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Effects of plant age on antioxidant activity and endogenous hormones in *Elymus sibiricus* in Alpine region of the Tibetan Plateau

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Elymus sibiricus L. shows rapid and substantial reductions of aboveground biomass and seed yield after 3 or 4 years of growth, and has an accelerated aging process.To understand possible mechanisms of aging, we planted *E. sibiricus* seeds in triplicate blocks in 2012, 2015, and 2016, respectively, and harvested samples of aboveground biomass (leaves) and roots at the jointing and heading stages in 2018 and 2019 for determinations of antioxidant enzyme activities and endogenous hormone s. The results showed that the fresh and hay biomass and seed yield declined substantially in plants aged 3, 4, and 5 years. The superoxide radical generation rate in leaves and roots at the jointing and heading stages did not show any apparent pattern with plant age. There was an increasing trend of the malondialdehyde concentration with plant age, particularly in leaves and roots at the heading stage in 2019. The superoxide dismutase (SOD) activity appeared declining with plant ages in roots, but not in leaves; the peroxidases activity declined with plant age in both leaves and roots; whereas the catalase activity declined with plant age in leaves at the heading stage in 2018. Overall, the concentrations of plant hormones, auxin (IAA), gibberellin (GA), zeatin (ZT), and abscisic acid (ABA) were many-fold lower in roots than in leaves. The IAA concentration presented different patterns with plants age between leaves and roots. The ZT concentration in roots declined with plant age. The changes of the GA concentration with plant age varied between the physiological stages and between years. The ABA concentrations appeared increasing with plant age particularly in leaves. In conclusion, the aging process of *E. sibiricus* was apparently associated with an increase of oxidative stress, a decrease of ZT and an increase of ABA, particularly in roots. However, these plant age-related trends were influenced significantly by plant physiological stages and year-to-year variations, which need to be carefully minimized in future studies.

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ABSTRACT

Elymus sibiricus L. shows rapid and substantial reductions of aboveground biomass and seed yield after 3 or 4 years of growth, and has an accelerated aging process. To understand possible mechanisms of aging, we planted *E. sibiricus* seeds in triplicate blocks in 2012, 2015, and 2016, respectively, and harvested samples of aboveground biomass (leaves) and roots at the jointing and heading stages in 2018 and 2019 for determinations of antioxidant enzyme activities and endogenous hormones. The results showed that the fresh and hay biomass and seed yield declined substantially in plants aged 3, 4, and 5 years. The superoxide radical generation rate in leaves and roots at the jointing and heading stages did not show any apparent pattern with plant age. There was an increasing trend of the malondialdehyde concentration with plant age, particularly in leaves and roots at the heading stage in 2019. The superoxide dismutase (SOD) activity appeared ~~declining~~ with plant ages in roots, but not in leaves; the peroxidases activity declined with plant age in both leaves and roots; whereas the catalase activity declined with plant age in leaves at the heading stage in 2018. Overall, the concentrations of plant hormones, auxin (IAA), gibberellin (GA), zeatin (ZT), and abscisic acid (ABA) were many-fold lower in roots than in leaves. The IAA concentration presented different patterns with ~~plants~~ age between leaves and roots. The ZT concentration in roots declined with plant age. The changes ~~of~~ the GA concentration with plant age varied between the physiological stages and between years. ~~The ABA concentrations appeared increasing with plant age particularly in leaves.~~ In conclusion, the aging process of *E. sibiricus* was apparently associated with an increase of oxidative stress, a decrease of ZT and an increase of ABA, particularly in roots. However, these plant age-related

trends were influenced significantly by plant physiological stages and year-to-year variations, which need to be carefully minimized in future studies.

Subjects Agricultural Science, Plant Science, Ecosystem Science

Keywords: *Elymus sibiricus*, Aging, Growth stage, Antioxidant system, Endogenous hormone

INTRODUCTION

Perennial herb *Elymus sibiricus* L. belongs to *Poaceae* genus and is an important species in the alpine region of the Tibetan Plateau and the steppe region of northern Eurasia（*Ma et al., 2009*; *Xiong et al.,2009*）. *E. sibiricus* has many prominent characteristics that can be used for the new grassland and restoration of deteriorated grasslands for livestock farming (Yan *et al.,2007*; *Ma et al.,2008*). However, *E. sibiricus* populations in grasslands are vulnerable to decline and the yield reduces with increasing plant age, which prevents its use for long-term plantation in large areas (*Jin, 2021*). Recent studies indicate that physiological burdens, such as the changes of phytohormones in the aging process, age-induced oxidative stress and age-related changes in water relations and photosynthesis are responsible for reduced growth as plants age (*Munné-Bosch & Lalueza*, *2007*). It seems obvious that with the increase of planting years for *E. sibiricus*, the intrinsic changes of physiology and biochemistry metabolism will affect the population stability and productivity reduction. Therefore, studying the aging-related physiological mechanism of occurrence and regulation of *E. sibiricus* has important theoretical significance and a practical application value.

Plant aging is a highly complex process ~~influences~~ by the metabolism of plants and environmental factors (*Munné-Bosch & Lalueza*, *2007*). For plant aging regulation, many mechanisms have been proposed, such as nutrient deficiency, the excessive free ~~radical related~~ aging process, and plant hormones changes(*Ashok & Ali, 1999; Jibran, Hunter & Dijkwel, 2013; Kraj, 2016*). The excessive accumulation of free radicals and the disturbance of endogenous hormone profile in the cells can cause an oxidant stress and deteriorate plant growth and metabolism (*Ashok & Ali, 1999, Rustin et al., 2000; Chen, et al.,2020*). ROS are by-products of many metabolic processes, and ROS accumulation is a key feature of plant senescence （*Ashok & Ali, 1999*）. With the onset of plant senescence，ROS such as superoxide anion (O^{2-}) and other free radicals are excessively produced（*Jing et al.,2008*）, which results in peroxidation of membrane lipids, damage of macro molecules, and even programmed cell death (*Breusegem & Dat, 2006*).

Plants have a variety of defense strategies, such as antioxidant enzymes and non- enzymatic antioxidants, to cope with ROS stress (*Shri et al., 2009*; *Farooq et al.,2015*). Super-oxide dismutase (SOD) is the first line of ~~antioxidant~~ enzyme to scavenge ROS by converting O_2^- to oxygen (O_2) and H_2O_2. Then, H_2O_2 is reduced rapidly to H_2O and O_2 catalyzed by catalase (CAT) and peroxidase (POD) (*Noodén, Guiamet & John, 1997*; *Palma et al.,2006*). Malondialdehyde (MDA) is a primary end-product of lipid peroxidation in plants, and its concentration is usually used to indicator the severity of oxidative stress. For example, plant tissues under abiotic stress had an increase ~~of~~ MDA content (*Duan et al.,2014*; *Suzuki et al.,2012*). The role of oxidative stress in plant senescence and aging has been demonstrated especially in annual and biennial

(*Quirino, Normanly & Amasino, 1999*)

Previous research has also shown that, in general, senescence promoters encompass ethylene (Eth) and abscisic acid (ABA) promote senescence in the aging process of perennial plants. Ethylene and ABA are recognized as key hormones in plant aging, stress-induced ethylene and ABA production have been reported to involve in the generation of reactive oxygen species and were also closely associated with the ROS generation (*Zakari et al.,2020*). It has been shown that endogenous ABA concentrations in 7-year-old *Cistus clusii* plants were higher than 2-year-old (*Munné-Bosch & Lalueza*, *2007*). In this case, 2-year-old plants had already reached the mature stage, in which the growth rate was delayed (*Finkelstein, Gampala & Rock, 2002*). In contrast, retardants include cytokinin (CK), auxin (IAA), gibberellin (GA) and their related compounds have been well known to delay plant aging, respectively (*Saniewski et al., 2020*). The ability of newly emerged leaves to produce auxins and cytokinins declines with plant aging in conifers, thus supporting a link between the reduced growth and decreases of auxin and cytokinins levels during aging process in perennials (*Valdés*, *Fernández & Centeno*, *2004*; *Aldés*, *Centeno & Fernández, 2004*). It is also important to mention that these phytohormones do not work alone, and they are often functioning concomitantly to achieve the regulation of plant senescence (*Noodén & Leopold, 1988*). Currently, researches on the hormone regulation and mechanisms of perennial plants focus mainly on trees and crops(*Chen et al.,2020; Cui et al., 2020*), and little information is available for the hormonal changes in various tissues of perennial grasses during plant aging process. Specifically, research on the regulatory mechanism of the aging process has been focused mainly in annual plants such as *Arabidopsis* and rice (*Zakari et al., 2020; Xiao et*

al., 2020), and the information about the aging regulation in perennial herbs is limited.

In this study, we used *E. sibiricus* Qingmu 1 (a novel variety) planted in the Tibet Plateau region for 3, 4, 5, 7, and 8 years and determined the changes in the antioxidant system and endogenous hormones in leaves and roots at jointing and heading stages to investigate their roles in the process of plant aging. The study was repeated in two years.

MATERIALS & METHODS

Field site

The experiment was carried out at the Haiyan Research Station of Qinghai Province, China (E100°85', N36°45') from June to October 2018 and repeated in 2019. The averaged altitude of the location is 3,159 m, with mean monthly temperatures ranging from −33.8℃ in January to 30.5℃ in July and the mean annual temperature at 0.6℃. The average annual precipitation is about 369-403 mm, mostly occurring during the plant growing season (July to September). The average annual evaporation is 1435 mm, the sunshine duration 2985 h, and the frost-free period about 30 days. The distributions of monthly rainfall and mean temperature in 2018 and 2019 are shown in Fig.1. The changes of temperature and rainfall synchronized throughout the year, and the hot season from July to September had higher rainfall. The soil is the chernozem soil type (Chinese classification), and the chemical properties of soil are shown in Table1.

Experimental design and field management

E. sibiricus seeds were planted in 2012, 2015 and 2016 respectively in three replicate plots each

year. Each plot sized 4 m × 5 m with 0.5m distance between two plots. Seeds were sown in rows with 30 cm space between rows, sown depth 3 cm, and the seeding rate of 4.5 g/m^2. Fertilizer was applied before sowing, and no fertilizer applied afterwards. There was no irrigation system for the plots. Weeds were removed manually and regularly throughout the experimental periods. ~~In 2018 and 2019, plants were sampled in late June (for the jointing stage of the grasses) and late July (for the heading stage of the grasses) respectively for various measurements and analyses, so the plant ages were 3, 4, 5, 7, and 8 years respectively, as shown in Table 2.~~

Samples

More than 100 ~~uniformly~~ growth tiller branches at the jointing and heading growth stages, respectively, were randomly selected in each plot. Leaves of similar parts were collected from the first to third leaves (counting from the tip of each branch) on each ~~of the branches~~. The leaves were immediately separated from stems. The roots within 20 cm-deep soil were fully dug out and cleaned with water. All samples were immediately snap-frozen in liquid nitrogen and stored in -80℃ for subsequent analyses.

The aboveground biomass was harvested in flowering period from 1 m^2 quadrats per plot in 2019. Samples were weighed and oven-dried at 65℃ for 48 h to determine dry matter content.

At the late stage of seed maturity of each plant age, the reproductive branches were cut from randomly selected 1 m^2 quadrats per plot. After natural drying, the seeds were threshed, selected, and weighed, and the average value was used to calculate the seed yield per unit area (kg/hm^2).

136

137 **Sample processing and assays**

138 Samples were cut into smaller pieces and well-mixed. The concentration of superoxide anion
139 (O_2^-) in leaves and roots were determined according to the hydroxylamine oxidation method with
140 some modifications (*Hao, Kang & Yu, 2007*). One g of leaves was ground in 3 mL of 50 mM
141 potassium phosphate buffer (pH 7.8) solutions. The reaction mixture comprised of 0.5 mL of the
142 extracts, 0.5 mL of 50mM potassium phosphate (pH 7.8) buffer and 1 mL of 10mM
143 hydroxylamine and was incubated at 30℃ for 1h. Subsequently, 1 mL of 17mM sulfanilic acid
144 (water preparation) and l mL of 7mM α-naphthylamine were added, and the mixture was kept at
145 30°C for 30 min. Then O_2^- concentration was determined at 530 nm against a calibration curve
146 with known concentrations of nitrite as the standard.

147 The concentration of MDA was measured according to the method of Qiu et al. (*2008*) with
148 some modifications. Briefly, 0.5 g frozen leaf or root was homogenized in 10 mL of phosphate
149 buffer (pH: 7.8) on an ice bath and centrifuged at 15,000 ×g and 4°C for 20 min. One milliliter of
150 supernatant was mixed with 2 mL of 0.6% thiobarbituric acid solution, incubated at 95℃ in a
151 water bath for 15 min, quickly cooled for 2 min to room temperature and the mixture was
152 centrifuged at 5000×g for 10 min at 25°C. The absorbance of the solution was determined at 450,
153 532, and 600 nm (A_{450}, A_{532} and A_{600}) respectively using UV-2450 spectrophotometer
154 (Shimadzu, Japan).

155 To determine anti-oxidant enzymes activities, 0.5 g frozen leaves or roots were grounded

using liquid N, and added 2 mL of phosphate buffer (0.05M, pH 7.8, a mixture of Na_2HPO_4 and NaH_2PO_4).The mixture was centrifuged at 11,000 ×g for 20 min at 4°C and the supernatant was used to determine the activities of antioxidant enzymes. For the SOD activity, 100 μL supernatant was added into 4 mL of the reaction mixture that consisted of 2 mL of 0.05M phosphate buffer, 0.5 mL of 104 mM methionine, 1 mL of 300μM nitroblue tetrazolium, and 0.5 mL of 0.3mM disodium ethylenediaminetetraacetic acid (EDTA-Na_2). The solution was placed under 4000 lx fluorescent lamps for 10 min and the absorbance was recorded at 560 nm. The CAT activity was determined using Zhang's method (*2004*). 100 μL of the supernatant was mixed with 3.4 mL of the reaction mixture that consisted of 2.8 mL Na_2HPO_4 and NaH_2PO_4 (0.05M pH 7.8) buffer and 100 μL 0.1M of H_2O_2 solution and 0.5 mL of 2mM EDTA. The absorbance at 240 nm was recorded for 3 min and the attenuation of the absorbance of was used to calculate the CAT activity against a calibration curve generated with H_2O_2. For the control group, 100 μL of 0.05M pH 7.8 phosphate buffer was used instead of the crude enzyme solution. Absorbance at 240 nm was recorded.

