

Original Research Paper

Space Distribution of Highland Barley GNS and its Relationship with Environmental Factors in the Qinghai-Tibet Plateau

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Abstract: In order to study the space distribution of Grain Number per spike (GNS) and its relationship with the environmental factors in the Qinghai-Tibet Plateau, 83 data samples from different ecological areas were selected for GNS investigation. Meanwhile, the seeds of highland barley were harvested and planted in the farm of Xizang Agriculture and Animal Husbandry College; the varieties without genetic differences were selected and their GNS was analyzed with the local geography and climate factors. The results shows that: (1) In the horizontal direction, GNS forms the high value area of Central and South Tibetan taking Qiongjie and Longzi as the center; (2) In the vertical direction, GNS appears with double high value areas at the altitude of 3900m-4200m and above 4500m respectively and the corresponding GNS is 52.9 ± 17.6 and 57.2 ± 0.2 respectively; (3) the environmental factors influencing GNS are in the order that soil total nitrogen>soil available nitrogen>the average precipitation at heading-filling stage>altitude>soil organic matter>soil total phosphorus>the average rainfall at jointing-heading stage>soil available potassium>the average rainfall at tillering-jointing stage.

Keywords: Qinghai-Tibet Plateau, Highland Barley, Grain Number Per Spike, Distribution Pattern, Environmental Factor

Introduction

The Qinghai-Tibet Plateau is the largest plateau in China and the highest plateau of the world with complicated ecological environment. It is called "the roof of the world" and "earth's third pole" (Cheng *et al.*, 2017) and is one of the origin centers of pioneering crop barley (*Hordeum vulgare* L.) (Dai *et al.*, 2014; 2012). Its main planting crop is highland barley, also known as naked barley and hulless barley (*Hordeum vulgare* Linn. var. nudum Hook.f.) and the Tibetan language is called "Nai". Highland Barley is one of the important foods of Tibetans in Xizang, Qinghai, Gansu and Sichuan with a long planting history and large planting area, up to more than 80% of the crop planting area (Ma, 2000). Grain Number per Spike (GNS) is an important component factor of highland barley yield, its affecting factors are more and with complicated mechanism (Gesangzhuoma, 1995). Li *et al.* (2006) thought barley GNS was associated with genetic factor; Du *et al.* (2002) thought the temperature of highland barley at booting-heading stage and the precipitation at flowering-milk stage in

Xigaze of Xizang had a significant effect on the GNS; Chalak *et al.* (2015) thought the rainfall before and after the flowering had a great influence on barley GNS; Prystupa *et al.* (2004) thought early Nitrogen(N) influenced GNS; Abbate *et al.* (1995) used the ratio of PAR obtained before and after the flowering and the average temperature to describe GNS linearly. Abeledo *et al.* (2008; Garcia *et al.*, 2003) thought GNS was the result of environment and species interactions. Zou *et al.* (2009) combined the genetic factors and environmental factors (light, temperature, water and fertilizer) organically and established the mathematic model of the barley yield to make corresponding prediction for GNS.

The above study is given priority to skin barley with small area coverage, less sample quantity; the geographical factors, for example, the effects of altitude, latitude and longitude on the GNS were not researched. The Qinghai-Tibet Plateau crosses more than 10 latitudes and 30 longitudes and the altitude is from 200 m to over 6 000 m, which provides a natural model for the large-scale and

three-dimensional highland barley GNS research (Wang *et al.*, 2014). Taking the sample data in the large-scale scope of Qinghai-Tibet Plateau as support, the paper adopts the research methods with the combination of agricultural ecology and geography through the establishment of stepwise regression and advantages (dominant) model to reveal the space distribution characteristics of highland barley GNS in the horizontal and vertical directions and the influence of different environmental factors on it, to guide the highland barley breeding and cultivation.

Materials and Methods

Study Area

The study area stretches the mountain steppe, alpine meadow steppe, alpine scrub meadows, mountain coniferous forest, mountain scrub grassland, mountain desert and semi-desert, involved in Sichuan, Gansu, Qinghai and Tibet. The sunshine hours in growth period are 1 642-3 393 h, the temperatures in growth period are -0.6~12.9°C, the rainfall in growth period is 150-890 mm.

