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Comprehensive evaluation of the allelopathic potential of Elymus nutans

Xiaolong Quan[1], youming qiao[1], Mengci Chen[1], zhonghua duan[1], and huilan shi[1]

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April 20, 2021

Abstract

Drooping wildryegrass (Elymus nutans) has been widely planted together with other perennial grasses for rebuilding degraded alpine meadow atop the Tibetan Plateau. However, the rebuilt sown pastures begin to decline a few years after establishing. One of the possible causes for degradation of sown grassland may come from allelopathy of planted grasses. The purpose of this study was to examine allelopathic potential of drooping wildryegrass. Three types aqueous extracts from drooping wildryegrass and its root zone soil were prepared and 5 highland crops and 5 perennial grasses were used as recipient plants. Drooping wildryegrass exhibited strong allelopathic potential on germination and seedling growth of 5 crops germination and growth, but different crops or perennial grasses respond to the extracts differently. The pieces extract has stronger inhibition than others. Hulless barley, oat and Chinese fescue were the most affected, while quinoa and Siberian wildryegrass were the least affected. Drooping wildryegrass presented less influence on Kentucky bluegrass and crymophylla bluegrass than on Chinese fescue. It is recommended that the species combination of mixture for restoration should consider allopathic effects of the co-seeding and decrease the seeding rate ratio of drooping wildryegrass. The annual dicot crop quinoa and rape seeds can be used as alternative subsequent crop for seed field of drooping wildryegrass monoculture.

Comprehensive evaluation of the allelopathic potential of *Elymus nutans*

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Drooping wildryegrass (*Elymus nutans*) has been widely planted together with other perennial grasses for rebuilding degraded alpine meadow atop the Tibetan Plateau. However, the rebuilt sown pastures begin to decline a few years after establishing. One of the possible causes for degradation of sown grassland may come from allelopathy of planted grasses. The purpose of this study was to examine allelopathic potential of drooping wildryegrass. Three types aqueous extracts from drooping wildryegrass and its root zone soil were prepared and 5 highland crops and 5 perennial grasses were used as recipient plants. Drooping wildryegrass exhibited strong allelopathic potential on germination and seedling growth of 5 crops germination and growth, but different crops or perennial grasses respond to the extracts differently. The pieces extract has stronger inhibition than others. Hulless barley, oat and Chinese fescue were the most affected, while quinoa and Siberian wildryegrass were the least affected. Drooping wildryegrass presented less influence on Kentucky

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Keywords

Allelopathy; Autotoxicity; Gramineae; Alpine meadow; Grassland degradation; Mixed sowing

1 INTRODUCTION

Ecological projects are essential for the adaptation and restoration of ecosystems in response to environmental changes and human disturbances (Cai et al., 2015; Dong et al., 2020). Revegetation not only contribute to the naturalization of the landscape, but also reduce soil erosion, and increase carbon capture and recreational functions of the territory (Lasanta et al., 2015). Degradation of alpine meadow atop Qinghai-Tibetan Plateau (QTP) has been one of great concerns of academy community, pastoralists, and government officials over past four decades (Qin, 2014; Dong et al., 2020). One of characteristics of degraded alpine meadow is that the sedge-dominant vegetation is replaced by unpalatable or poisonous forb-dominated vegetation (Santonja et al., 2019). The severely degraded alpine meadows are characterized by fragmented turf with denudated patches. Many trials have been conducted to select suitable forages that can be used in the alpine meadow region and turned out only some gramineous perennial grass can adapt to the local natural condition (Zhang et al., 2014; Zhang et al., 2017). No suitable perennial legumes have been selected to apply to the ecological restoration of degraded meadow (Shang et al., 2017).

Due to the impossibility to restore degraded alpine meadows with native dominant *Kobresia* plants, mix-seeding(mixture) gramineous perennial grass has been regarded as an ideal option for revegetation of degraded alpine meadow on QTP because it increases the diversity and stability of the planted community(Ma et al., 2002; Shi et al., 2009), though the pristine alpine meadow vegetation communities are mainly composed plant species of *Kobresia* family, such as *Koresia pygmaea* , *Koresia humilis* etc.(Qiao & Duan, 2016). Planting perennial grasslands on degraded alpine meadow can not only increase the land utilization rate and restore the degraded grassland vegetation as soon as possible, but also ease the grazing pressure on natural grasslands and prevent grassland degradation and desertification.

The primary grasses being planted for revegetating severely degraded alpine meadow are limited to few graminoid varieties, such as drooping wildryegrass (*Elymus nutans*), crymophila bluegrass (*Poa crymophila*), Kentucky bluegrass (*Poa pratensis*) and Chinese fescue (*Festuca sinensis*). Among these germplasms, drooping wildryegrass is the major seed material for ecological restoration and the ratio of seeding rate of drooping wildryegrass in mixture accounts for 50%. This reflects drooping wildryegrass' relative ease of establishment, high forage production and sufficient seed supply.

However, the established grassland with perennial grasses begin to decline within 2 to 4 years and causing economic and ecological loss (Dong et al., 2007). Some sown grassland becomes more severe degraded land (bare land), challenging the sustainable use of revegetated grassland in alpine regions (Shang et al., 2006). It is recorded that most monocropped perennial grasses can be utilized for around ten years in low altitude areas, but the yield of monoculture of perennial grasses in plateau area begin to decline 3 years after planting and much shorter than expected (Dong et al., 2010).