The peroxidase (POD) activity was determined according to the method described by Zhang's method (*2004*). 3 mL of reaction solution contained 1 mL 0.3% H_2O_2, 0.95 mL 0.2% guaiacol, 1 mL 50mM phosphate buffer (pH 7.0) and 0.05 mL enzyme extract, and the reaction was started with the addition of the enzyme extract. For the control group, 50 μL of 0.05M phosphate buffer (pH 7.8) was used instead of the crude enzyme solution. The change in absorbance at 470 nm was recorded for 1 min.

176

177 **Determination of plant hormones**

178 The concentrations of endogenous hormones, including IAA, ABA, GA, and zeatin (ZT) in
179 leaves and roots were determined according to the methods reported previously (*Marasek-*
180 *Ciolakowska et al.,2021)*. 2.5 g of frozen leaves or roots was ground to powder in liquid nitrogen,
181 then the powder was quickly transferred into a 50mL centrifuge tube and extracted with 20 mL
182 of 80% methanol at 4℃ overnight. The extract was centrifuged at 12000 ×g at 4℃ for 15 min.
183 Supernatant was transferred into a clean 50mL centrifuge tube. The residue was ultrasonically
184 extracted with 15 mL 80% methanol at room temperature for 30 min and centrifuged at 12000 ×*g*
185 at 4℃ for 15 min. Two supernatants were pooled, and concentrated to 20 mL in a rotary
186 concentrator at 40℃. Then, decolorization of the concentrate was performed by adding and
187 discarding 15 mL petroleum ether twice. The volume of the solution was further concentrated to
188 near dry, and 2 mL of 80% methanol was added and mixed. The concentrations of the
189 endogenous hormones were determined in a HPLC-MS/MS system (Agilent Infnity 1260,
190 Agilent, Germany).

191

192 **Statistical Analysis**

193 All data for each sampling year (i.e., 2018 and 2019) were subjected to one-way analyses of
194 variance (ANOVA) of SPSS 20.0 statistical software package for Windows. Plant age was the
195 fixed factor and the plot was a random factor. LSD multiple comparisons were performed to

196 distinguish the differences between the means. Data are present as the least square means and
197 standard error of means (SEM). Statistical significance was declared with P values < 0.05.
198

199 RESULTS

200 Plant phenotypes, the aboveground biomass and seed yield

201 Plant phenotypes were observed and photographed in 3- and 4-year old plants (Fig. 2).
202 In 3-year old plants, the central "rotten" phenomenon started appearing in plant clusters, and it
203 became obvious in 4-year old plants. The rotten part expanded gradually from the center to the
204 peripheral parts of plant clusters year after year, and in 7- and 8-year old plants, plant vegetation
205 was very scarce on the ground (photos lost). The aboveground biomass and seed yield for 3-, 4-,
206 and 5- year old plants in 2019 are shown in Table 3. The aboveground fresh and dry weights and
207 seed yield declined substantially and continuously with plant ages: the fresh biomass of 4- and 5-
208 year old plants declined by 34.22% and 52.45% respectively compared with 3-year old plants,
209 and the seed yield declined by 12.72% and 34.17%, respectively.
210

211 O_2^- generation rate in leaves and roots

212 The superoxide radical generation rate in leaves and roost are shown in Fig. 3. In year 2018,
213 leaves of 7-year old *E. sibiricus* had lower O_2^- generation rate than leaves of 3- and leaves 4-year
214 old plants at the jointing stage ($P < 0.05$), and also lower than leaves of 3-year old plants at the
215 heading stage ($P < 0.05$). In year 2019, the O_2^- generation rate was lower in leaves of 4-year old

plants than in leaves of 5-year and 8-year old plants at the jointing stage ($P < 0.05$), but did not differ between 5-year and 8-year old plants ($P > 0.05$). The concentration for 5-year old plants was lower than that for 4- and 8-year old plants at the heading stage ($P < 0.05$) (Fig.3A).

In roots (Fig.3B) in 2018, the O_2^- generation declined continuously in plants aged 3-, 4-, and 7-years at the jointing state ($P < 0.05$), and at the heading stage, the O_2^- generation was lower for 4-year plant than those for 3-year and 7-year old plants ($P < 0.05$). In year 2019, the O_2^- generation declined continuously in plants aged 4-, 5-, and 8-years at the jointing state ($P < 0.05$), but did not differ at the heading stage between 5-year and 8-year old plants.

MDA concentration in leaves and roots

The MDA concentrations in leaves and roots are shown in Fig. 4. In year 2018 (Fig. 4A), the concentration in leaves was low for 4-year old plants at the jointing stage, and higher for 3-year old plants ($P < 0.05$) and further higher for 7-year old plants ($P < 0.05$). At the heading stage, the concentration for 3- and 7-year old plants was higher than that for 4-year old plants ($P < 0.05$). In year 2019, the MDA concentration showed an increasing trend with the ages of plants at both the jointing and heading stages.

For roots in year 2018 (Fig. 4B), the MDA concentration for 3-year old plants was lower than those for 4- and 7-year old plants at the jointing stage ($P < 0.05$), and at the heading stage, the concentration was higher for 7-year old plants than those for 3- and 4-year old plants ($P < 0.05$). In year 2019, the MDA concentration increased continuously with the increases of plant ages at

the jointing stages, and the MDA concentration was higher for 5-year old plants than those for 4- and 8-year old plants at heading stage ($P < 0.05$), but no difference was found between 4- and 8-year old plants ($P > 0.05$).

SOD, CAT, and POD activities in leaves and roots

SOD activity

As shown in Fig. 5, in year 2018, the SOD activity in leaves (Fig. 5A) was higher for 7-year old plants than those for 3- and 4-year old plants at the jointing stage ($P < 0.05$)， and was lower for 3- and 4-year old plants at the heading state. In year 2019, the SOD activity was lower for 3-year old plants than those for 5- and 8-year old plants at the jointing stage ($P < 0.05$), and at the heading stage, the activity increased with the age of plants.

For roots in year 2018 (Fig. 5B), the SOD activity declined continuously with the increases of plant ages at the jointing stage ($P < 0.05$), and at the heading stage, the SOD activity was higher for 4-year old plants than those for 3- and 7-year old plants ($P < 0.05$).

In year 2019, the SOD activity was lower for 4-year old plants than those for 5- and 8-year old plants at the jointing stage ($P < 0.05$), and also lower for 5- and 8-year old plants than those for 4-year old plants ($P < 0.05$).

POD activity

In year 2018, the POD activity in leaves of 4- and 7-year old plants at the jointing stage was

lower than that for 3-year old plants ($P < 0.05$); at the heading stage, the activity was lower for 4-year old plants than that for 3- and 7-year old plants ($P < 0.05$). In 2019, the POD activity declined continuously with the ages of plants at the jointing stage, but at the heading stage, it increased for 8-year old plants compared with 4- and 5-year old plants (Fig. 6A).