Highland Barley Collection and Sample Points Arrangement

In July-September of 2014 and July-September of 2015, 83 sample points were set in the different planting areas (northern latitude 27 -38°C, east longitude 79-104°C) of Qinghai-Tibet Plateau and a total of 83 varieties (promotion) were collected from the local peasant. Among them, there are 67 peasant varieties and 16 promotion varieties. The specific

geographic location was shown in Fig. 1. When the samples were collected, 20 strains of highland barley were randomly pulled out for GNS investigation.

Soil Samples Collection and Analysis

When highland barley was collected for GNS investigation at each sample point, 5-6 sample quadrats was set; 3 soil samples in the plough layer (0-30 cm) were collected randomly from each sample quadrat (the area was not less than 20 m²) and put them into the soil bag. After drying in the lab, the soil was grinded fine for 1mm sieving to determine total n, total phosphorus, total potassium, available n, available phosphorus, available potassium, pH and soil. Among them, determination of the total nitrogen of soil and available nitrogen adopted semi-micro kjeldahl method (Feng *et al.*, 2014); determination of total phosphorus and available phosphorus of soil adopted Vanadium molybdate blue colorimetric method (Lu, 1991); determination of total potassium and available potassium adopted flame photometry; determination of organic matter used potassium dichromate oxidation-heating method (Shuang and Nisana, 2016) and pH value was determined by potentiometry.

Test Design

83 varieties with different germplasm were planted in the farm of Xizang Agriculture and Animal Husbandry College (March-August, 2015 and March-August, 2016). The area was 6 m ×4 m = 24 m² with random block arrangement with 3 times repeat. The management was the same with local field. After harvest; each community chose 10 strains for GNS statistics.

