Article

Genome Size Unaffected by Variation in Morphological Traits, Temperature, and Precipitation in Turnip

Supriyo Basak [1,2,3] iD **, Xudong Sun** [1,2,3] iD **, Guangyan Wang** [1,2,3] **and Yongping Yang** [1,2,3,*]

[1] Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China; basak@mail.kib.ac.cn (S.B.); sunxudong@mail.kib.ac.cn (X.S.); wangguangyan@mail.kib.ac.cn (G.W.)

[2] Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

[3] Institute of Tibetan Plateau Research at Kunming, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

* Correspondence: yangyp@mail.kib.ac.cn; Tel.: +86-871-6522-3234

check for updates

Featured Application: The current work describes the adaptive evolution of turnip in the Qinghai Tibetan Plateau and its surrounding regions which will provide valuable information for genetics and plant breeding studies of *Brassica* vegetables.

Abstract: Genome size (GS) was proposed as proxy for gross phenotypic and environmental changes in plants. GS organismal complexity is an enigma in evolutionary biology. While studies pertaining to intraspecific GS variation are abundant, literatures reporting the adaptive significance of GS are largelymissing. During food shortage, *Brassica rapa* var. *rapa* (turnip) is used as food and fodder for sustaining the livelihood of residents in the Qinghai Tibetan Plateau (QTP), which is also known as "the roof of the world". Thus, climatic extremities make this region a natural environment to test adaptive significance of GS variation in turnip landraces. Therefore, from the QTP and its adjacent regions (the Hengduanshan and the Himalayas), we investigated adaptive evolution of GS in turnip landraces. Tuber diameter of turnip landraces was found to be significantly correlated with most of the environmental factors. GS was also shown not to be associated with morphological traits, temperature, and precipitation. Moreover, principal component analyses based on the whole dataset trisected the landraces into three distinct populations based on landrace usage—Hengduanshan, QTP, and the Himalayas. Nonetheless, our cumulative dataset showed evidence of adaptation of turnip landrace to different environments throughnonassociated genomic and phenomic plasticity.

1. Introduction

Quantification of phenotypic and genotypic changes in landrace requires an easily quantifiable character. Genome size (GS), being an indicator of evolutionary distance [1], is an important biodiversity trait [2] for molecular characterization of living organisms at a rapid pace. Many authors have proposed adaptive significance of GS variation [3–8]. Hence, GS is considered as proxy for understanding elementary biological processes at different spatial scales [9]. For instance, GS is positively correlated with cell size [10]. GS is also correlated with leaf mass per area [11]. A positive correlation between GS and seed weight (SW) was speculated [12] after studying a large number of congeneric taxa,

but a nonsignificant correlation of GS vs. SW [5] and GS vs. seed size (SS) [13] were investigated recently. Furthermore, no correlation of GS with SW was shown as evidence for noninvasiveness in Acacia [5]. Moreover, lack of association of GS with morphological traits (SW) between wild and cultivated Amaranthusspecies exhibited the signature of incomplete domestication [14]. Therefore, the knowledge of adaptive significance of GS variation for different plants from different environmental conditions is still in its infancy [15]. Unfortunately, to date, studies addressing adaptive evolution of GS are rare and these contrasting hypothesis remains to be corroborated experimentally in the Qinghai–Tibetan Plateau (QTP) and its neighboring regions (the Himalayas and the Hengduanshan) (Figure 1) which may unravel the adaptive evolution of landraces from these important agroecological environments.

Landraces are important constituents of agriculture and a source of farmer's income [16]. Understanding the climatic resilience of landraces requires the knowledge of adaptation under natural selection [17]. Although the last two decades saw considerable attention to conserve these genetic resources on farm, the gross estimation of diversity present on farms at a global and regional scale is scarce; there is a concern for erosion of genetic diversity that sustains feeding the world [18]. Landraces may adapt to a rapidly changing climate either in genotypic changes or phenotypic modification or by combination of both genotypic and phenotypic traits [19]. Domestication, a slow evolutionary process regulated by human selection, governs GS variation of wild and cultivated species [8,13,20]. For instance, highly domesticated dwarf coconut (*Cocosnucifera*) varieties show significantly lower GS compared to less domesticated taller coconut varieties [13]. The relationship of GS with plant traits under a varying environment has been the topic of recent biotechnological research interests [3,7,8,21]. Morphological traits of archaeobotanical samples were taken as proxy for reconstruction of ancestral GS. For example, using leaf guard cell size as proxy, the ancestral GS of early angiosperms (Magnoliaceae) was calculated at ~1 pg, suggesting a small genome size might be a typical feature of early diverging angiosperms [22]. The QTP, the world's highest plateau, is considered as the least hospitable climate for agriculture requiring clarification of many issues involving crop evolution [23]. For example, the knowledge of domestication is still in the immature stage for crops of the QTP [23]. This region witnessed massive climatic disturbances during the past 50 million years [24]. Forests were transformed into grasslands and deserts due to formation of a dry, cool, and windy climate [25]. Landraces adapted in these hilly slopes of the QTP are unable to escape these climatic fluctuations. On the other hand, landraces adapted in the Himalayas and Hengduanshan regionsare untapped reservoirs of genetic resources on farms. Thus, from the QTP and its adjacent region, investigation of the relationship of GS with phenotypic traits and environmental factors may unravel the knowledge of adaptive evolution of genetic resource on farm.

Animal husbandry (approximately 21 million head of livestock from a recent estimate) strengthens the QTP economy along with its agriculture [26]. Although the QTP is gaining self-sufficiency in feeding its people through its own cultivation, the production of fodder crops within the limited arable land is becoming an uphill task [27]. Turnip (*Brassica rapa* var. *rapa*), maize (*Zea mays*), pea (*Pisumsativum*), broad bean (*Viciafaba*), vetch (*Viciasativa*), and lucerne (*Medicago sativa*) are commonly used as a fodder crop of the QTP [28]. Turnip is locally named as "Man Jing" in China, "YuanGen" and "Niu Ma" in QTP, and "Salgam" in the Himalayas. It is an herbaceous plant from Brassicaceae, cross pollinating and biennial in nature. Turnip is also used as food during food scarcity in the QTP. Storage of seeds for next year sowing is the tradition of the QTP agriculture which led to the accumulation of locally adapted landraces. Usage of turnip as fodder is not conceptualized yet in the Himalayas, whereas China (Hengduanshan) enjoys a monopoly in turnip production. In our previous research, we have shown that GS of turnip landraces from the QTP were smaller than turnip landraces from the Yunnan Plateau [29]. In this research article, we show that the adaptation of turnip landraces to different environments is governed by nonassociated genotypic and phenotypic plasticity, this is supported by the experimental results of the following addressed questions; (1) what is the relationship between GS and morphological and reproductive traits for landraces adapted to different

environments? (2) What did this information reveal on the potential ecological and evolutionary forces that act on turnip landraces from the QTP, the Himalayas, and the Hengduanshan region?
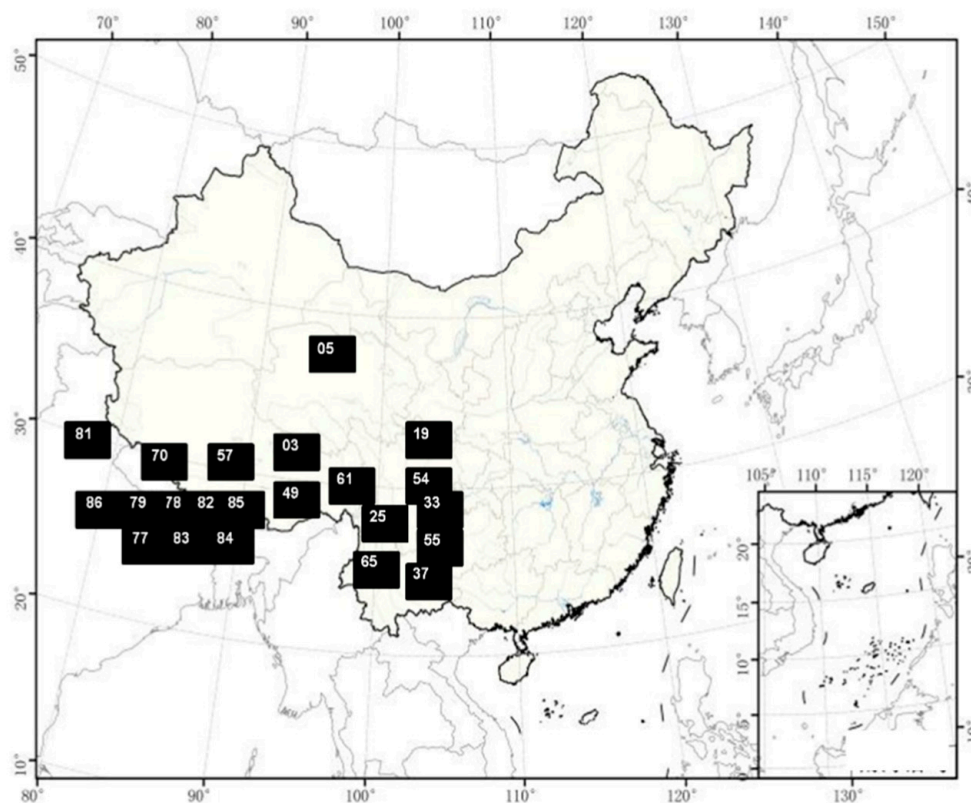

Figure 1. Geographic distribution of landraces. Locations are not exact geographic locations because location data were given as country province. Landraces are represented by their accession numbers in this figure and detailed information of the collection sites are listed in Supplementary Table S1. The three environments are Henduanshan (KTRG-B-25, KTRG-B-33, KTRG-B-37, KTRG-B-54, KTRG-B-55, KTRG-B-61, and KTRG-B-65),Qinghai–Tibetan Plateau (KTRG-B-03, KTRG-B-05, KTRG-B-19, KTRG-B-49, and KTRG-B-57), and the Himalayas (KTRG-B-70, KTRG-B-77, KTRG-B-78, KTRG-B-79, KTRG-B-81, KTRG-B-82, KTRG-B-83, KTRG-B-84, KTRG-B-85, and KTRG-B-86). KTRG represents the Kunming Turnip Research Group.

2. Materials and Methods

2.1. Plant Materials

Twenty-two turnip landraces were collected from the farmers of different regions. Seven landraces were collected from China (Mountain of Hengduanshan), five landraces were collected from QTP region, and ten landraces were collected from the Himalayas. The location information was listed (Supplementary Table S1). During the time of collection of the samples, data were recorded about the morphology of the plants along with the method of cultivation (use of manures or pesticides). The collection of seeds from the farmers was carried out during the time of harvesting (February–March). The seeds collected from the farmers were the representative samples of those particular agroclimatic environments. The geographical coordinates were recorded using a satnav (satellite navigation instrument) and altitude of each collection places was determined with help of a digital altimeter. The seeds were deposited in the germplasm bank of wild species—the Kunming Institute of Botany. Seeds were grown in the agricultural field of Ninglang, Yunnan province all at the same time to full maturity. Sandy loam soil of pH between 6 and 7 was used for the propagation of turnip. Separation between the seedlings was maintained 15 cm. During time of flowering, turnip flowers were netted to prevent introgression. Morphological observation was carried out from the

on-field data. The plant materials were photographed during the time of harvesting for their tuber dimension which was already documented in our previous study [29]. After transfer to the laboratory, seeds were stored at 15 °C and 15% relative humidity for 1 week to reduce moisture content to prevent mildew. Seeds harvested from landraces grown in the same agricultural field of Ninglang were used for measurement of seed characteristics and at least seeds from 15 individuals were mixed randomly and best seeds were selected for phenotypic measurements. Immature leaves were transferred to Kunming for GS investigation from Ninglang and all GS estimates were carried out within 5 days of arrival of the materials.

2.2. Measurement of Morphological Characteristics

Morphological traits were recorded at the maturity cycle of varieties. Leaf length (LL), weight of above ground biomass (ABM), tuber diameter (TD), and weight of below-ground biomass (UGB) are important attributes for understanding the genome–phenome relationship. As these four parameters were shown to correlate significantly with the GS, we measured only four phenotypic characteristics. The morphological measurement for each landrace was carried out on ten individual plants. Leaf length was measured for the third leaf of each plant after flowering stage was reached. Tuber diameter (TD), weight of above ground biomass (ABM), and weight of below-ground biomass (UGB) were measured after the harvesting of turnip root [30].

2.3. Measurement of Seed Characteristics

To investigate variations in turnip's SS and SW, 1000 seeds per landrace (at random) were selected for measurement. The measured seeds were divided into five groups, each of which was comprised of 100 seeds. Seed diameter (SS) was measured with a digital caliper (Deli, Shanghai, China). The seed groups were weighed on an electronic balance (Longteng, Shenyang, China) to calculate hundred SW of different turnip landraces. The data represented in this manuscript was one-thousand SW, which is calculated as the ten times the data of hundred SW [30].

2.4. GS Measurements

In this investigation we measured holoploid GS (2C nuclear DNA content) of turnip races. GS was estimated by using Glycine max 'Polanka' (soybean) as internal standard (2C = 2.50 pg) [31] in a flow cytometer (Partec GmbH, Münster, North Rhine-Westphalia, Germany). The sample preparation for GS investigation was as described previously [32]. In short, leaves of standard and test species were co-chopped in 800 μL of WPB buffer [32] and filtered through 30 μm nylon filters (CellTrics®Partec, Görlitz, Germany). The filtrate was stained with a solution containing propidium iodide (PI) (2 mg·mL^{-1}) (Sigma, St. Louis, MO, USA) and RNase A (50 μg·mL^{-1}) (Sigma) and acquired in flow cytometer. The resulting histogram was analyzed in FlowJo version 7.6.1 (FlowJo, