biological herbicides.

Microbial herbicide refers to a kind of microbial preparation for weeding, which is directly developed and prepared for individual microorganisms. Weed biocontrol microorganisms (mainly fungi) are mostly collected from nature, with the characteristics of low toxicity and long acting. It is an important source for the development of biological herbicides. With the increasing attention paid to the research of microbial herbicides, more and more microorganisms with biocontrol effects have been developed (Pomella et al., 2007; Liu et al., 2013). The literature has reported that 40 genera and more than 80 species of microorganisms, including fungi and bacteria, can control weeds (Chen and Qiang 2015). At present, voluminous research has identified microorganisms with herbicidal potential. For example, Devine is the first successfully registered fungal herbicide; its product is a chlamydospore suspending agent of

Phytophthora palmivora Butler, mainly used to control Morinia odorata in citrus orchards (Kenney 1986; Smith 1993). Fungus Collego has an obvious control effect on weeds in soybean and paddy fields (Bowers 1986). Curvularia eragrostidis and Fusarium chlamydosporum are used to control Digitaria sanguinalis(Zhu and Qiang 2010). Culvularia lunata and Helminthosporium gramineum are used to control Echinochloa crusgalli (Jamil et al., 1987; Geng et al., 2009). Tang et al. (2010) reported for the first time that Sclerotium rolfsii is isolated from the diseased Solidago canadensis, which brought the dawn to the control of this weed. Using plant pathogenicfungal or secondary metabolites as biological herbicides has potential advantages, which can provide a reference for future research and development of new biological pesticides.

Based on the idea of controlling weeds by microorganisms, a candidate biocontrol fungus HY-041 was screened out from diseased plants of C. setosum in Huangyuan county, Qinghai province, China in this study. The taxonomic status of the strain HY-041 was determined by morphological characteristics and molecular biological identification techniques. The herbicidal activity of this strain to 9 weeds were determined in *vitro* and potted weed inoculation tests. the safety to main crops in Qinghai was evaluated by pot bioassay. The ultrastructural changes of *A. fatua* infected by HY-041 were observed for further tissue pathological changes after infection by scanning electron microscope. The findings provide an important basis for the research, development, and utilization of new and efficient microbial herbicides.

Materials and Methods

Strain testing

The diseased samples of C. setosum were collected from the farm fields in Bohang town, Huangyuan, Qinghai(36°66'31"N, 101°21'11"E). HY-041 was obtained after separation and purification and stored in the comprehensive pest control laboratory of Plant Protection Institute, Qinghai Academy of Agriculture and Forestry Sciences.

Weeds and crops for testing

Common broad-leaved weeds in Qinghai include *Chenopodium album* L., *Elsholtzia densa Benth*., *Malva verticillate* L. var. crispa, *Galium aparine* L., *Polygonum lapathifolium* L., *Avena fatua* L., *Amaranthus retroflexus* L., *Thlaspi arvense* L. and *Polygonum aviculare* L., The main crops in Qinghai Province: *Triticum aestivum* L., *Pisum sativum* L., *Hordeum vulgare*, *Vicia faba L*. and *Brassica napus* L.

Isolation and purification of strain

This study recorded the symptoms of infected weeds by taking photos with a camera (Canon, EOS, 550D). We carried out strain isolation according to Fang (1998) method of pathogen isolation. Weed disease samples were cleaned with sterile water on a clean bench and left to dry naturally. Scissors were

used to cut the boundary between diseased and healthy leaves. The weed disease samples were soaked in 1% sodium hypochlorite for 5 min, 75% alcohol for 1 min, and washed with sterile water three times. The water on the leave surface of leaves was absorbed by filter paper. Then, the samples were clamped by tweezers on the PDA plate, and 4-6 pieces of diseased samples were connected to each culture dish. The plate was inverted and cultured in a constant temperature incubator at 25℃. After 3-5 days of culture, hyphae grew on the surface of the diseased tissue, which was transferred to a new PDA plate in time. After single spore separation, purified strains were obtained, the strains were then purified and numbered.

Morphological identification

The purified strain HY-041 was transferred to the PDA medium and cultured at 25 ℃. The colony growth rate was observed regularly, and the changes in colony morphology and color were recorded. The hyphae, molecular spores, and conidiophore were observed under the optical microscope and photographed. According to the classification method of Fusarium by (Gerlach and Nirenberg 1982; Merjan 2017), the isolated strain was preliminarily identified.

Molecular identification and phylogenetic tree construction

The total DNA of pathogenic fungal was extracted by the CTAB method (Murray and Thompson 1980). PCR amplification was carried out with two sets of universal primers separately: ITS1 (5'-TCCGTAGGTG-AACCTTGG-3' and ITS4 5'-TCCTCCTCCGCTTTTGG-ATATGC-3') (Glass and Donaldson 1995); EF1-728F 5'-CATCGAGAGTTCGAAGG-3' and EF1-986R 5'- TACTTGAAGGAACCTTACC-3' (Lawrence et al. 2013). Primers were synthesized by Shanghai Sangon Biotech Co., Ltd. PCR reaction system was (25 μL): 0.5 μL of positive and negative primers (10 μmol/L), 0.5 μl of DNA template, 2.5 μL 10 × PCR buffer, 0.2 μL Taq enzyme, supplemented by ddH2O. PCR cycle settings are as follows: pre-denaturation at 94 ℃ for 5 min, denaturation at 94 ℃ for 45 s, annealing at 55 ℃ for 45 s, extension at 72 ℃ for 1 min, 30 cycles, extension at 72℃ for 10 min, and heat preservation at 4 ℃; after amplification, electrophoresis detection was carried out, and the purified product was recovered by SanPrep column DNA J gel recovery kit (SK8131, Shanghai Sangon) and sent to Shanghai Sangon for two-way sequencing. The obtained sequencing results were corrected by chromas.exe software, and the DNA sequence with the same homology as the strain HY-041 sequence was searched by NCBI Blast. Clustal X 1.8 and MEGAX 7.0 were used for comparative analysis of sequences, neighbor-joining (NJ) was used for cluster analysis of rDNA-ITS and EF-1α sequences, and a phylogenetic tree of polygenic sequences was constructed (Rahjoo et al.2008). Moreover, the bootstrap method was used to test, repeated 1,000 times, to analyze the genetic relationship between the strain and different strains of the same genus.