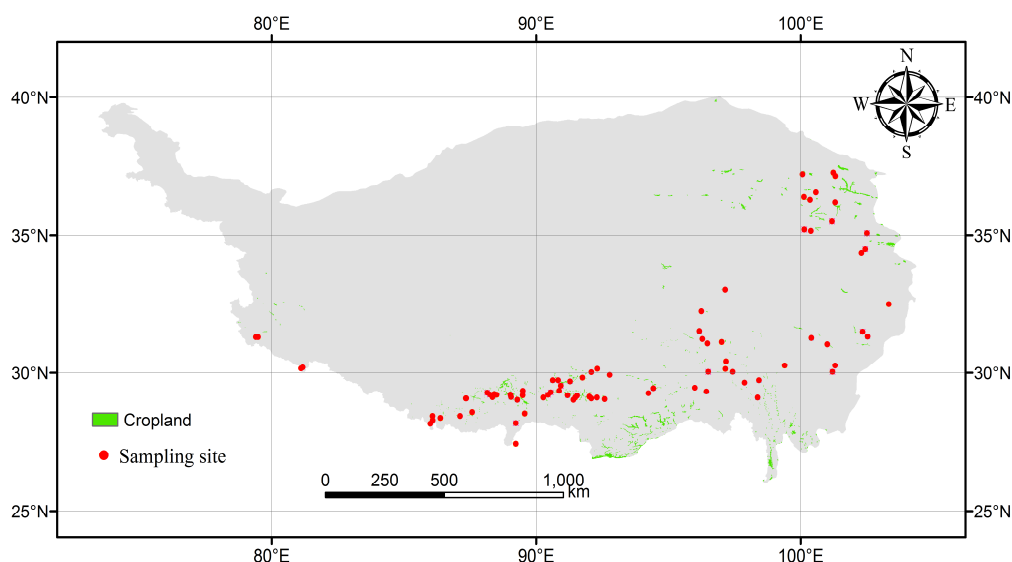


Fig. 1: Distributions of sampling points

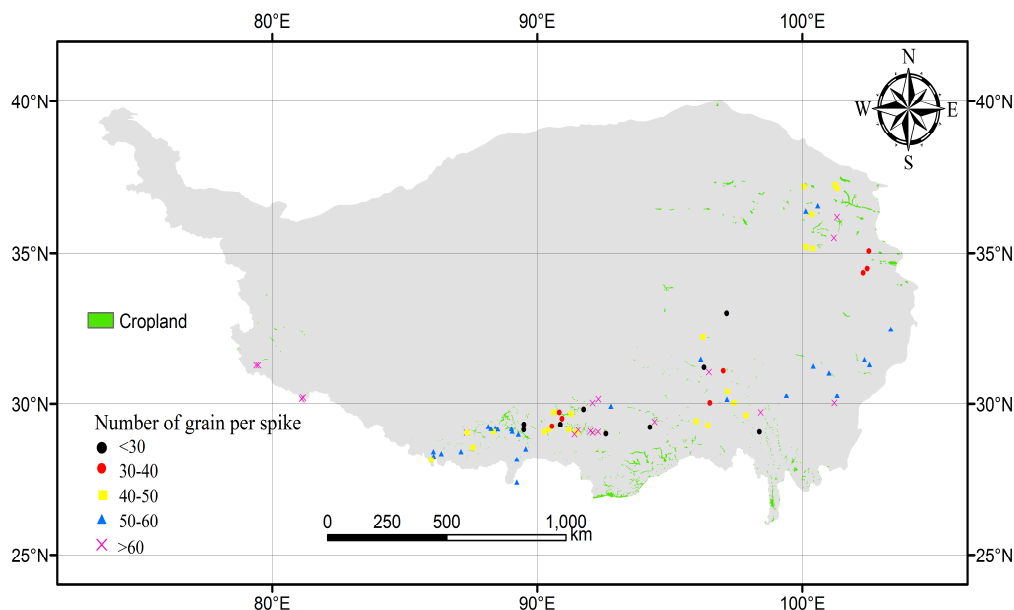


Fig. 2: Distribution of highland barley GNS in the Qinghai-Tibet plateau

Data Analysis and Processing

(1) Using ARCGIS9.3 software made space distribution (shown in Fig. 2) of highland barley GNS for the 83 sample points. (2) In order to study the effect of environmental factors on highland barley GNS, the interference of variety factor (genetic factor) should be eliminated. In view of the dozens of latitude and longitude and thousands of meters of altitude gradient of Qinghai-Tibet Plateau, we chose dozens of sample points and tens of varieties for planting experiment. This study was mainly that different highland barley varieties in different sample points were planted in the same environment conditions. Through the variance analysis and multiple comparisons, the samples (eliminate the interference of variety factor) without significant GNS difference are chosen and using the GNS data of origin and geography, soil and climate data of origin (note: Climate data comes from China's national meteorological information center) to establish data model and compare the contribution of each environmental factor to GNS.

Results

Distribution Characteristics of Highland Barley GNS in the Qinghai-Tibet Plateau

The Horizontal Distribution Characteristics of GNS

As shown in Fig. 2, the high value area of highland barley in the Qinghai-Tibet Plateau is mainly in longitude 91.0-92.5° and north latitude 29.0-31.0° and the high value area of central and southern Tibet taking

Qiongjie, Longzi, Jiacha and Shangri county of Xizang, where the average GNS is around 71.9±4.0.

The Vertical Distribution Characteristics of GNS

Vertically, GNS presented "bimodal curve" type distribution pattern with the increasing of altitude (Table 1). That is, under the altitude of 3000-3300 m, GNS changes smoothly with the change of altitude. Above the altitude of 2700-3000 m, highland barley GNS increases with the increasing of altitude; it reaches a higher value area at altitude of 3900-4200 m and the average GNS is 52.9±17.6. And then, GNS decreases with the increasing of altitude and reaches the least value at the altitude of 4200-4500 m. After that, GNS increases gradually with the increase of altitude and reaches the peak value at above 4500 m with 57.2±0.2. In the area that the altitude is less than 2700 m and more than 4200 m, the variation coefficient of highland barley GNS is small; in the area that the altitude is 2700-4200 m, the variation coefficient of highland barley GNS is large (Table 1)