For roots (Fig. 6B) in year 2018, the POD activity was lower for 4- and 7-year old plants than that for 3-year old plants at the jointing stage ($P< 0.05$), and at the heading stage, the POD activity declined continuously with the increase of plant age. In year 2019, the POD activity declined continuously with the increases of plant ages at both the jointing and heading stages.

CAT activity

The CAT activity in leaves and roots are shown in Fig.7. In year 2018, the activity in leaves was lower for 7-year old plants at the jointing stage than those for 3- and 4-year old plants ($P < 0.05$), and at the heading stage, the activity declined with the increase of plant age. In year 2019, however, no difference was found in the CAT activity for plant aged of 4, 5, and 8 years at both the jointing and heading stages ($P > 0.05$), except for the lower CAT activity for 8-year old plants at the heading stage (Fig. 7A).

For roots in year 2018 (Fig. 7B), the CAT activity was lower for 4- and 7-year old plants at the jointing stage than that for 3-year old plants ($P < 0.05$), and at the heading stage, the activity was lower for 4-year old plants than those for 3-year and 7-year old plants. In year 2019, no difference was found in the CAT activity for plants aged of 4, 5, and 8 years at both the jointing and heading stages ($P > 0.05$).

277

278 **Endogenous hormones in leaves and roots**

279 IAA concentration

280 The IAA concentrations in leaves and roots are shown in Fig.8. In year 2018, the IAA
281 concentration in leaves increased continuously with the increase of the plant age at both the
282 jointing and heading stages (Fig. 7A). In year 2019, this increasing trend of the concentration
283 with plant age was also present in leaves of 4-, 5-, and 8-yer old plants at the jointing stage, as
284 well as for 4- and 5-year old plants at the heading stage. The IAA concentration dropped
285 substantially for 8-year old plants (Fig. 8A).

286 For roots in year 2018, the IAA concentration was much higher for 3-year old plants at the
287 jointing stage compared with those for 4- and 7-year old plants ($P < 0.05$), and the concentration
288 showed no difference between plant ages at the heading stage ($P > 0.05$). In year 2019, the IAA
289 concentration was much lower for 4-year old plants than those for 5- and 8-year old plants at the
290 jointing stage ($P < 0.05$), and the concentration was higher for 8-year old plants than those for 4-
291 and 5-year old plants at the heading stage ($P < 0.05$) (Fig.8B).

292

293 **ZT concentration**

294 The zeatin (ZT) concentrations in leaves and roots are shown in Fig.9. In year 2018, the ZT
295 concentrations in leaves of 3-year old plants at the jointing stage was higher than those for 4- and
296 7-year old plants ($P < 0.05$), and at the heading stage, the concentration declined continuously
297 with the increase of plant age. In year 2019, the concentration was lower for 4-year old plants

than those for 5- and 8-year old plants at the jointing stage, and the concentration showed no difference between plant ages at the heading stage ($P > 0.05$) (Fig.9A).

For roots in year 2018 (Fig. 9B), the ZT concentration declined continuously with the increase of plant age at the jointing stage, and at the heading stage, the concentration was higher for 3-year old plants than those for 4- and 7-year old plants, but no difference between 4- and 7-year old plants. In year 2019, the concentration showed a decline trend with plant age at both the jointing and heading stages.

GA concentration

The GA concentration in leaves and roots are shown in Fig. 10. For leaves in year 2018 (Fig. 10A), the GA concentration showed an increasing trend with the increase of plant age at the jointing stage, and also at the heading stage, but no difference in the concentration between 4- and 7- year old plants. In year 2019, the GA concentration was lower for 4-year old plants at the jointing stage than those for 5- and 8-year old plants, and at the heading stage ($P < 0.05$), the concentration was lower for 8-year old plants than those for 4- and 5-year old plants.

For roots in year 2018, the GA concentration was higher for 4-year old plants at the jointing stage than those for 3- and 7-year old plants ($P < 0.05$), and no difference between 3- and 7-year old plants ($P > 0.05$). No difference was found in the GA concentration for plants aged of 3, 4, and 7 years at the heading stages ($P > 0.05$). In year 2019, the GA concentration was lower for 4-year old plants than those for 5- and 8-year old plants at the jointing stage ($P < 0.05$), and at the

heading stage, the concentration was lower for 8-year old plants than those for 4- and 5-year old plants ($P < 0.05$)(Fig.10B).

ABA concentration

The ABA concentration in leaves and roots are shown in Fig.11. In leaves in 2018 (Fig. 11A), the ABA concentration was the lowest for 4-year old plants at the jointing stage, followed with that for 7-year old plants, and 3-year old plant had the highest concentration. At the heading stage, the ABA concentration was higher for 7-year old plants than those for 3- and 4-year old plants ($P< 0.05$). In year 2019, the concentration showed an increasing trend with plant age at both the jointing and heading stages.

The ABA concentrations in roots (Fig. 11B) were much lower compared with those for leaves. For roots in 2018, the ABA concentration showed an increasing trend with plant age at the jointing stage, and at the heading stage, the concentration was almost negligible. In year 2019, the ABA concentration was much higher for 5-year old plants, followed with that for 8-year old plants, and 4-year old plants had the lowest ABA concentration at the jointing stage. At the heading stage, the ABA concentration was higher for 4-year old plants compared with 5- and 7-year old plants, the concentration of which was much low.

The effects of physiological stages on all measures

337 The significance (P values) of the effects of physiological stages of plants on the antioxidant
338 indicators and plant hormones are shown in Table 4. The physiological stage did not affect the
339 MDA concentration in leaves, CAT activity in roots in 2019, ZT concentration in roots in 2018,
340 and GA concentration in 2019 ($P > 0.05$), whereas the effects on the other measures were
341 significant ($P < 0.05$).

342

343 **DISCUSSION**

344 Rapid deterioration of vegetation status and declines of aboveground biomass with the age of
345 *Elymus sibiricus* plants were observed in this study, indicating that *Elymus sibiricus* aged at a
346 high rate, which has been commonly found in other reports (*Jin et al., 2021; Yang et al., 2021*).
347 Plant production capacity, seed reproduction capacity decreased with the increase of plant age,
348 which affect the maintenance and regeneration of the plant population (*Kuai, 2014*). Likely,
349 increase of plant senescence contributes to these declines. Senescence occurs at different stages
350 and at different levels (plants, organs, tissues, cells) of plant (*Leopold, 1961*). In the process of
351 senescence, a series of changes occur in the external morphological characteristics of each part
352 of the plant (*Van Doorn & Woltering, 2004*).These changes mainly include plant height, leaf
353 number and biomass, and the changes of these morphological features on the surface ultimately
354 reflect the changes of physiological and biochemical processes and material transport inside the
355 plant will eventually affect the yield and quality of the seed (*Kuai, 2014; Song, 1998*).