Inoculation experiments on different weeds in *vitro*

The purified strain was screened by in *vitro* leaf test, and the plate of the purified strains was punched with a hole punch (Φ = 8 mm) in a sterile operating table after disinfection. Weed *C. album*, *E. densa*, *P. lapathifolium*, *M. verticillata*, *G. aparine*, *P. aviculare, A. retroflexus, A. fatua* and the leaves of *T. arvense* were placed in a Petri dish (Φ = 90 mm) covered with filter paper, soaked with sterile water to provide a humid environment. Then, the fungal mycelium was placed on the leaves and cultured in a constant temperature incubator at 25℃. Each treatment was repeated three times, and PDA without fungal was used as the control. After 7 days, the diseased spot area was observed: the diseased spot area = 1/4 × length × width × 3.14. According to reference(Zhu et al.2004). the degree of the disease was classified as follows: Grade 0: the leaves have no disease spots; Grade 1: diseased spots on leaves; Grade 2: the lesion spreads to 1/3-2/3 of the leaves; Grade 3: more than 2/3 of the leaves are necrotic; Grade 4: all leaves are dead and discolored.

Herbicidal activity of HY-041 on different weeds in *vivo*

Nine field-grown weeds (*C. album*, *E. densa*, *Galium aparine*, *M. verticillata*, *P. lapathifolium*, *A. retroflexus*, *Thlaspi arvense*, *Avena fatua*, and *P. aviculare*) were transplanted into flowerpots (Φ = 15 cm) and cultured in greenhouses until they grew normally. On the fungal colony cultured for 7 days, the fungal mycelium with a diameter of 8 mm was taken with a sterile hole punch and then cultured in 250 mL of sterilized PDB solution for 5-7 days. The cultured fermentation filtrate was diluted to 1.0 × 105 CFU/mL 1.0 × 107 CFU/mL. The filtrate was poured into a watering can sterilized with 75% alcohol, 3 mL of Tween 80 was added, and the filtrate was sprayed and inoculated to six kinds of weeds that grew normally. The inoculation amount was controlled to 20-25 mL/pot. Taking the healthy plants exposed to sterile water as the control, repeating each treatment three times, using plastic film for moisturizing culture within 24 h after inoculation, and observing the disease of plants after 7 days (Puja and Sumitra 2013).

The herbicidal activity of the strain HY-041 was evaluated according to the following formula described by Fang (1998):

Disease Incidence (%) = (Number of diseased plants/Number of investigated plants) ×100.

Fresh weight inhibition (%) = [1- (Fresh weight of treatment/Fresh weight of Control)] ×100.

Disease index = (number of sick leaves × number of corresponding grades)/(total number of leaves investigated × number of leaves in highest grade) × 100%.

Safety evaluation of HY-041 on major crops

Five main crops in Qinghai (*Triticum aestivum* L., *Pisum sativum* L., *Hordeum vulgare*, *Vicia faba* L. and *Brassica napus* L.) were planted in Φ =12 cm pots and cultured indoors for use. The HY-041 strain was diluted and inoculated into three to four leafy plants with three repetitions per treatment. The method of inoculation was consistent with that of the HY-041 strain for different potted weeds. After 7 days of

inoculation, the incidence of the tested crops was observed, and the incidence degree of the crops was recorded (Zhu et al. 2017). The safety evaluation criteria of crops were as follows: NS means that the plant is asymptomatic (no disease spots, normal growth of the plant); LS indicates slight influence (scattered patches on the leaves, slightly controlled growth and development); MS means moderate susceptibility (1/5-1/4 of the leaf area has disease spots and growth is inhibited); SS stands for severe disease (a large number of plants die and their growth and development are severely controlled).

Observation of pathogenic process of *A. fatua* leaves by strain HY-041

The filter paper and weed leaves were putted in a sterilized Petri dish (Φ = 90 cm), the filter paper was soaked with sterile water to provide a humid environment, and placed the mycelial block(Φ = 8 mm) in the center of the leaves with a hole punch, with sterile PDA medium cake as the control. Each treatment and control were repeated three times. The experiment was carried out at 25-28 ℃. After mycelium blocks of the strain were inoculated for 1-7 days, 3-6 leaf segments (0.5-1.0 cm) were prepared with fresh shaving blades of each inoculated leaf, fixed in 2.5% (volume/volume) glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) at room temperature for 2 hours under vacuum condition, and fixed at 4℃ in the same buffer. The leaf samples were washed three times with 0.1 M phosphate buffer (pH 7.2) and dehydrated for 30 minutes in each gradient by graded ethanol series (70%, 80%, 90%, and 100%). Drying the sample with liquid CO2 at the critical point. The material was coated with a layer of 10 nm gold/palladium and observed by a scanning electron microscope.

Statistical analysis

During this study, all of the inoculation treatments involving herbicidal activity tests in *vivo* and in *vitro* and the safety evaluations were conducted in triplicate. The values are the means of each treatment. Data parameters in a year regarding the disease incidence, disease index and fresh weight inhibition were analyzed within a single factor design using DPS 7.05 software at a 5% level of probability and compared using Duncan's multiple range test. The standard error was also calculated and is represented in all the figures in this study.