The Relationship of Highland Barley GNS in the Qinghai-Tibet Plateau and Environmental Factors

After the planting of 83 sample points on the farm of Xizang Agriculture and Animal Husbandry College, their GNS was for variance analysis. The results showed that the amplitude of highland barley GNS was 22.2-86.9, the average was 49.7±17.0 and the variation coefficient was 34.2%, different sources of highland barley varieties GNS were extremely significant different (Table 2). After multiple comparisons to the 83 varieties from the 83 sample points, it can be found that 47 varieties had no significant difference.

Table 1: The distribution of highland barley GNS in different elevation gradient

Altitude (m)	Number of sites	Average (%)	CV (%)
<2700	2	45.3±6.9	15.29
2700~3000	6	44.2±14.9	33.74
3000~3300	17	47.8±14.4	30.03
3300~3600	9	49.5±19.0	38.32
3600~3900	27	49.4±19.0	38.39
3900~4200	14	52.9±17.6	33.20
4200~4500	6	48.7±4.8	9.92
>4500	2	57.2±0.2	0.30
Total	83	49.4±16.1	32.71

Table 2: F-test of highland barley variety GNS from different sources

Variation	SS	DF	MS	F	p
Inter-blocks	524.4046	2	262.2023	2.597	0.0776
Inter-treatments	54397.4563	82	663.3836	6.569	0.0023
Error	16561.1688	164	100.9827		
Total CV	71483.0296	248			

The Relationship of Highland Barley GNS and Geographical Factors

Taking geographic longitude (X_1), latitude (X_2) and altitude (X_3) as the independent variables, highland barley GNS(Y) as the dependent variable to establish the regression equation as follows:

$$Y = 66.4289 + 0.400X_1 - 0.3416X_2 - 0.5615X_3$$

$$\left(\begin{array}{l} N = 47 R = 0.3399 R_1 = 0.1399 \\ R_2 = -0.4614 R_3 = -0.5416^* \end{array} \right) \quad (1)$$

It can be seen from Equation (1) that highland barley GNS (Y) has positive correlation with geographic longitude (X_1), has negative correlation with geographic latitude (X_2) and altitude (X_3); among them, the effect of altitude on highland barley GNS is prominent. For the reason that the regression equation does not make F test, it shows that the combination of each geographical factor has no significant effect on highland barley GNS.

The Relationship of Highland Barley GNS and Climatic Factors

Taking highland barley GNS(Y) as the dependent variable, sunshine hours in growth period(X_1), evaporation in growth period (X_2), accumulated temperature $\geq 0^\circ\text{C}$ in growth period (X_3), accumulated temperature $\geq 10^\circ\text{C}$ in growth period (X_4), temperature in growth period(X_5), the relative humidity in growth period (X_6), rainfall in growth period (X_7), the average temperature at emergence-tillering stage (X_8), the

average temperature at tillering-jointing stage (X_9), the average temperature joining-heading stage (X_{10}), the average temperature at heading-filling stage (X_{11}), the average temperature filling-mature period (X_{12}), daily range of the average temperature at emergence-tillering stage (X_{13}), daily range of the average temperature at tillering-jointing stage (X_{14}), daily range of the average temperature in jointing-heading period (X_{15}), daily range of the average temperature at heading-filling stage (X_{16}), daily range of the average temperature in filling-mature period (X_{17}), the average relative humidity at emergence-tillering stage (X_{18}), the average relative humidity at tillering-jointing stage (X_{19}), the average relative humidity in jointing-heading period (X_{20}), the average relative humidity at heading-filling stage (X_{21}), the average relative humidity in filling-mature period (X_{22}), the average precipitation at emergence-tillering stage (X_{23}), the average precipitation at tillering-jointing stage (X_{24}), the average precipitation at jointing-tillering stage (X_{25}), the average precipitation in tillering-filling period (X_{26}) and the average precipitation in filling-mature period (X_{27}) as the independent variables, the equation was established as follows through stepwise regression:

$$Y = 65.7023 - 0.0056X_4 + 0.2892X_6 + 0.4644X_{12}$$

$$+ 0.3139X_{19} - 0.9923X_{22} + 0.2530X_{24} - 0.3657X_{25}$$

$$+ 0.5302X_{26} - 0.1890X_{27} \quad (2)$$

$$\left(\begin{array}{l} N = 47 R = 0.6737 R_4 = -0.2078 R_6 = 0.3549 \\ R_{12} = 0.1517 R_{19} = 0.2752 R_{22} = -0.4206 R_{24} = -0.4392^* \\ R_{25} = -0.5898^{**} R_{26} = 0.4916^* R_{27} = -0.2219 \end{array} \right)$$

It can be found from Equation (2) that the main climate factors effecting highland barley are X_4 , X_6 , X_{12} , X_{19} , X_{22} , X_{24} , X_{25} , X_{26} and X_{27} . The effects of sunshine hours in growth period (X_1), evaporation in growth period (X_2) and accumulated temperature $\geq 0^\circ\text{C}$ in growth period (X_3) are small. Meanwhile, it can also be found from Equation (2) that highland barley GNS and the partial correlation coefficient of X_{26} reach the difference level of positive correlation; highland barley GNS and X_{24} and X_{25} reach the difference level of significant and extremely significant negative correlation; highland barley GNS and the partial correlation coefficient of X_4 , X_6 , X_{12} , X_{19} , X_{22} and X_{27} do not reach the difference level of significance ($p < 0.05$). F test of the regression equation was not significant, which indicates that the combination of climate factors on the impact of barley GNS not reached significant level ($p < 0.05$).

The Relationship of Highland Barley GNS and Soil Factors

Taking highland barley GNS (Y) as the dependent variable, soil organic matter (X_1), total N (X_2), total phosphorus (X_3), total potassium (X_4), soil available N (X_5), available phosphorus (X_6), available potassium (X_7) and soil PH as the independent variable, the equation is as follows through stepwise regression:

$$Y = 37.0299 - 2.4259X_1 + 49.4526X_2 + 167.4792X_3 - 0.0222X_5 - 0.0386X_7 \quad (3)$$

$$\left(\begin{array}{l} N = 47 R = 0.9060^{**} R_1 = -0.5688^{**} R_2 = 0.9032^{**} \\ R_3 = 0.6034^{**} R_5 = -0.6895^{**} R_7 = -0.4535^{**} \end{array} \right)$$

It can be found from Equation (3) that the main soil factors influencing highland barley GNS (Y) are X_1 ,

X_2 , total phosphorus X_3 , X_5 and X_7 ; while, the effect of X_4 , X_6 and X_8 is small. It can also be seen from Equation (3) that highland barley GNS has extremely significant positive correlation with X_2 and X_3 and has extremely significant negative correlation with X_1 , X_5 and X_7 . The regression equation passes F test, which indicates that highland barley GNS increases with the increasing of X_2 and X_3 and the decreasing of X_1 , X_5 and X_7 .

The Relationship of Highland Barley GNS and Comprehensive Factors

According to the above analysis, highland barley GNS and the partial correlation coefficient of the average precipitation at heading-filling stage (X_{26}), total N (X_2) and total phosphorus (X_3) reaches the difference level of significant or extremely significant positive correlation. Highland barley GNS and the partial correlation coefficient of the average precipitation at tillering-jointing stage (X_{24}), the average precipitation at jointing-tillering stage (X_{25}), soil organic matter (X_1), available N (X_5), available potassium (X_7) and altitude (X_8) reach the difference level of significant or extremely significant negative correlation. Therefore, we use advantages (leading) analysis to make importance analysis to the 9 variables (soil organic matter $X(1)$, total N of soil $X(2)$, soil total phosphorus $X(3)$, soil available N $X(4)$, soil available potassium $X(5)$, the average rainfall at tillering-jointing stage $X(6)$, the average rainfall at jointing-heading stage $X(7)$, the average rainfall at heading-filling stage $X(8)$, altitude $X(9)$) affecting highland barley GNS significantly, the order is total N of soil > soil available N > the average rainfall at heading-filling stage > altitude > soil organic matter > soil total phosphorus > the average rainfall at jointing-heading stage > soil available potassium > the average rainfall at tillering-jointing stage (Table 3).