It is undeniable that re-degradation of sown pasture on the plateau may be caused by a combination of biotic and abiotic stresses. The interspecies (intraspecies) competition maybe an important cause (Lin et al., 2018). The authors argue that the decline or re-degradation of vegetated grassland resulted from interspecies competition between mix-seeding (co-seeding) plants, and between grasses and unpalatable forbs. The degradation of drooping wildryegrass monoculture may resulted from autotoxicity.

Given the fact that a lot of research on plant allelopathy focused on farmland crops and turfgrasses, and little information about allelopathy of perennial gramineous forage grass is available, we focus our attention to allelopathic effects of drooping wildryegrass on seed germination and seedling growth of mix-seeding plants

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and itself. We suppose that the concentration of allelopathicals is below the threshold value of inhibiting in the early stage of the establishment of community or monoculture and are conducive to plants that are invaded or co-planted. With the growth year, the allelopathicals accumulate in the soils, drooping wildryegrass not only outcompete co-seeding grasses but also release allelopathicals which enter the soil by leaching and inhibit the germination and the growth of seedlings of itself. The competitiveness of *Elymus nutans* is weakened, which provides opportunities for invasion of unplatable plants and further accelerates the degradation of seeded pasture and replacement of unwanted vegetation.

The objectives of this study were: (1) to evaluate the allelopathical potential of wildryegrass on highland crops, co-seeding grasses and wildryegrass itself; and, (2) to reveal the potential cause of sown grassland degradation and provide reference for sustainable use and management of revegetated grassland in alpine region.

2 MATERIALS AND METHODS

2.1 Plant and soil materials

Grass seeds of drooping wildryegrass, crymophila bluegrass, Kentucky bluegrass, Chinese fescue and Siberian wildryegrass (*Elymus sibiricus*) were harvested in September 2019 and provided by Tongde Forage Seed Production Base (35°15'N, 100deg38'E) of Qinghai Province, which is located at the eastern Tibetan Plateau. Crop seeds of hulless barley (*Hordeum vulgar* var. *nudum*), oat (*Avena sativa*), wheat (*Triticum aestivum*), quinoa (*Chenopodium quinoa*) and rape (*Brassica napus*) which have been harvested in 2019 were acquired from the Academy of Agriculture and Forestry Sciences, Qinghai University. These crops can grow well in the agricultural area of the province. All seeds were stored in the refrigerator at 4degC before experiment. Plants of drooping wildryegrass were collected in early August 2019, when the plant growth was in peak period. Robust whole drooping wildryegrass plants with roots were dig out with a small shovel. The plants were cut into aerial parts and below ground parts on site. The root zone soils were collected by shaking the roots parts. All plant and soil materials were put in plastic bags and kept in a refrigerator and taken back to the laboratory for experiment.

2.2 Preparing of extracts

Two kinds of plant extracts were prepared. The whole plant extracts were prepared by soaking whole fresh aboveground parts in distilled water (w/v, 1:4). The aqueous pieces extracts were prepared by cutting plants into 1-2 cm pieces and soaking them in distilled water (w/v, 1:4). The containers of soaked plant materials were shaken at 500 rpm for 5 minutes per 12 hours in 72 hours at room temperature. Soil extracts were prepared by soaking fresh root zone soil in distilled water (w/v, 1:2) and shaken at 200 rpm for 24 h in gyratory shaker at room temperature.

The resulting mixtures were passed through qualitative filter paper and 0.45 μm aqueous membrane, respectively. And thus 0.25 g·mL^{-1} plant extracts and 0.5 g·mL^{-1}root zone soil extracts were obtained. The solutions were stored at 4°C until use.

2.3 Germination experiment

All seeds were surface sterilized by soaking in 0.5% sodium hypochlorite solution for 10 min and rinsed several times with distilled water. Five replicates of 50 seeds were placed in Petri dishes (90×15 mm) lined with 2-layer filter paper. The seeds and filter paper were wetted with 3 mL distilled water or extract solution. To maintain moisture, the Petri dishes were put in plastic bags and placed in a growth chamber at temperature of 20/16°C for 12/12 h in light and dark period. The light intensity was 3000 lux. A seed was considered germinated when the root protruded [?]2 mm. Germination was counted at 24 h intervals over 15 days, and the first count was carried out on the fourth day.

2.4 Seedling growth experiment

To evaluate the effects of different extracts on seedling growth, a completely randomized block design with three replications was applied to conduct seedling growth tests on growth plate (5x8 cups; O 40 mm; height

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80 mm). The media was a mixture vermiculite/pearlite (50:50, v/v). Three pre-germinated seeds (5 days old) were transplanted into each cup, and ten cups were used as a replicate. The growth plates were place in growth chambers with temperature of 20/16degC for 12/12 h in light and dark period. The light intensity was 3000 lux. The moisture content of the media was maintained at a ratio of 1.5:1 solution or water to media on weight basis by spraying different plant extracts every three days. After 10 days of growth, the seedling with roots were carefully washed from media. Ten seedlings of a replicate were selected and the plumule length and radical length of each seedling were measured. The seedlings were oven-dried to constant weight at 65degC and weighed to obtain dry weights (mg*10 plants^{-1}).

2.5 Date analysis:

Germination force (GF), germination percentage (GP) and germination index (GI) were calculated according to the following equation (Wang, 2019):

GF (%) = Number of germinated seeds in first one-third of evaluation days / Total number of Seeds x100

GP (%) = (N_1+ N_2 + N_3... + N_{n-1}+ N_n)/ Total number of Seeds x100

GI = (N_1)/1+ (N_2)/2+(N_3)/3+ ... + (N_{n-1})/(n-1) + (N_n)/n

where, N_1, N_2, N_3, . . ., N_n: the number of germinated seeds in the first, second, and final count, and 1, 2, . . ., n was the first, second, and final evaluation days.

The allelopathic effect response index (RI) and comprehensive allelopathic effect response index (CE) were calculated using the Equation suggested by Williamson (1988):

RI_n =1 - C_n /T_n (T[?]C) or C_n /T_n– 1(T¡C)

CE= (RI_1+RI_2+RI_3+...+RI_n) / n

where T is the treatment value and C is the corresponding value of control. Positive values indicate stimulating effects, while negative ones indicate inhibitory activity of the aqueous extracts.

Experimental data were subjected to one-way analysis of variance (ANOVA). Statistical significant difference was assumed at the probability level of 0.05 and means were separated by using LSD.

3 RESULTS

3.1 Allelopathic potential of *Elymus nutans* to alpine crops

Germination force of the tested crops seeds to different extracts were dependent on crop varieties and extract types (Figure1a). Compared to control, the whole extract of drooping wildryegrass had no significant effect on the germination force of hulless barley, oat and rape, but decreased germination force of wheat and increased that of quinoa. Significant inhibitory effects of pieces extract on the germination force of hulless barley, oat, wheat, and rape were detected except quinoa. Root zone soil extracts reduced the germination force of hulless barley, oat, wheat and rape and increased that of quinoa.

All the three kinds of extract significantly reduced the germination percentage of tested crops as shown in Figure 1b. Oat, wheat and rape presented similar pattern and their germination percentage decreased by three kinds of extract compared to control, but no differences among the extract treatments. Pieces aqueous extracts of drooping wildryegrass significantly inhibited the germination of hulless barley and quinoa. Hulless barley germination was sensitive to pieces aqueous extracts.

Comparably, the three extracts have less effect on the germination index of rape and quinoa, in contrast, but they had greater impact on that of barley, oats and wheat (Figure 1c). The root zone soil extracts could improve the germination index of quinoa, but no effects on rape. Both whole plant extract and pieces extract could significantly decrease the germination index of hulless barley and oat. The effects of and the three extracts on the germination index of wheat varied with extracts.

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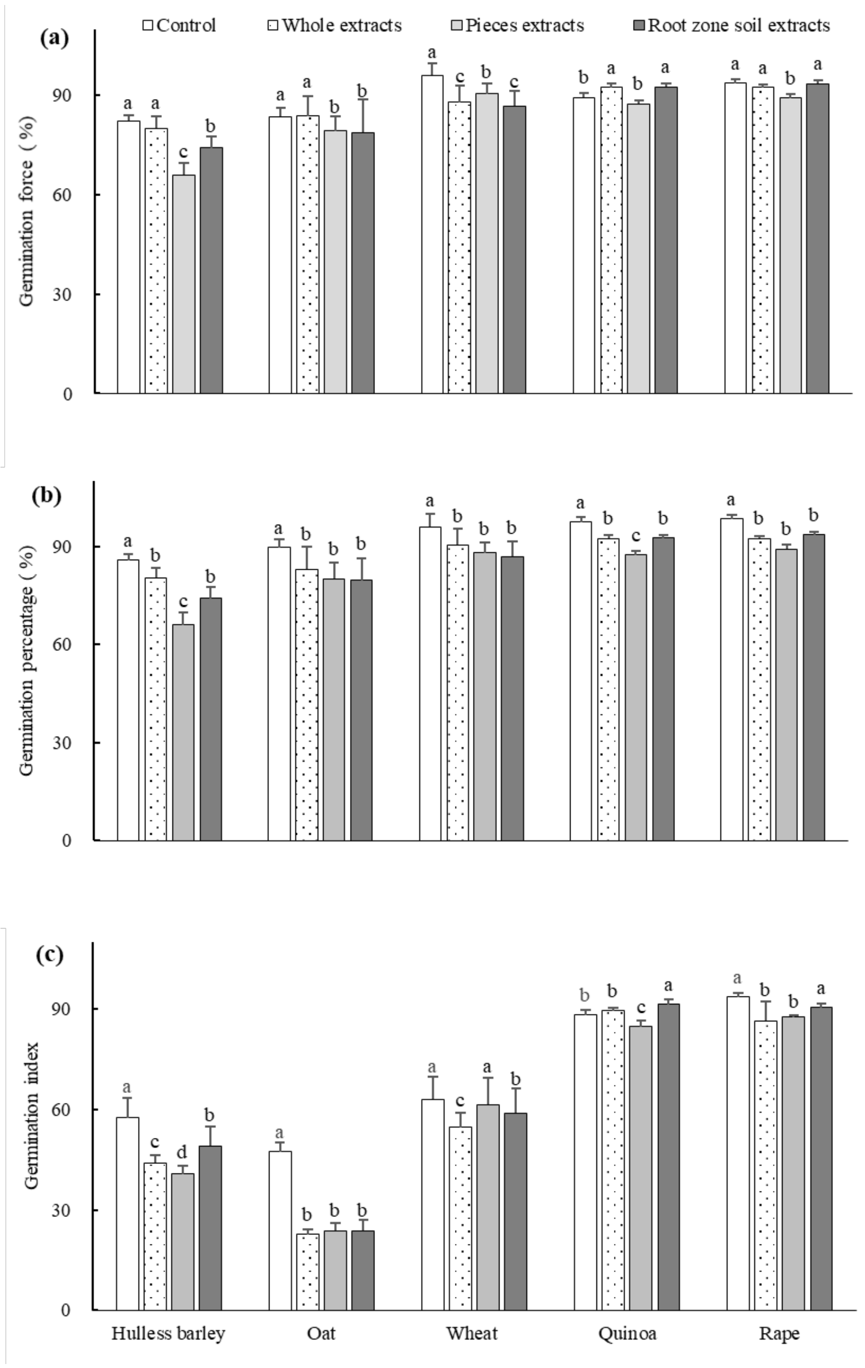

FIGURE 1 Effects of *Elymus nutans* extracts on germination of highland crops. Values are mean ± SD(n=5). Different letters denote significant differences between treatments with a > b > c > d, the same below.

Root length of hulless barley, oat, wheat and rape showed significant differences under three kinds of extracts, while there was no significant effect on quinoa (Figure 2a). The whole extracts exhibited stronger inhibitory effects than pieces and root zone soil extracts on root growth of hulless barley and wheat. No significant difference of root growth was detected among the three extracts treatments for oat and rape.

The shoot growth of hulless barley, oat and wheat were impeded by the three kinds of extracts, whereas quinoa and rape shoot were not sensitive to the extract (Figure 2b). There was no significant difference in the shoot growth of hulless barley among whole, pieces and root zone soil extracts. Oat shoot proved to be sensitive to the whole extracts of donor plants. The plant whole, pieces and root zone soil extracts exhibited 14.55%, 11.32% and 7.71%, allelopathic inhibitory on shoot growth of wheat.

Similar to the effects of extracts on shoot length and root length, seedling dry weight of hulless barley, oat, wheat and rape to three kinds of extracts showed differences with crops and extracts (Figure 2c). No significant difference was detected in dry weight of quinoa among different extract treatments. The whole and root zone soil extracts exhibited higher inhibition than pieces extract on seedling dry weight of hulless barley and wheat.

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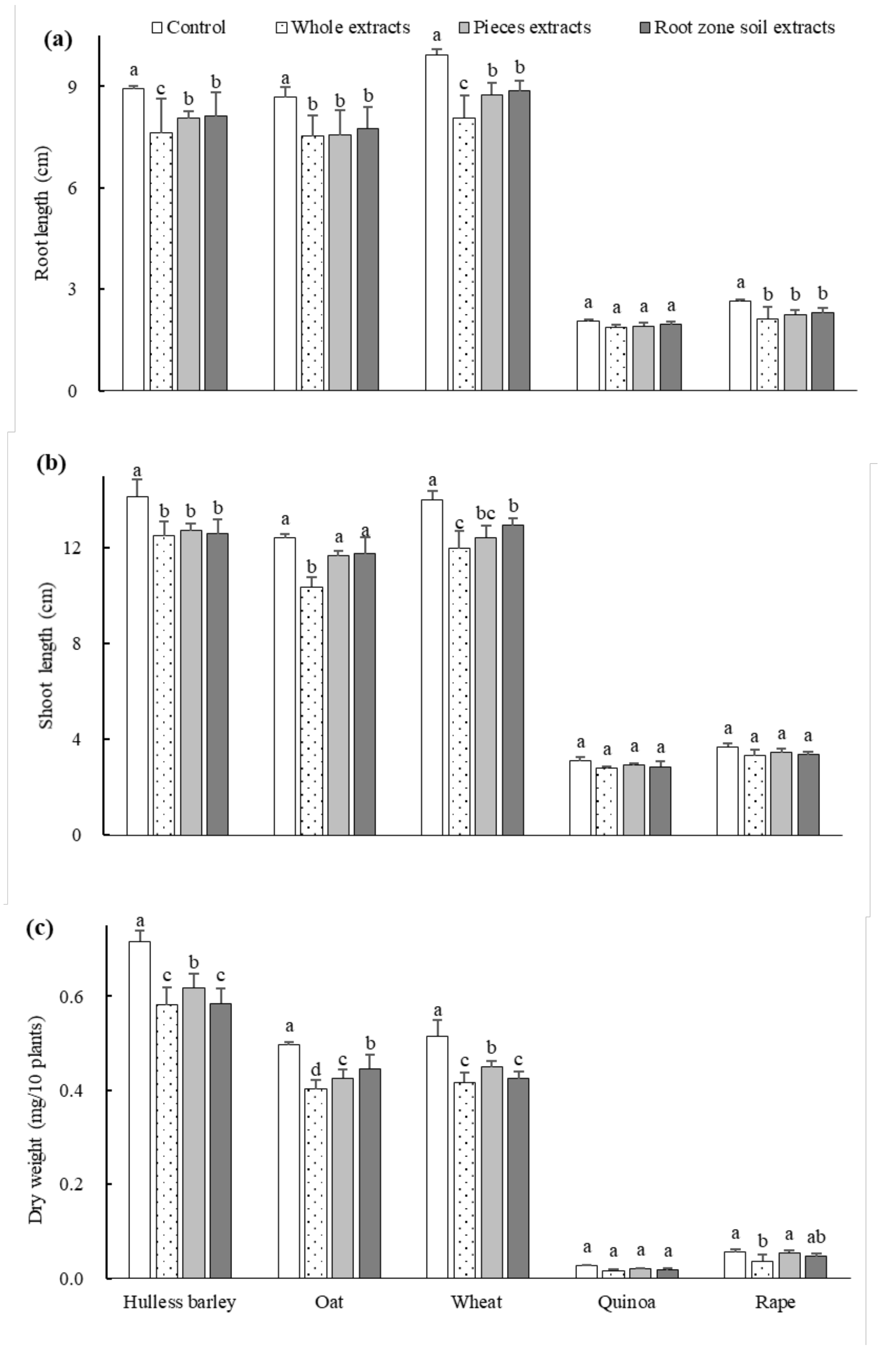