Results and analysis

Morphological identification

In Fig. 1, after strain HY-041 was cultured on PDA medium for 7 days, its colony was slightly round and raised, its surface was like cotton, the edge was neat, the hyphae were dense, and the front was pink or magenta. The aerial hyphae was dense, and growed rapidly; the colony diameter reached 8.5 cm after 7 days (Fig. 1A and D). Under the microscope, HY-041 microspore was oval, with 0-1 septa, and its size was

3.24-9.96 μm × 1.58-3.63 μm; the macroconidia were sickle-shaped or long cylindrical, with a size of 9.72-15.82 μm × 3.75-6.86 μm and 3-6 septa (Fig. 1B, C and E, F). According to the morphological observation, the pathogen was preliminarily identified as Fusarium sp according to the above characteristics.

Molecular biological identification and phylogenetic tree construction

The rDNA-ITS and EF1a gene sequences of strain HY-041 were analyzed by PCR amplification, sequencing, and homologous comparative analysis, resulting in two gene segments of 384 bp (ITS) and 289 bp (EF1α), respectively. The login numbers are OP799841 and OP805349 respectively. The BLAST comparison in NBCI found that the sequence similarity between this strain and *F. avenaceum* (KU852640/JF278604) was 99%, and 46 strains with different similarities were selected. MEGA7.0 was used to construct phylogenetic tree, and *Alternaia brassicae* was used as the external group to construct phylogenetic tree. It is found that the support rate of the two gene sequences with *F. avenaceum* was as high as 99%, and they clustered into one branch on the phylogenetic tree (Fig. 2). Combined with morphological characteristic, the strain was identified as *F. avenaceum*.

Pathogenicity of *F. avenaceum* HY-041 to weeds leaves

As shown in Fig. 3 and table 1, after strain HY-041 was inoculated with in *vitro* leaf 7d, 9 kinds of weed leaves showed different degrees of pathogenicity. The largest spot produced on *P. lapathifolium* was 7.15 cm2, followed by *C. album* at 6.76 cm2, with a large number of white mycelium growing on the inoculated part of the *C. album* leaf and wilting of the leaf; Penetrating growth of mycelium on the reverse side of the leaf of *E. densa*, covering the back of the leaf and extending over the entire leaf, resulting in dark brown and decaying leaves; *P. lapathifolium*, *M. verticillat*a, *A. fatua* and *P. aviculare* had yellowing extending along the veins at the inoculum site and large numbers of white mycelium growing on the leaves of four weed species, with severe necrotic symptoms on the leaves; Infestation and spot production on the inoculated areas of *A. retroflexus*, *G. aparine* and *T. arvense* are surrounded by yellowish, greenish symptoms with gradual yellowing and wilting at a later stage. The pathogenicity of HY-041 mycelia blocks to different detached leaves after inoculation of the strain HY-041 for 7 days was as follows: *P. lapathifolium* > *C. album* >*M. verticillata* > *A. fatua* > *E. densa* > *P. aviculare* >*G. aparine* > *A. retroflexus* > *T. arvense*.

Table 1 symptoms of leaves of 9 weeds after inoculation with strain HY-041

Weeds leaves	Incidence area	Incidence characteristics
C. album	6.76±0.37	Plenty of powdery white mycelium was produced at the inoculation site of the leaf, and the leaf faded and wilted
E.densa	4.35±0.46	2/3 of the leaf blade wilted and blackened with a few mycelium attached to the leaf surface
P. lapathifolium	7.15±0.15	Yellowing and wilting of the leaves, with a large number of mycelium attached to the leaf surface and brown spots produced
M. verticillata	6.26±0.11	Yellowing of the leaves, production of large amounts of white mycelium at the inoculation site, fading of the leaves to yellowish-brown
A. retroflexus	3..56±0.71	Leaf blade inoculation sites with spots and rot.
G. aparine	0.87±0.31	White mycelium production at inoculation site, followed by chlorosis of leaves
A. fatua	6.27±0.22	Most of the leaves faded green to yellowish-brown, leaves covered with large amounts of white mycelium
T. arvense	0.92±0.16	Large amount of white mycelium attached to the inoculation site, leaves fade to green and then wilt
P. aviculare	4.80±0.13	Produces irregular yellow-brown spots with mycelium causing yellowing and crinkling of the entire leaf

Potting Pathogenicity Determination of *F. avenaceum* HY-041

In Fig. 4 and Table 2, the fermentation filtrate of strain HY-041 was sprayed onto the transplanted healthy weed plants. The results showed that after 7 days of inoculation, *C. album* and *E. densa* had the most serious disease, and a large area of dark brown spots appeared after inoculation. In the later stage, the spots gradually spread to all leaves, and all the plants withered and died, with the morbidity and disease indexes reaching 94.7% and 92.83%, respectively, for *C. album* and 89.13%, 93.17%, respectively, for *E. densa*. The inhibition rates of HY-041 on the fresh weight of two weeds were both over 90.0%. *P. lapathifolium* showed that a large number of disease spots appeared on the leaves, and the bottom leaves withered and fell off, with the morbidity and disease indexes of 72.93% and 62.57%, respectively. The leaves of *M. verticillat*a plants were curled, the stems and leaves were yellow and dry, and the symptoms at the edge of leaves were aggravated. Two-thirds of leaves of *A. fatua* showed yellow lesions, and finally the whole pot died, with the incidence rate of 93.13%. *A. retroflexus, G. aparine, T. arvense*, and *P. aviculare* showed slight disease, and some leaves were curled and yellowed. Combined with the comprehensive analysis of incidence rate, fresh weight control effect, and fresh weight control effect

(Table1), the strain HY-041 demonstrated the best control effect on *C. album*, *E. densa*, and *A. fatua*, followed by *P. lapathifolium*, but it had no obvious control effect on *M. verticillata*, *A. retroflexus*, *G. aparine*, *T. arvense*, and *P. aviculare*.