Table 3: The contribution of decision factor in each order regression model

Models	$X(1)$	$X(2)$	$X(3)$	$X(4)$	$X(5)$	$X(6)$	$X(7)$	$X(8)$	$X(9)$
Single factor	0.0278	0.3619	0.0338	0.0465	0.0075	0.0028	0.0253	0.0998	0.0728
One order interaction	0.0740	0.4541	0.0795	0.1012	0.0494	0.0185	0.0389	0.0968	0.0711
Two orders interaction	0.1094	0.4593	0.1130	0.1371	0.0838	0.0599	0.0748	0.1019	0.0864
Three orders interaction	0.1369	0.4321	0.1373	0.1613	0.1123	0.1065	0.1170	0.1221	0.1013
Four orders interaction	0.1624	0.3840	0.1577	0.1796	0.1385	0.1433	0.1502	0.1455	0.1232
Five orders interaction	0.1840	0.3242	0.1755	0.1932	0.1624	0.1675	0.1713	0.1656	0.1457
Six orders interaction	0.1938	0.2600	0.1889	0.1985	0.1807	0.1814	0.1825	0.1795	0.1587
Seven orders interaction	0.1915	0.1979	0.1910	0.1919	0.1901	0.1901	0.1902	0.1899	0.1786
Eight orders interaction	0.1350	0.3592	0.1346	0.1512	0.1156	0.1088	0.1188	0.1376	0.1213
Nine orders interaction	0.0278	0.3619	0.0338	0.0465	0.0075	0.0028	0.0253	0.0998	0.0776
Ten orders interaction	0.0740	0.4541	0.0795	0.1012	0.0494	0.0185	0.0389	0.0968	0.0745
Total	0.1094	0.4593	0.1130	0.1371	0.0838	0.0599	0.0748	0.1019	0.1011
Percentage (%)	10.7100	28.4900	10.6800	11.9900	9.1700	8.6300	9.4200	10.9200	10.8200

Discussion

This study results show that soil factors interact with climate factors, in particular, the effect of soil factors on the Qinghai-Tibet Plateau has a great influence on highland barley GNS. Among them, the geographical factor effecting highland barley GNS is altitude gradient; soil factor is the content of total n and available n; the climate factor is the average precipitation at heading-filling stage. Influenced by these environment factors, highland barley GNS appears a patch shape distribution pattern in the geographical horizontal directions, forming high value areas of highland barley GNS taking Tibet Qiongjie, Longzi, Jiazha and Sangri County as the center; In the vertical direction, the change of highland barley appears "bimodal curve" distribution pattern and forms two high value areas of highland barley above the altitude of 3900-4200 and 4500 m. Causes of this phenomenon may be related to the unique plateau climate factors and the effect of soil difference in the geographical area of Qinghai-Tibet Plateau. That is consistent with the research results that most of scholars thought although barley GNS is mainly controlled by variety characteristics, i.e., control of genetic factors and environmental factors also have obvious influence on it (Abeledo *et al.*, 2008; Garcia *et al.*, 2003; Zou *et al.*, 2009).