FIGURE 2 Effects of *Elymus nutans* extracts on growth of highland crops. Values are mean ± SD (n=5). Different letters denote significant differences between treatments with a > b > c > d, the same below.

The allelopathic effect response index and comprehensive allelopathic effect response index of drooping wildryegrass on the tested crops presented in Figure 3. The inhibition of the extracts on hulless barley and oat occurred in germination with germination allelopathic index of -0.391 and -0.210, respectively. Wheat, quinoa and rape occurred in seedling growth with growth allelopathic index of -0.173, 0.097 and -0.177, respectively. Oat exhibited the largest comprehensive allelopathic index of -0.265, and quinoa presented the smallest one -0.068.

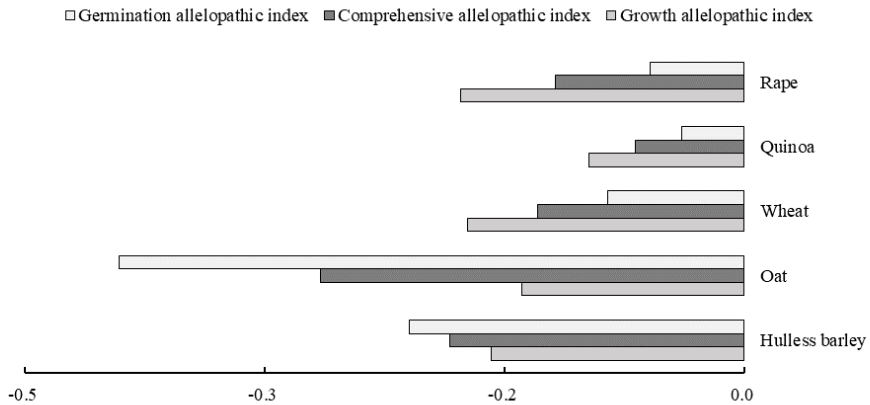

FIGURE 3 Comprehensive allelopathic effect index of*Elymus nutans* extracts on highland crops.

3.2 Autotoxicity of *Elymus nutans* and its allelopathic potential on perennial grasses

The effects of the three types of extracts on germination force of perennial grasses were shown in Figure 4a. The control's germination force of Siberian wildryegrass was greater than 65%, and that of other four grasses were less than 50%, suggesting Siberian wildryegrass seed has strong vitality and the emergence is uniform. The pieces extract treatment resulted significant decrease of germination force of all tested perennial grasses, with a biggest decrease by 58.8% of Chinese festuca and smallest decrease by 14.6% of Kentucky bluegrass compared to control. The pieces extracts treatment leads to significant decrease of germination force of Siberian wildryegrass, Chinese fescue and Kentucky bluegrass than those of whole extracts treatment. Root zone soil extracts had no significant allelopathic effect on germination force of Kentucky bluegrass and crymophila bluegrass, but significantly lowered the germination force of Siberian wildryegrass, Chinese fescue, and drooping wildryegrass itself.

The germination percentage of the control of the five perennial grasses were less than 70%. The germination percentage of perennial grasses was decreased by the three kinds of extracts (Figure 4b). The whole extracts decreased the germination percentage of drooping wildryegrass, Chinese fescue, Kentucky bluegrass and crymophila bluegrass except Siberian wildryegrass. The pieces extracts and root zone soil extracts showed significant inhibitory on germination percentage of Chinese fescue and crymophila bluegrass compared to control and whole extracts.

The germination indexes of all control were less than 40, indicating lower seed vigor of the tested perennial grasses (Figure 4c). The reduction of germination index by the whole extracts varied with donor plants. The biggest drop occurred in drooping wildryegrass and crymophila bluegrass. The pieces extracts reduced Siberian wildryegrass (38.1%), Chinese fescue (47.3%), and crymophila bluegrass (40.2%), significantly. The root zone soil extracts increased the germination index of Kentucky bluegrass by 24.3%, but decreased that of other four grasses.

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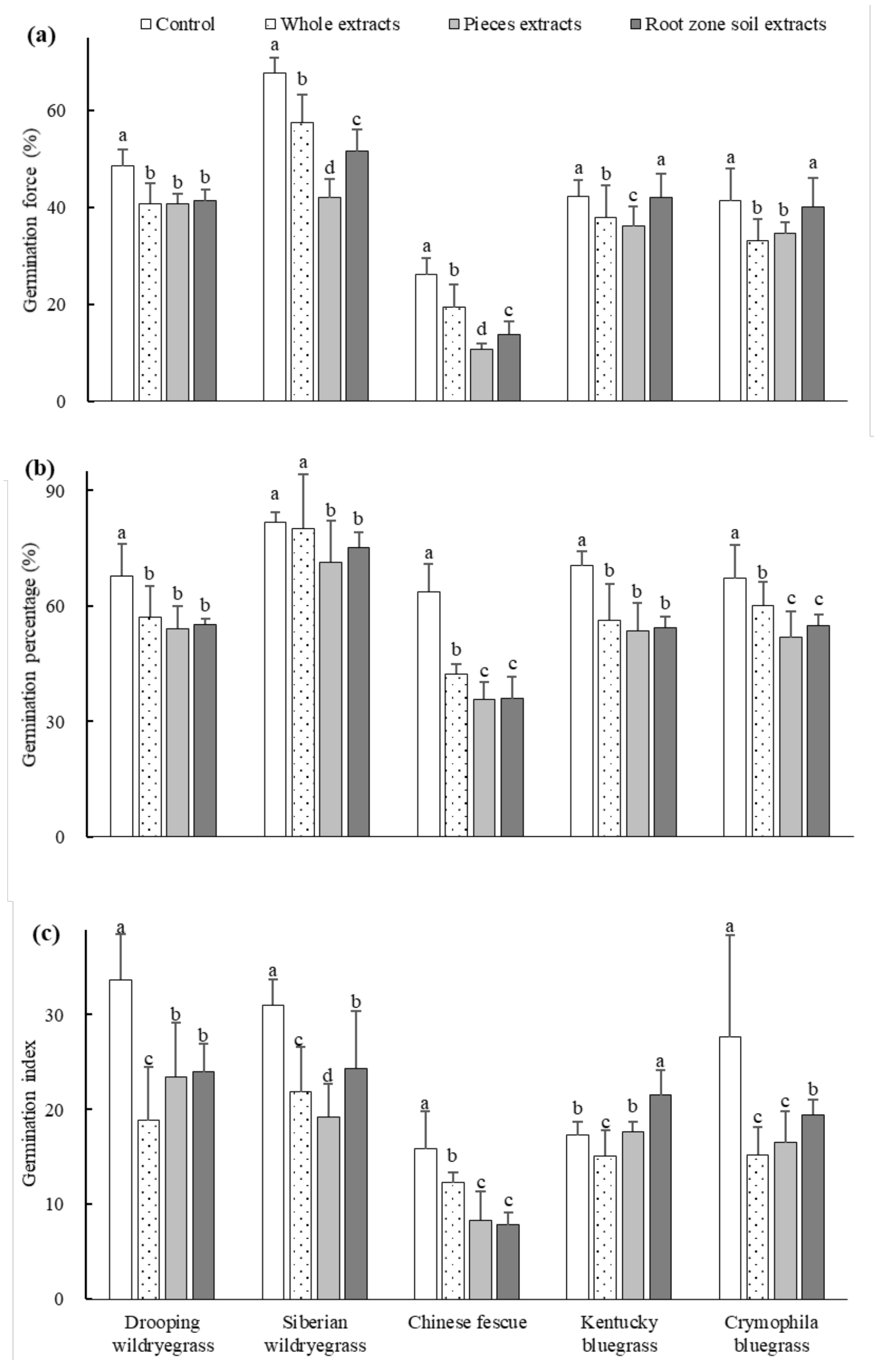

FIGURE 4 Effects of*Elymus nutans* extracts on germination of highland grasses. Values are mean ± SD(n=5). Different letters denote significant differences between treatments with a > b > c > d, the same below.

The root growth of drooping wildryegrass and Siberian wildryegrass was significantly inhibited by the three kinds of extracts, and the differences among extract treatments were not significant (Figure 5a). The effects of the three extracts on the root length of Chinese fescue, Kentucky bluegrass and crymophila bluegrass exhibited similar pattern, i.e. pieces extracts and root zone soil extracts presented stronger inhibition than

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whole extracts, but a slight difference in Kentucky bluegrass.

Except for Kentucky bluegrass, the three kinds of extracts significantly affected hypocotyl length of other four donor grasses (Figure 5b). The pieces extracts exhibited the strongest shoot growth inhibition on Chinese fescue, crymophila bluegrass, Siberian wildryegrass and drooping wildryegrass , and resulted in shoot length reduction by 42.9%, 31.1%, 21.0% and 25.6%, respectively.

The overall influence of different treatments on seedling dry weight of donor grasses was inhibition, varied with grasses and extracts (Figure 5c). The pieces extracts lead to significant seedling dry weight decrease of Chinese fescue, crymophila bluegrass, Kentucky bluegrass, Siberian wildryegrass and drooping wildryegrass with 42.9%, 34.7%, 30.2%, 27.1% and 26.9%, respectively. The root zone soil extracts presented similar effects of pieces extracts on Kentucky bluegrass, but similar effects of whole extracts on other four grasses.