Table 2 Pathogenicity of fermented filtrate of HY-041 strain on different potted weeds

Weeds species	Disease incidence/%	Disease index	Fresh weight inhibition /%
C. album	94.7±1.45a	89.13±2.70ab	92.83±2.11a
E. densa	95±2.41a	93.17±1.81a	92.7±1.26a
P. lapathifolium	72.93±5.35b	62.57±3.43bc	83.4±2.53a
M. verticillata	53.3±3.85c	64.17±2.28bc	49.47±2.96bc
A. retroflexus	58.4±3.53c	50.17±3.17c	40.2±1.63c
G. aparine	51.7±2.74c	46.5±1.56c	52.03±4.21bc
A. fatua	93.13±3.03a	56.1±10.4c	69.1±15.67ab
T. arvense	57.13±2.64c	69.3±19.65abc	81.5±15.57a
P. aviculare	60.47±3.97c	52.13±2.40c	48.03±2.64bc

Note: The data in the table are averages ± standard deviations. Different lowercase letters after the same column of data indicate that the P<0.05 levels are significantly different from those tested by Duncan's new compound polarity method.

Safety evaluation of *F. avenaceum* HY-041 on major crops

As shown in Figure. 5 and Table 3, The fermentation filtrate of strain HY-041 was inoculated continuously for 2 days. after 7 days, the fermentation broth of strain HY-041 had no pathogenicity to *P. sativum* and *V. faba*, regardless of the crop growth and plant height. Compared with the blank control plant, it showed no susceptibility (NS). *B. napus* had moderate pathogenicity; 7 days after inoculation, 45% of leaves were infected, their growth was inhibited, and they wilted and lodged, showing moderate susceptibility (MS). It infected *T. aestivum* and *H. vulgare*, and there were a few needle-shaped black spots on the inoculated top leaves, with plant height inhibition rates of 4.6% and 2.1%, respectively. In the later stage, the disease spots did not expand, showing light susceptibility (LS).

Table 3 Susceptibility testing of the test crop to strain HY-041

Crops tested	Inhibition rate of height	Disease rate	Disease severity
20. *aestivum*	4.6	5	LS
2. *napus*	37.2	45	MS
V. faba	0	0	NS
16. *sativum*	0	0	NS
8. *vulgare*	2.1	3	LS

Note:Table NS indicates that the plant is asymptomatic (no spots, normal growth); LS represents a slight influence (scattered patches on the leaves, and growth and development are slightly controlled); MS means moderate susceptibility (1/5-1/4 of the leaf area has disease spots and growth is inhibited).

Electron microscope scans of the pathogenic process of *A. fatua* by strain HY-041

Fig. 6 shows that the strain HY-041 infected *A. fatua* leaves and their ultrastructure. The cell structure of uninoculated healthy *A. fatua* leaves was normal, and the tissues were arranged in order (Fig. 6-A). One day after inoculation of *A. fatua* strains, hyphae invaded the stomata, and the tissues were not damaged (Fig. 6-B). After 2-3 days, there were hyphae on the tissue surface of *A. fatua* penetrating the stomata, and many hyphae were distributed around the stomata (Fig. 6-C and D). After 4-5 days, the tissue surface was slightly damaged, hyphae and spores parasitized on the tissue surface to absorb nutrients, and the plants showed symptoms. It is speculated that the strain HY-041 entered the stomata on the surface of *A. fatua* leaves to complete the infection process. After infection, plant tissues were diseased, and a large number of spores were visible on the surface of the damaged tissues (Fig. 6-E and F). After 6-7 days, with the infection of pathogens, the metabolism of leaf tissues of *A. fatua* became disordered, and the infected cells gradually died (Fig. 6-G and H).

This study isolated an strain HY-041 from the leaves of naturally infected *Herba Epimedii,* and identified the pathogen as *F. avenaceum* by morphological and molecular biology (rDNA-ITS, EF1a). Inoculating isolated leaves *withfungall* cake was selected to explore the herbicidal activity of strain HY-041 in the early stage. The method confirmed that the strain has different pathogenicity to nine weeds: *C. album*, *E. densa*, *G. aparine*, *M. verticillata*, *P. lapathifolium*, *A. retroflexus*, *A. fatua*, *T. arvense* and *P. aviculare*. In the pot experiment, live weeds were inoculated with their fermentation filtrate. The results showed that HY-041 fermentation filtrate controls *C. album*, *E. densa* and *A. fatua.* The crop safety test showed that the

strain is moderately susceptible to B. napus, slightly infects *H. vulgare* and *T. aestivum*, and is relatively safe to P. sativum and *V. faba*.

Discussion

The classification system based on morphology makes it challenging to accurately classify and identify these difficult species, and sometimes it is necessary to identify them by using biology and molecular biology. Hsuan et al. (2010) Establishing phylogenetic species based on molecular biology makes up for the deficiency of morphological identification and reflects the phylogenetic relationship of Fusarium more scientifically. Guadet et al. (1989) first identified Fusarium by molecular biology. In recent years, the emerging methods of evolution have improved the accuracy and scientificity of the classification and identification of Fusarium, such as through the methods of internal transcribed spacer (rDNA-ITS) sequence in ribosomal DNA, translation elongation factor (TEF-1α) gene sequence, the second largest subunit (RPB2) gene sequence of RNA polymerase II, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique, random amplified polymorphic DNA analysis (PCR-RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat polymorphism (ISSR), and other nucleotide sequence analysis techniques and labeling techniques (Vaux et al. 2020; Suvi et al. 2019). For example, Geiser et al. (2004) established a database of Fusarium based on partial sequences of the TEF-1α gene, where researchers can easily identify strains to species by using their gene sequences. Meanwhile, the method of using molecular markers or single-copy genes to design species-specific primers to quickly identify related species in Fusarium has also been obtained. Mulè et al. (2004) amplified Fusarium spp. isolated from corn in Europe and America with three pairs of species-specific PCR primers, and identified three species: *F. subglutinans, F. proliferatum* and F. verticillioides. Nicholson et al. (1998) used RAPD technique to find and design species-specific PCR primers to identify *F. culmorum* and *F. graminea-rum* in wheat. Yang et al. (2012) further identified the isolated strains using specific primers FOF1/FOR1 and fungi rDNA-ITS, and the results showed that *F. oxysporum* is the pathogen of tomato, eggplant, and pepper. The present study examined the species identification and phylogenetic problems of the strain HY-041 isolated from *C. setosum* by combining the morphological characteristics of rDNA-ITS and EF-1α gene sequences. Thus, it is easier to understand the genetic characteristics and evolution of *F. avenaceum* strain at the molecular level.