Partial correlation analysis shows that highland barley GNS has positive correlation with the average precipitation at heading-filling stage and soil total n content; and highland barley GNS has negative correlation with the average precipitation at tillering-heading stage, altitude, the content of soil organic matter and available potassium and has no correlation with sunshine hours in the growth period. This study is basically identical with the results of Du *et al.* (2002; Chalak *et al.*, 2015; Ugarte *et al.*, 2007). However, Guillermao *et al.* (2015) thought night temperature increased in the reproductive growth period of barley, GNS decreased; Reinhardt *et al.* (2013) thought the night temperature was about 30°C in the reproductive growth period of barley, GNS decreased significantly. The effect of climate factors on barley GNS is relatively complex, interaction of multiple factors makes a single factor analysis more difficult. In actual experiment, it is difficult to let other variables constant to analyze the effect of a single variable; this is also the limitations of partial correlation analysis. Night temperature increase influences the accumulation of dry matter; extreme high temperature influences pollination and spikelet differentiation and then affects GNS. The research results of Guillermao *et al.* (2015; Reinhardt *et al.*, 2013) have no contradiction; on the contrary, large temperature difference in the Qinghai-Tibet Plateau provides corresponding basis for the increase of highland barley GNS. Högy *et al.* (2013) thought that barley plant in

Stuttgart, Germany, temperature had no significantly effect on barley GNS, which is inconsistent with most research results. Temperature difference is small in Stuttgart, the average temperature all the year round is 10.7°C with less rainfall. Temperature is not the restrictive factor for barley growth, the conclusions of Högy *et al.* (2013) may be related to the special climate factors of Stuttgart.

Many scholars at home and abroad agree that N rate has positive correlation with barley GNS (Papastylianou, 1995b; Cantero-Martínez *et al.*, 2003; Papastylianou, 1995a; Le *et al.*, 1999; Prado *et al.*, 2013; Baethgen *et al.*, 1995) and the results are basically identical with them. Miralles and Richards (2000) though that with the increase of sunshine time, barley GNS in Arapiles increases with it. The study shows that barley GNS is not associated with the sunshine time in growth period. This may be due to Qinghai-Tibet Plateau is located in the low latitudes with longer sunshine time and sunshine time is not the restrictive factor of barley growth and development.

The GNS of barley is one of the most important contributing factors of the yield of barley and it is one of the important indicators of the genetic diversity of barley. According to the spatial distribution pattern of GNS in the Qinghai-Tibet Plateau, they are mostly distributed in the high-altitude areas of the high mountains and valleys in the south of Tibet and eastern Tibet, which is basically consistent with the origin center of barley reported by Ma (2000). Therefore, the spatial distribution regularity of GNS can provide help for the study of the origin and evolution of barley. The results of studies on the impact of environmental factors on the GNS of highland barley indicate that the amount of precipitation at heading stage and nitrogen fertilizer has an important effect on the increase of GNS of the highland barley. Therefore, it is necessary to increase nitrogen fertilizer and timely and reasonable irrigation in order to increase the yield of barley in Qinghai-Tibet Plateau. However, the soil types of the main cultivation areas in the Qinghai-Tibet Plateau are mainly shrub grassland and sub-alpine grassland soils (Hu, 1995). The main characteristics of this soil type are high sand content, poor water retention and fertility conservation. Therefore, reasonable fertilization and irrigation, increase of nitrogen fertilizer and water use efficiency are important issues that need to be resolved in the future.

Conclusion

Highland barley GNS in the Qinghai-Tibet Plateau appears a patch shape distribution pattern, mainly distributes in the north of Himalayan, central south and east of Tibet in the Transaction Mountain over 3900 m altitudes. It is located in the high mountain canyon of middle of the Yarlung Zangbo River, the Jinsha River, Nujiang River and Lantsang, is one of the distribution

centers of barley diversity with complicated and diverse geographical environments. Among them, soil n, altitude gradient, organic matter content and precipitation have great influence on highland barley GNS.

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Author's Contributions

Xi-Bo Feng: Conceived and designed the work that led to the submission, analyzed the data and wrote the paper.

Gai-Hua Wang: Performed the numerical experiments and revised the manuscript.

Jian-Lin Wang: Revised and approved the final manuscript.

Ethics

The authors declare their responsibility for any ethical issues that may arise after the publication of this manuscript.

Conflict of Interest

The authors declare that they have no competing interests. The corresponding author affirms that all of the authors have read and approved the manuscript.

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