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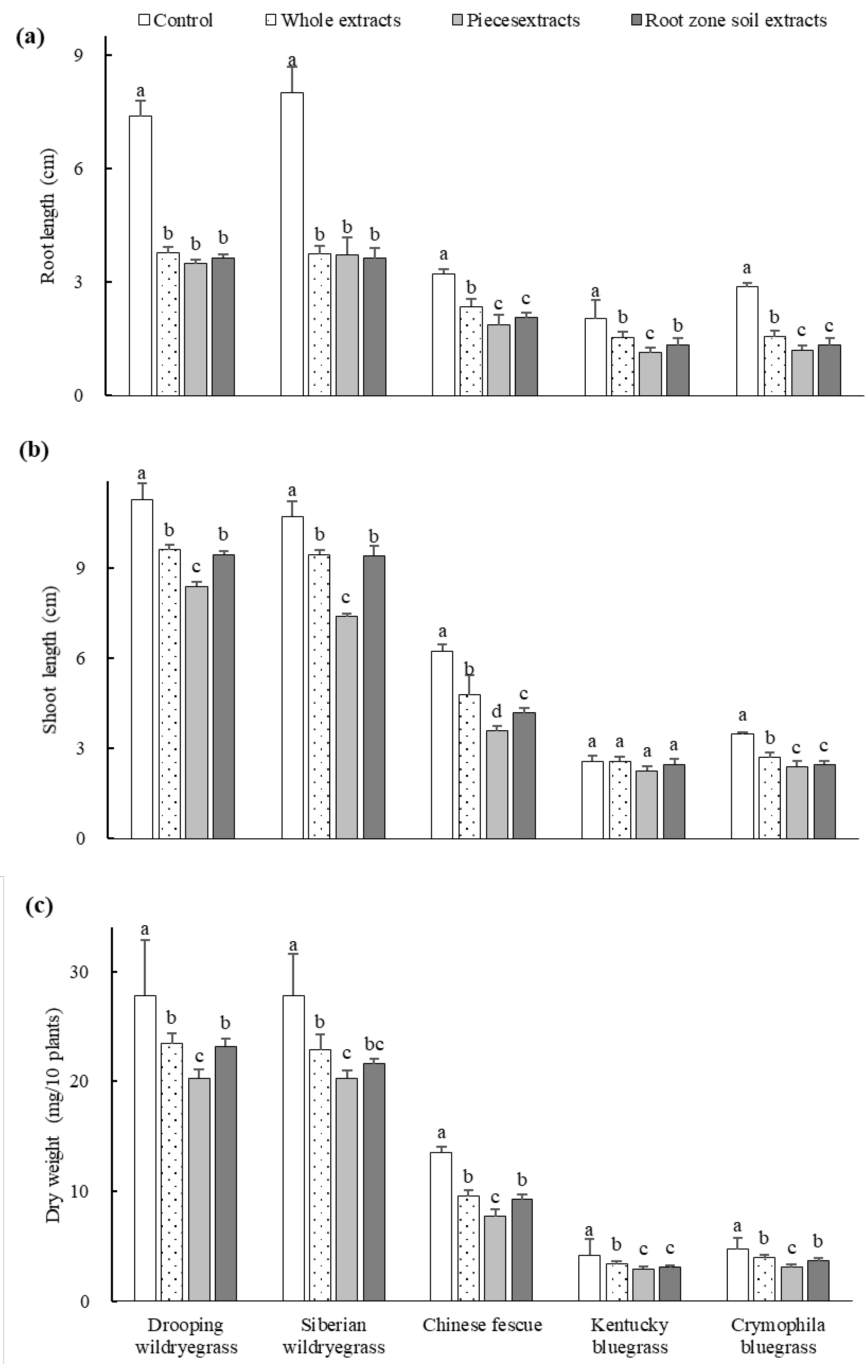

FIGURE 5 Effects of*Elymus nutans* extracts on growth of highland grasses. Values are mean ± SD (n=5). Different letters denote significant differences between treatments with a > b > c, the same below.

The comprehensive allelopathic effect index of drooping wildryegrass on the tested grasses see Figure 6. The maximum germination allelopathic index was noted for Chinese fescue (-0.772), and the minimum is Kentucky bluegrass (-0.120). The inhibition of the extracts on Chinese fescue was seed germination and on crymophila bluegrass, Kentucky bluegrass, Siberian wildryegrass and drooping wildryegrass were root and shoot growth.

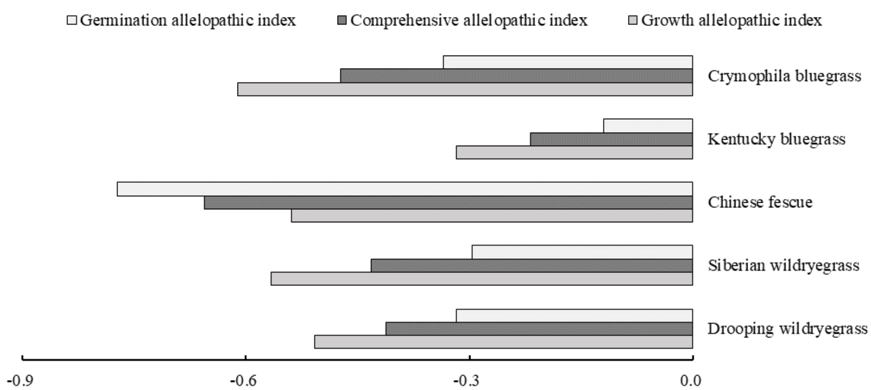

FIGURE 6 Comprehensive allelopathic effect index of *Elymus nutans* extracts on highland grasses

4 DISCUSSION

Plant allelopathy is a traditional subject and numerous studies have demonstrated that weeds can exhibit allelopathic effects on economic crops (Nichols et al., 2015). Some perennial grass, such as quackgrass (*Agropyron repens*), ryegrass (*Lolium perenne*), red fescue (*Festuca rubra*) and Kentucky bluegrass have also been proved to be allelopathic to other plants (Grummer 1961, Fales and Wakefield 1981). Recently, interest has developed in the allelopathic on agroecosystems, like cultivated land and commercial forest (Kural, 2020; Mushtaq, 2020). However, the role of allelopathic effects of perennial grass on the seeds and seedlings of crops and other grass species remain largely unknown. Our results indicated that the extracts from drooping wildryegrass plant materials and root zone soil did impact the germination and seedling growth of commonly cultivated crops on plateau area and perennial grasses of mix-seeded for vegetation restoration.