356 Plant aging is one of the most crucial and complex physiological phenomena in the

lifecycle of a plant, which often falls prey to environmental and biological stresses that leads to erratic growth. An increase of oxidative stress is one of biological stresses that is linked to plant aging (*Munné-Bosch & Alegre, 2002*). Oxidative stress can occur when the rate of scavenging free radicals is over-ride by the rate of free radical production in an organism. In the present study, we measured the superoxide radical generation rate in leaves and roots at the jointing and heading stages, and did not find any apparent pattern between the superoxide radical generation rate and plant age. The radical generation rates in both leaves and roots in the heading stage were higher than those in the jointing stage. There was also a large year-to-year variation in the superoxide radical generation rate. It should be noted that the year-to-year variation consists of the effect of plant age confounded by environmental changes (climate in particular) between years. Therefore, the results suggest that the superoxide radical generation rate was influenced strongly by environmental factors and physiological stage of plants.

The concentration of MDA is a good indicator to oxidative damage of lipids in plants (*Ozlem, 2022*). The results in the present study showed an increasing trend of the MDA concentration with plant age, particularly in leaves and roots at the heading stage in 2019, indicating an increase of lipid peroxidation with plant age. An increase of MDA concentration is resulted from damage to the membranes and accelerated aging, which leads to the metabolic dysfunction of plant cells and even leads to cell death directly (*Rysz et al., 2022*). Interestingly, it was noted that the physiological state had significant influence on the MAD concentration in roots but not in leaves (Table 4); also the year-to-year variation in the MDA concentration

377 appeared small. It seems that the MDA concentration was associated with the age of roots, as
378 well as the late physiological stage.

379 The antioxidative defense system comprise of several antioxidant enzymes such as SOD,
380 catalase, and POD that scavenge superoxide radicals, peroxides, and other free radicals in plants(
381 *Noctor & Foyer,1998*). The results in this study showed that the SOD activity appeared
382 declining with plant ages in roots, but not in leaves; the POD activity declined with plant age in
383 both leaves and roots; whereas the CAT activity declined with plant age in leaves at the heading
384 stage in 2018, but remained almost unchanged with plant age in both leaves and roots at the other
385 stages and years. Overall, the antioxidant capacity appeared becoming weak with plant aging,
386 particularly in roots. Aforementioned, the superoxide generation rate did not change much with
387 plant aging, so the weak of the antioxidant capacity could result in a risk of oxidative stress,
388 which agrees with the increase of the MDA concentration with plant aging in this study. These
389 results indicate that the overall oxidative capacity was affected by plant aging, which is in
390 agreement with previous studies (*Munné-Bosch & Lalueza, 2007*). It is also possible that
391 oxidative stress accelerated leaf senescence with plant aging, therefore, is regarded as an
392 adaptive strategy for plants to copy with environmental stresses (*Munné-Bosch, Jubany-Mańi &*
393 *Alegre, 2001*).

394 It has been reported that plant endogenous hormones are one of the important factors that
395 regulate plant senescence (*Jan et al., 2019*) . However, little is known about their roles in plant
396 aging process. In this study, we determined the concentrations of IAA, GA，ZT, and ABA in

both leaves and roots at the jointing and heading stages. Overall, the concentrations of these hormones were many-fold lower in roots than in leaves, particularly IAA, GA, and ABA. IAA is involved in the regulation of leaf expansion and newly emerged leaves to produce auxins (*Aldés, Centeno & Fernández, 2004*). ZT is a type of cytokinin participating in many physio-biochemical processes, including different cellular divisions and the senescence of leaves, thus regulates the ratio of shoot/root systems (*Azzam et al., 2022)*. A reduction of such cytokinins is associated with plant aging in conifers (*Valdés, Fernández & Centeno, 2003)*. GA is necessary for shoot and root elongation and generally associated with plants senescence (*Ptošková et al., 2022*). ABA regulates various developmental processes and serves as an inducer to trigger plants senescence (*Lim, Kim & Nam,2007*; *Asad et al., 2019*). In the present study, the IAA concentration presented different patterns with plants age between leaves and roots. In leaves, the concentration increased with plant age, except for the very low concentration in 8-year old plants, the most of which was dead vegetable; whereas in roots, there was no clear pattern between the IAA concentration with plant age. The ZT concentration in roots at both the jointing and heading stages declined with plant age. The changes of the GA concentration with plant age varied between the physiological stages and between years: increasing with plant age in leaves and roots at the jointing stage in 2018, otherwise, no clear pattern was seen. The GA concentration is usually low in roots, however, such a low concentration can maintain the root growth (*Ptošková et al., 2022*). A reduction of GA could decrease the capacity for growth as plants age (*Colebrook et al., 2014*). Previous studies showed that the ABA concentration in plant was regulated not only by ABA biosynthesis but also by its catabolism (*Zhang et al., 2018*). It is

418 known that root is an important site of ABA synthesis, and then ABA is transported from roots to
419 leaves through xylem vessel (*Wilkinson & Davies, 2002*; *Dodd, 2005*). The increase of
420 endogenous ABA can reduce transpiration of the plant by inducing stomatal closure, but also by
421 decreasing leaf area. The ABA concentrations in leaves and roots appeared increasing with plant
422 age particularly in leaves, albeit there were some scatted data such as ABA in roots of 2019 (the
423 reason is unknown).

424 Aging occurs usually throughout the lifetime of perennials at the tissue and organ levels
425 (*Jing, Hille & Dijkwel, 2003*)and in aboveground and underground parts. The stems and leaves
426 are the annual parts, while roots are perennial parts of the perennial plant. *Elymus sibiricus*, as a
427 typical perennial species, the differences in physiological character reflect not only the changes
428 within the growing season, but also the response to the growing years, especially roots whose
429 living conditions affect the growth of plants in the next season(*Wang,2014*). Leaf senescence has
430 been studied intensively. The information about the mechanisms of roots in plant aging has not
431 been well understood yet. It was proposed that root senescence is closely related to leaves
432 senescence, and the main possibly reasons are the root tip is the site to synthesize cytokinins and
433 gibberellins, which are transported upward through the stem and leaf to regulate the senescence
434 of stems and leave. As the vitality of roots decreases, so does the ability to synthesize hormones,
435 resulting in a decline in the anti-aging ability of the aboveground part, leading to aging (*Chen &*
436 *Brassard, 2013)*. Based on these literatures, we believe physiological and biochemical changes
437 in roots may play primary roles in plant aging process. Aforementioned, the age-associated

reduction of antioxidant capacity, particularly the SOD and POD activities, in roots could be one of the contributors; the decline of the ZT concentration and a tendency of increasing ABA concentration in roots could not be ruled out.

CONCLUSION

Elymus sibiricus grasses showed a rapid aging process with substantial reductions of aboveground biomass and seed yield with plant ages. The aging process appeared to be associated with the reduced nativities of SOD and POD in roots and the increase of oxidative stress as indicated by increased MAD concentration in roots and leaves. The plant hormone concentrations were many-fold lower in roots than these in leaves. Among hormones in roots, the ZT concentration appeared increasing while the ABA concentration tended to decline with plant age. However, these plant age-related trends were influenced significantly by plant physiological stages and year-to-year variations, likely due to climate differences between years. In future studies, these influences shall be carefully controlled and minimized.

ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

Juan Qi conceived and designed the experiments, analyzed the data, prepared tables, wrote the manuscript, and approved the final draft.

Zhaolin Wu conceived and designed the experiments, performed the experiments, analyzed the data, and approved the final draft.

Yanjun Liu performed the experiments, analyzed the data, and approved the final draft.

Xiangjun Meng conceived and designed the experiments, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

The raw measurements are available in the Supplementary File.

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Figure 1

Monthly mean temperature and cumulative rainfall in 2018 and 2019 at Haiyan County, Qinghai

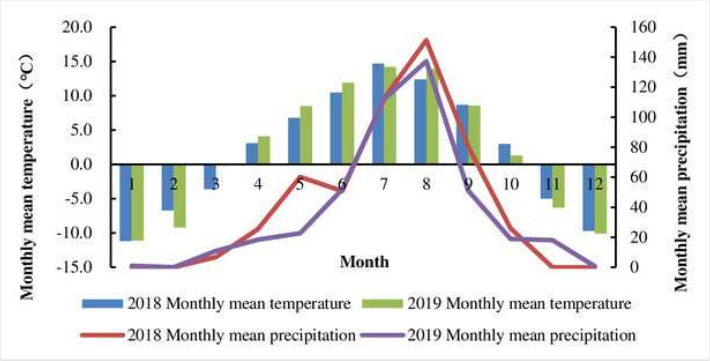
Monthly mean temperature (°C)
Monthly mean precipitation (mm)
Month
2018 Monthly mean temperature
2019 Monthly mean temperature
2018 Monthly mean precipitation
2019 Monthly mean precipitation

Figure 2

Plant phenotypes of 3- (A) and 4-year old plant (B) and roots of 4-year old plants (C)

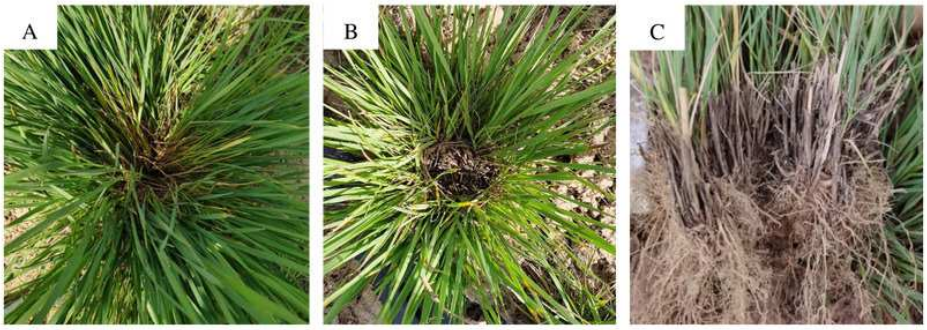
A
B
C

Figure 3

Superoxide radical generation rate in leaves (A) and roots (B) of *Elymus sibiricus* at the jointing and heading stages.

Different letters on the top of columns indicate significant differences between plants ages within the physiological state ($P < 0.05$).

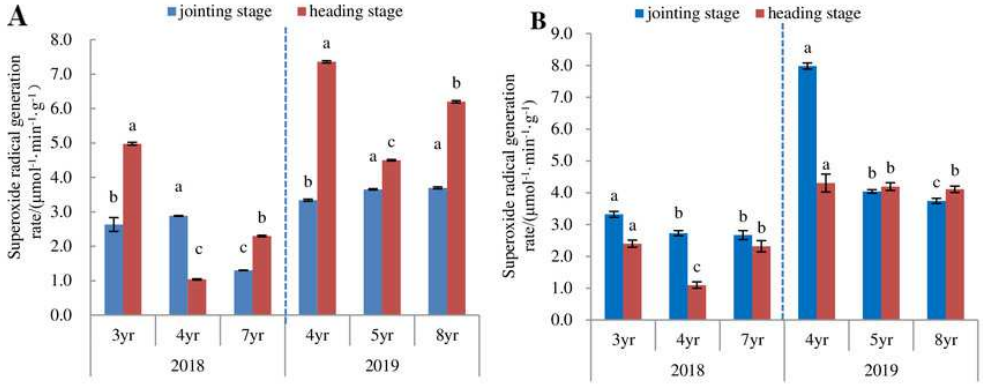
A
jointing stage
heading stage
Superoxide radical generation rate/(μmol·l⁻¹·min⁻¹·g⁻¹)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
3yr
4yr
7yr
4yr
5yr
8yr
2018
2019
B
jointing stage
heading stage
Superoxide radical generation rate/(μmol·l⁻¹·min⁻¹·g⁻¹)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
3yr
4yr
7yr
4yr
5yr
8yr
2018
2019

Figure 4

MDA concentration in leaves (A) and in roots（B）at the jointing and heading stages.

Different letters on the top of columns indicate significant differences between plants ages within the physiological state ($P < 0.05$).

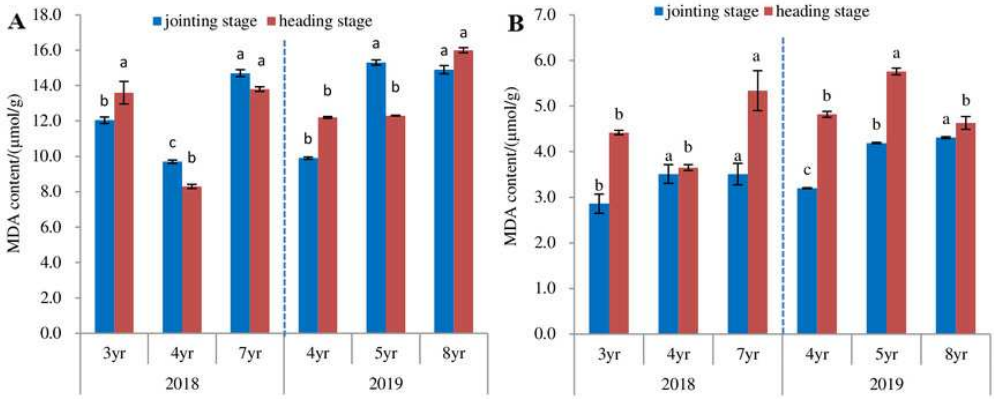
A
B
jointing stage
heading stage
MDA content/(μmol/g)
3yr
4yr
7yr
4yr
5yr
8yr
2018
2019

Figure 5

SOD activity in leaves (A) and roots (B) of *Elymus sibiricus* at the jointing and heading stages.

Different letters on the top of columns indicate significant differences between plants ages within the physiological state ($P < 0.05$).