Electron microscope technology is widely used in plant resistance physiology and plant histopathology after pathogen infection (Bello et al., 2017; Singh et al., 2014). In order to further understand the infection process of the strain HY-041 on *A. fatua*, this study observed the ultrastructural changes of *A. fatua* after infection by scanning the electron microscope and studied the pathogenic mechanism of interaction between *F. avenaceum* and *A. fatua* from a cytological point of view. Scanning electron microscope observation showed that the hyphae of HY-041 invade *A. fatua* tissue through stomata, parasitize, propagate in the tissue to produce spores, and gradually destroy the tissue. The infected wild *A. fatua* grows abnormally, resulting in disease. Ultrastructure showed that the mesophyll tissue of *A. fatua* is significantly damaged, indicating that the strain HY-041, as a potent pathogen of *A. fatua*, has the potential as a fungal herbicide to control *A. fatua.*

Fusarium is established based on *Fusarium roseum*, one of the most critical pathogens in crops, seriously affecting the growth and development of cereal crops and food production. There are many species of Fusarium, and more than 500 species of Fusarium have been reported so far. It is a kind of filamentous fungus parasitic in plants or soil, and it is a common soil habitant, which can cause many plant diseases described

by Kai et al. (2015). For example, F. verticillioides can cause sugarcane sheath rot (Lin et al., 2014), F. *solani* and *F. sambucinum* can cause potato dry rot (Khalil and Al-Mughrabi 2010), *F. oxysporum* can cause tomato wilt (Ramsey et al., 1992; Does et al., 2010), and *F. graminearum* can cause wheat scab (Wang et al., 2005). Besides, Fusarium is also widely used in biological control. (Wang et al. 1985) isolated *Fusarium orobanches* from natural diseased rhododendron and developed a biocontrol agent F798 to control rhododendron. (Heiko et al., 1999; Jyothi et al., 2010) showed that *F. oxysporum* could be used to control sunflower root parasitic weeds *Orobanche cumana* and *Echinochloa crusgalli*. Shao et al. (2019) isolated F. *equiseti* from the leaves of naturally infected Urtica *cannabifolia*. The strain's mycelium and crude toxin have a strong control effect on the malignant weed Urtica *cannabifolia*, and its weeding potential has been proved. There are few reports on applying *F. avenaceum* in biological weeding, which is of particular value for the isolation of *F. avenaceum* HY-041 and further study on weed control.

In this study, the pot experiment results showed that the *F. avenaceum* strain HY-041 significantly controls *C. album*, *E. densa*, and *A. fatua*. The crop safety experiment showed that the strain is moderately susceptible to B. napus, slightly infects *H. vulgare* and *T. aestivum*, and is safe to P. sativum and *V. faba*. It could be used as a candidate strain to control common weeds in Qinghai for further research. Our experiment preliminarily carried out the pathogenicity, crop safety evaluation, strain identification, and scanning electron microscope of isolated leaves and living potted plants. In the next step, we systematically studied the biological characteristics, weeding mechanism, field verification, herbicidal spectrum, dosage form research, and isolation and identification of active substances of pathogenicfungal, which provides alternative resources for the research and development of natural herbicides.

Declarations

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Conflict of Interests Statement

The authors declare that they have no conflict of interest.

Data Availability Statement

All data used for analyses are available from the corresponding author upon request. Both sequences in this manuscript were uploaded to the National Biotechnology Information Center under the login numbers OP799841 and OP805349.

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Figures

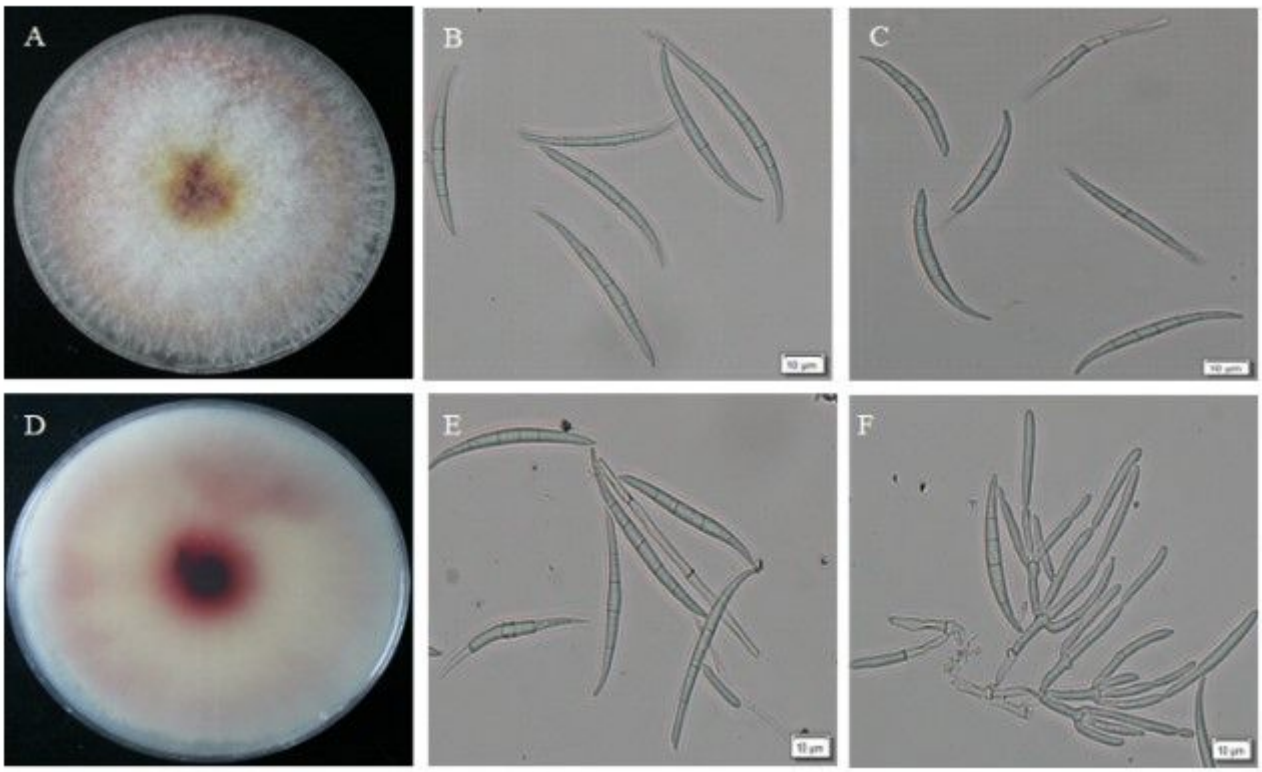


Figure 1

Morphological characteristics of strain HY-041

A and D, strain HY-041morphology; B, C, E, and F, HY-041 conidia and conidia stalk

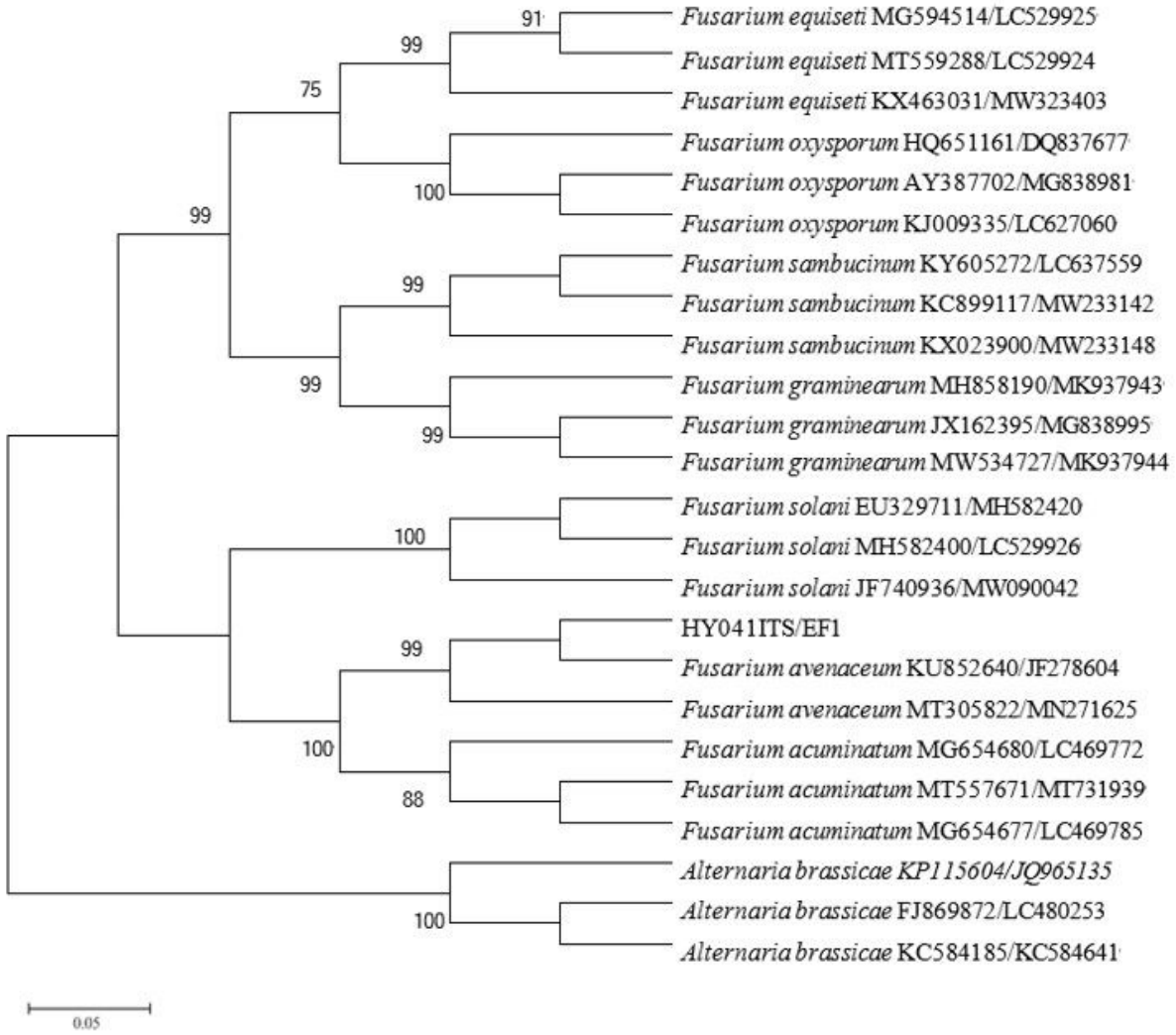


Figure 2

NJ phylogeny tree based on the combination of rDNA-ITS and EF-1a gene sequences

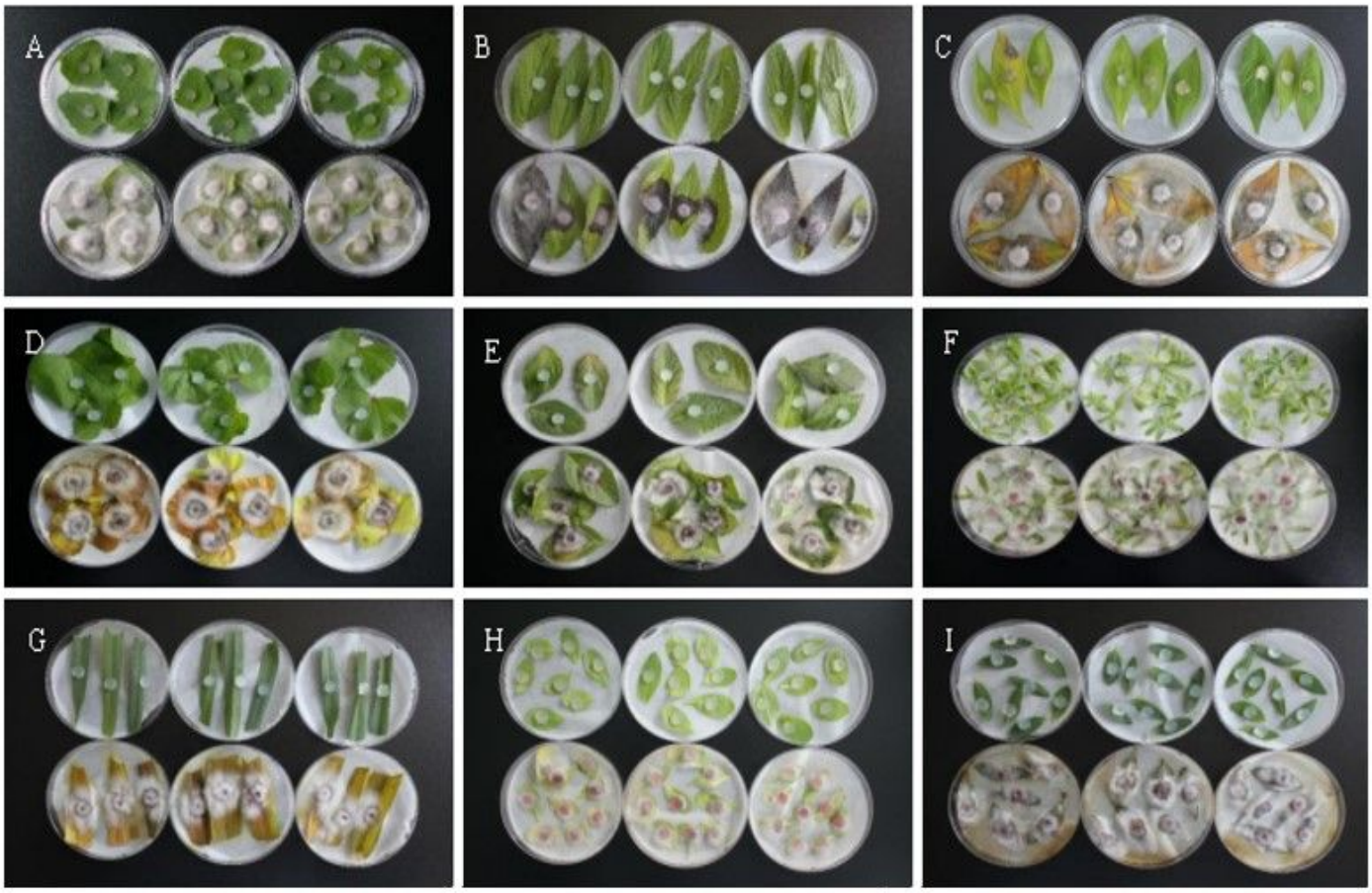


Figure 3

pathogenicity of strain HY-041to isolated leaves of 9 weeds (7 days after inoculation)

A, *C. album*; B, *E. densa*; C, P. lapathifolium; D, M. verticillata; E, A. retroflexus; F, *G. aparine*; G, *A. fatua*; H, T. arvense; I, P. aviculare;

Figure 4

Pathogenicity of strain HY-041 to potted weeds (7 d inoculation)

A, *C. album*; B, *E. densa*; C, *P. lapathifolium*; D, *M. verticillata*; E, *A. retroflexus*; F, *G. aparine*; G, *A. fatua*; H, *T. arvense*; I, *P. aviculare*;

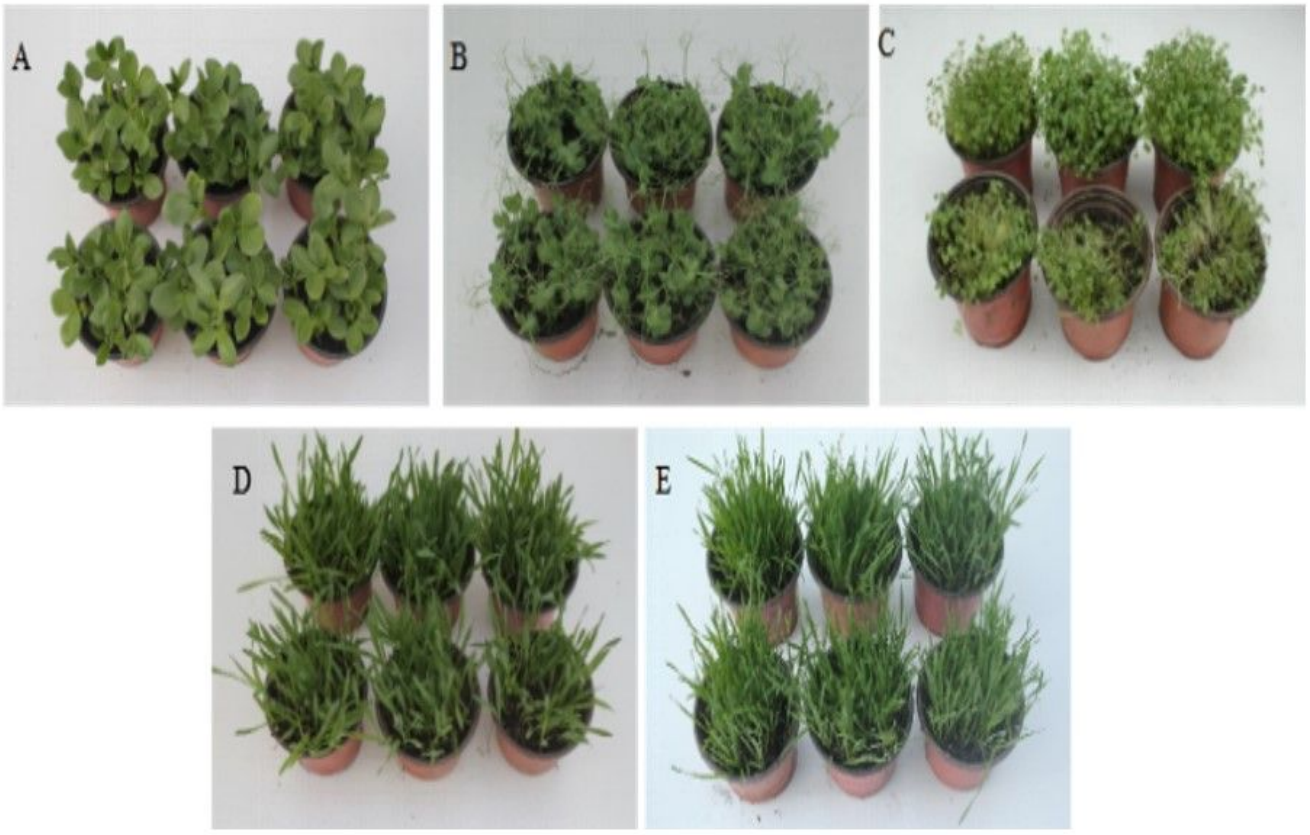
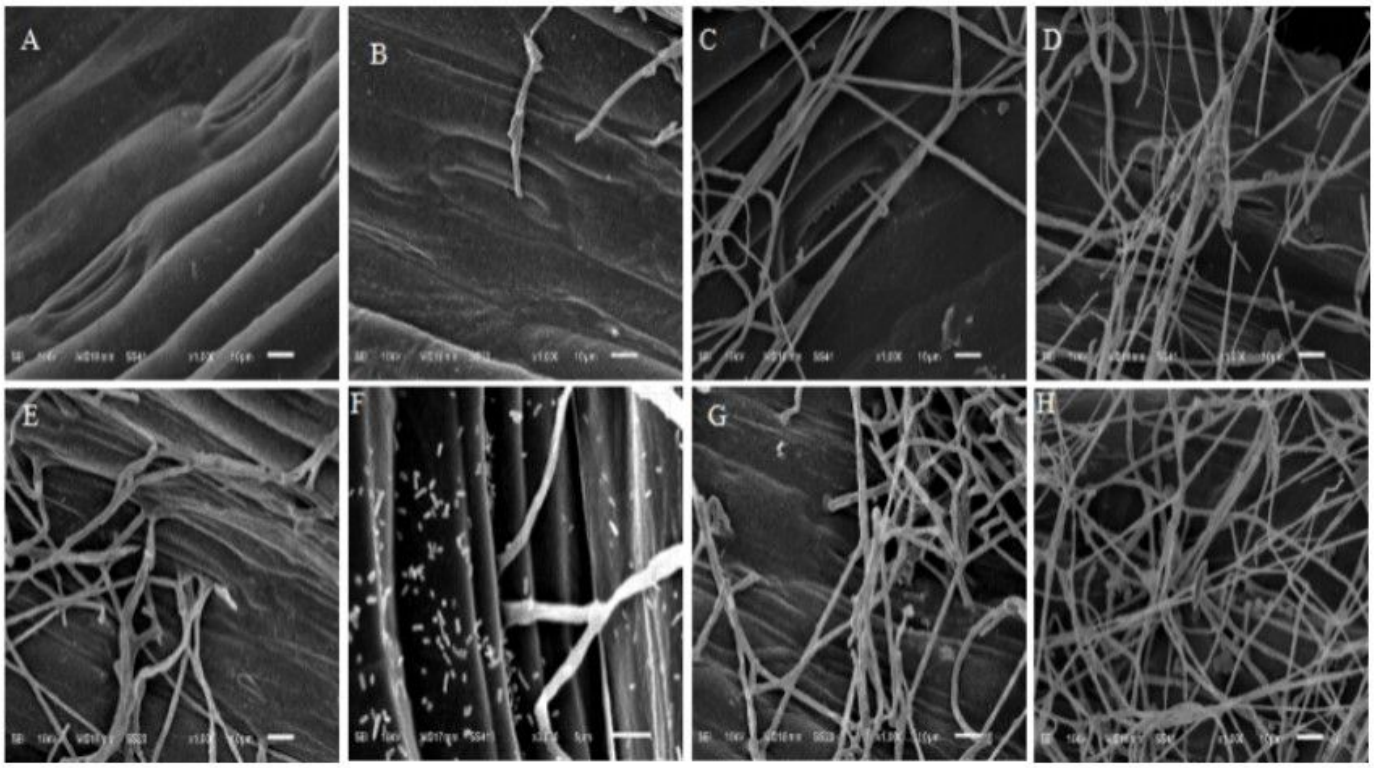


Figure 5

Crop safety test of strain HY-041 (7 d after inoculation)

A: *Vicia faba*; B: *Pisum sativum*; C: *Hordeum vulgare*; D: *Triticum aestivum*; E: *Brassica napus*



A.CK, B. 1d, C. 2d, D. 3d, E. 4d, F. 5d, G. 6d, H. 7d

Figure 6

Scanning electron microscope observation of the characteristics of strain HY-041 invading the leaf tissue *A. fatua*