The effects of different extract treatments on germination varied with crops and extract types. The annual monocots were more susceptible to inhibition of the extracts, while the annual dicots tended to be more tolerant or be promoted. The effects of drooping wildryegrass plant materials and root zone soil extract on the growth of seedlings were mainly manifested in inhibiting the growth of roots and shoots, and the impact on monocot crops is greater than the impact on dicot crops. Out results suggested that the three type extracts had no significant inhibition or promotion on the seedlings growth of quinoa.

All tested grasses in this study were perennial, different from the aforementioned crops. The highest germination force of control was 67.8% and the lowest was only 26.2%, reflecting the disuniformity of germination. The germination rates of control of the five grasses ranged from 63.8% to 81.8%. Except for Siberian wildryegrass which was greater than 80%, the germination rate of other four species were all less than 70%. The control germination index of the five grasses were all less than 35%, reflecting the lower vitality of tested perennial grass seeds.

Except that the root zone soil extract did not have a significant effect on the two bluegrasses, other extract treatments caused significant decrease in the germination force, germination rate and germination index of the five grasses. The strongest inhibitory effect was the pieces extract. Among the five grass species, the least affected was Siberian wildryegrass, and the most affected was Chinese fescue, which was consistent with previous reports (Liang et al., 2020). The effects of the extracts on the growth of grass seedlings were mainly manifested in the inhibition of the growth of roots, especially the growth of drooping wildryegrass and Siberian wildryegrass. The three kinds of extracts had no significant effect on the seedling growth of*Kentucky bluegrass* , but other extracts had inhibitive effects on the growth of roots and shoots, as well as the dry weight. Particularly, the effects of the pieces extract were more apparent. From allelopathic perspective, these maybe reasonable explanations of the degradation of mix-seeded pasture in alpine area.

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Previous studies on autotoxicity of forage were mainly focused on alfalfa and less reports of autotoxicity about Gramineae (Ghimire, 2019). The present study clearly demonstrated that drooping wildryegrass had autotoxicity during its germination and seedling growth. This may help to explain rapid decline of seed yield and above ground biomass of drooping wildryegrass monoculture after 3 years.

The differences in the same index for the same crop and forage should be attributed to different extracts. The composition of the whole plant extract was analogous to that of rain leaching under natural conditions. The plant pieces extract contained more components than whole plant extract because sample ground destroyed the internal tissues of plants and some enzymes, amino acids, inorganic salts, and nitrogen-containing substances could solve in the extracts. The soil extract from the root zone of drooping wildryegrass contained root exudates, leachate from above-ground parts, as well as residues from the decomposition of dead roots in the soil, and soil microorganisms related substances, although the amount may be a little. The composition differences of extracts might contribute to the indicator differences of the same crop or forage.

5 CONCLUSIONS

Drooping wildryegrass does have allelopathic potential on germination and seedling growth of highland crops or perennial grasses and the overall effect is inhibition. Different crops or perennial grasses respond differently, and some are sensitive and some are tolerant. The germination force (>80%) and germination rate (>85%) of the control of five crops were relatively higher, reflecting their good germination uniformity. Of the five crops, hulless barley and oat are susceptible and quinoa is tolerant. Of the five perennial grasses, Sibiricus wildryegrass is the least affected, and Chinese fescue is the most affected. The responses of seed germination and seedling growth of the same crop or grasses varies with the extracts from different sources. The pieces extract has stronger inhibition than others.

Drooping wildryegrass has less allelopathic effects on Kentucky bluegrass and crymophylla bluegrass than on Chinese fescue. It is recommended that the species combination of mixture for restoration should consider allopathic effects of the co-seeding and decrease the seeding rate of drooping wildryegrass in mixture. The subsequent crop or grass followed seed production of drooping wildryegrass monoculture should take planting annual dicots into consideration.

ACKNOWLEDGEMENTS

This research was financially supported by the grants from the National Natural Science Foundation of China (31760691), National Key R&D Program of China (2017YFC0504803), the Programme of Introducing Talents of Discipline to Universities (D18013) and Youth Foundation of Qinghai University (2020-QNY-2). The authors would like thank the anonymous reviewers for their helpful comments.

CONFLICT OF INTEREST

None declared.

AUTHORS' CONTRIBUTIONS

XLQ and YMQ designed the experiment. XLQ, MCC, ZHD, and HLS collected the plant seeds. XLQ performed the experiments, analyzed the data and led the writing of the manuscript. YMQ helped revising the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY STATEMENT

Xiaolong Quan (2021), Comprehensive evaluation of the allelopathic potential of *Elymus nutans* , Dryad, Dataset, https://doi.org/10.5061/dryad.tht76hdzv

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`FIGURE 1-6.pdf` available at https://authorea.com/users/408984/articles/518787-comprehensive-evaluation-of-the-allelopathic-potential-of-elymus-nutans