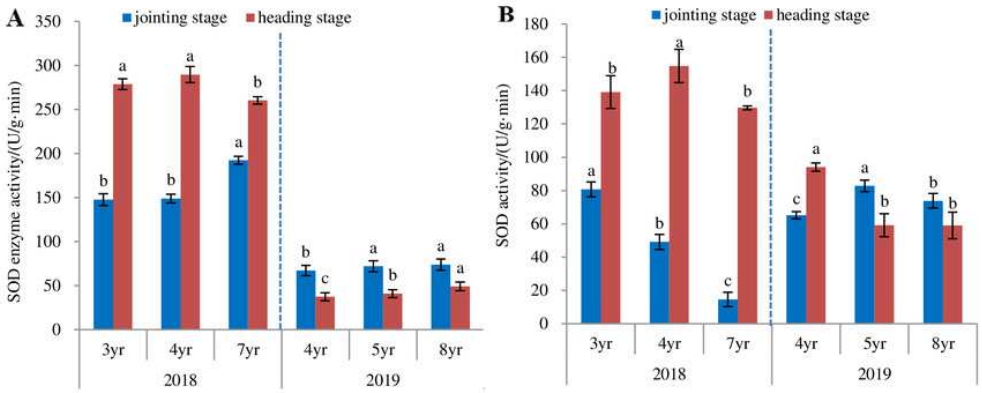
A
jointing stage
heading stage
SOD enzyme activity/(U/g·min)
350
300
250
200
150
100
50
0
3yr
4yr
7yr
4yr
5yr
8yr
2018
2019
B
jointing stage
heading stage
SOD activity/(U/g·min)
180
160
140
120
100
80
60
40
20
0
3yr
4yr
7yr
4yr
5yr
8yr
2018
2019

Figure 6

The POD activity in leaves (A) and roots (B) of *Elymus sibiricus* at the jointing and heading stages.

Different letters on the top of columns indicate significant differences between plants ages within the physiological state ($P < 0.05$).

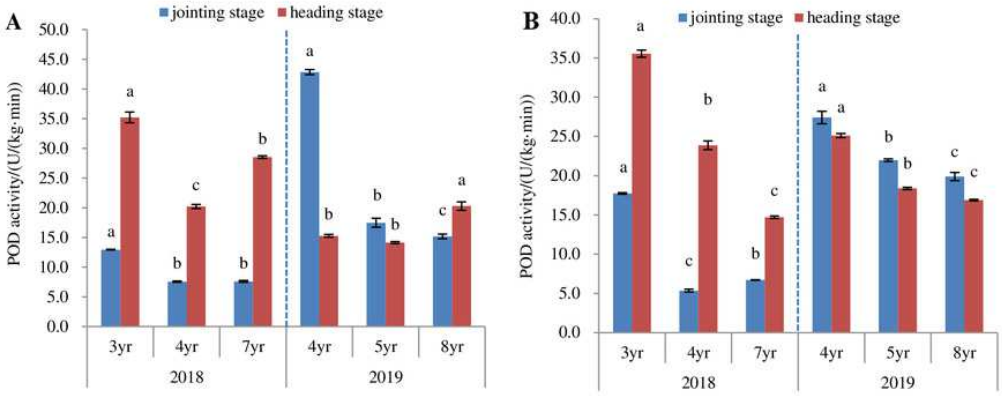
A
jointing stage
heading stage
POD activity/(U/(kg·min))
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
a
a
b
c
b
b
a
b
b
b
c
a
3yr
4yr
7yr
4yr
5yr
8yr
2018
2019
B
jointing stage
heading stage
POD activity/(U/(kg·min))
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
a
a
c
b
b
c
a
a
b
b
c
c
3yr
4yr
7yr
4yr
5yr
8yr
2018
2019

Figure 7

The CAT activity in leaves (A) and in roots (B) of *Elymus sibiricus* at the jointing and heading stages.

Different letters on the top of columns indicate significant differences between plants ages within the physiological state ($P < 0.05$).

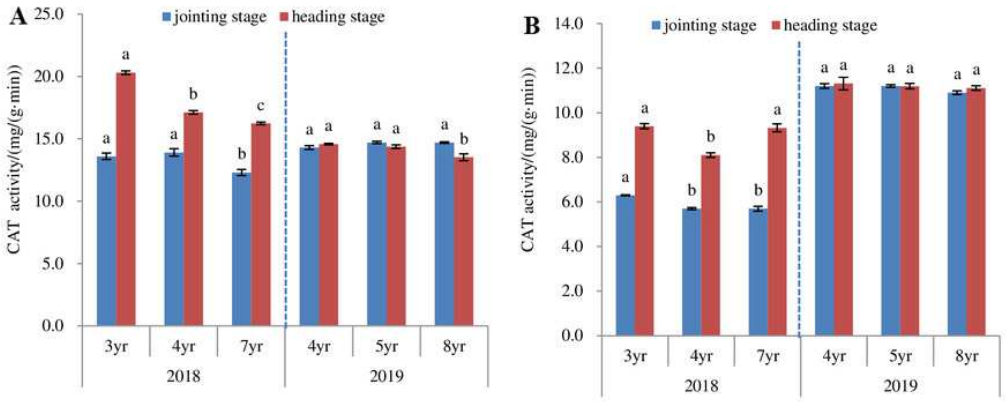
A
B
jointing stage
heading stage
CAT activity/(mg/(g·min))
3yr
4yr
7yr
4yr
5yr
8yr
2018
2019

Figure 8

IAA concentration in leaves (A) and in roots (B) of *Elymus sibiricus* at the jointing and heading stages.

Different letters on the top of columns indicate significant differences between plants ages within the physiological state ($P < 0.05$).

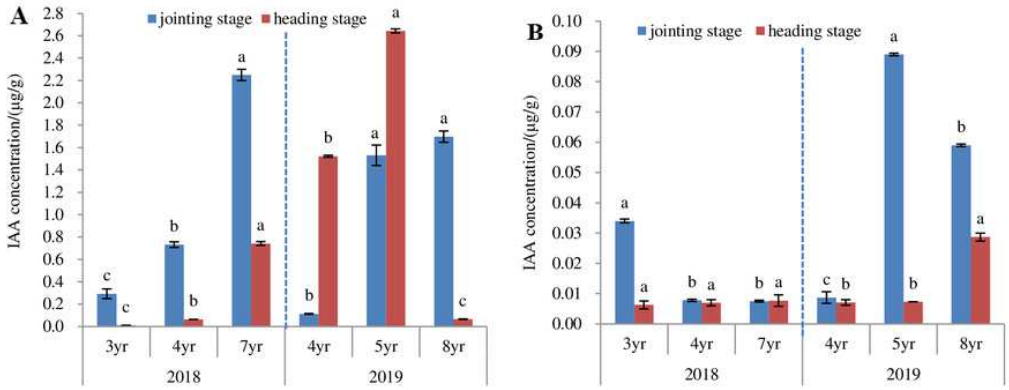
A
jointing stage
heading stage
IAA concentration/(μg/g)
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
3yr
4yr
7yr
4yr
5yr
8yr
2018
2019
B
jointing stage
heading stage
IAA concentration/(μg/g)
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
3yr
4yr
7yr
4yr
5yr
8yr
2018
2019

Figure 9

Zeatin (ZT) concentration in leaves (A) and in roots (B) of *Elymus sibiricus* at the jointing and heading stages.

Different letters on the top of columns indicate significant differences between plants ages within the physiological state ($P < 0.05$).

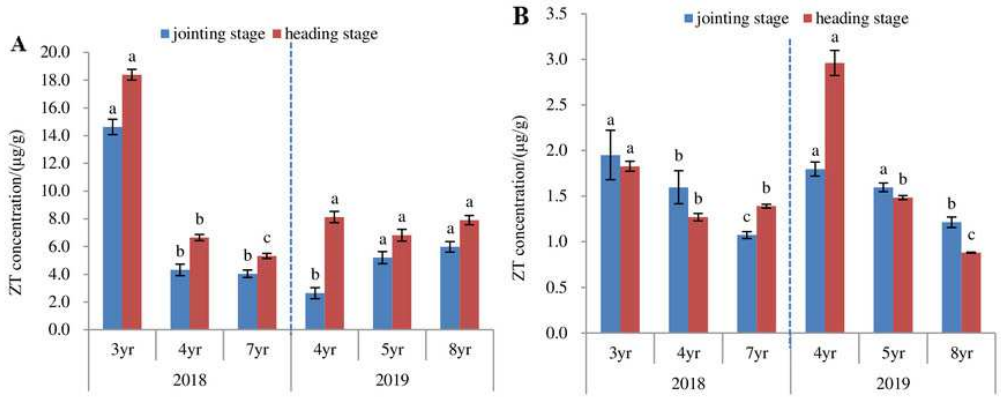
A
jointing stage
heading stage
ZT concentration/(μg/g)
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
3yr
4yr
7yr
4yr
5yr
8yr
2018
2019
B
jointing stage
heading stage
ZT concentration/(μg/g)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
3yr
4yr
7yr
4yr
5yr
8yr
2018
2019

Figure 10

Gibberellic acid (GA) concentration in leaves (A) and in roots (B) of *Elymus sibiricus* at the jointing and heading stages.

Different letters on the top of columns indicate significant differences between plants ages within the physiological state ($P < 0.05$).

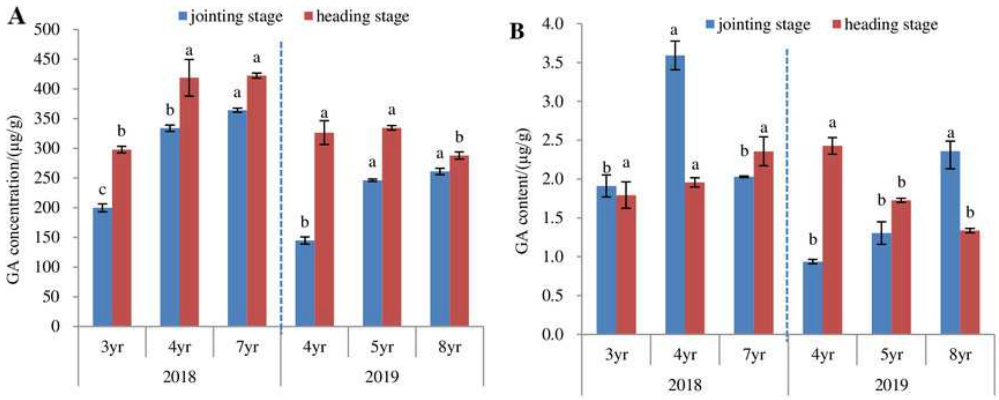
A
jointing stage
heading stage
GA concentration/(μg/g)
500
450
400
350
300
250
200
150
100
50
0
3yr
4yr
7yr
4yr
5yr
8yr
2018
2019
B
jointing stage
heading stage
GA content/(μg/g)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
3yr
4yr
7yr
4yr
5yr
8yr
2018
2019

Figure 11

ABA concentration in leaves (A) and roots (B) of *Elymus sibiricus* at the jointing and heading stages.

Different letters on the top of columns indicate significant differences between plants ages within the physiological state ($P < 0.05$).

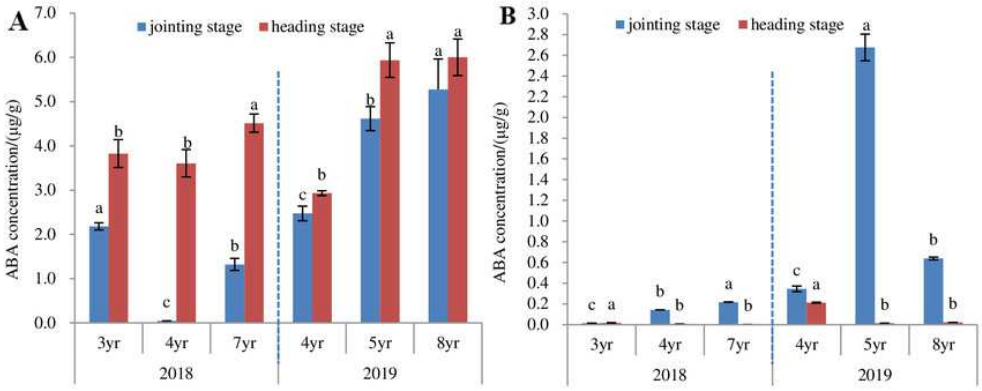
A
B
jointing stage
heading stage
ABA concentration/(μg/g)
3yr
4yr
7yr
4yr
5yr
8yr
2018
2019

Table 1(on next page)

Chemical properties of soil at Haiyan Research Station, Haiyan County, Qinghai Province

1 **Table 1 C**hemical properties of soil at Haiyan Research Station, Haiyan County, Qinghai Province

pH	OM	Total N	Total K	Total P	Available nitrogen	Available potassium	Available phosphorus
	%	g/kg	g/kg	g/kg	mg/kg	mg/kg	mg/kg
8	2.93	1.22	7.73	0.41	68.19	213.87	10.53

2

Table 2(on next page)

Plant age of *E. sibiricus* samples

1 **Table 2** Plant age of *E. sibiricus* samples

Year of sowing	Plant age	
	2018	2019
2016	3	4
2015	4	5
2012	7	8

2

Table 3(on next page)

Aboveground fresh and dry biomass and seed yield of *Elymus sibiricus*

1 **Table 3** Aboveground fresh and dry biomass and seed yield of *Elymus sibiricus*

Plant age	Fresh biomass (kg/ha)	Hay biomass (kg/ha)	Seed yield (kg/ha)
3	$15632^{a} \pm 162.80$	$7550^{a} \pm 163.28$	$809^{a} \pm 24.46$
4	$10283^{b} \pm 171.03$	$5823^{b} \pm 155.17$	$706^{b} \pm 40.54$
5	$7434^{c} \pm 133.18$	$4780^{c} \pm 162.88$	$532^{c} \pm 38.69$

2 Different superscripts within the column indicate significant different between plant ages ($P < 0.05$).

3

Table 4(on next page)

P values for the effects of plant physiological stages

Table 4. P values for the effects of plant physiological stages

Indicators	2018		2019	
	Leaves	Roots	Leaves	Roots
O_2^- generation rate	0.000	0.000	0.000	0.000
MDA	0.391	0.007	0.234	0.000
SOD	0.000	0.001	0.000	0.042
POD	0.000	0.000	0.000	0.000
CAT	0.000	0.000	0.006	0.483
IAA	0.000	0.000	0.000	0.000
ABA	0.000	0.000	0.002	0.000
ZT	0.000	0.698	0.000	0.002
GA	0.000	0.009	0.000	0.080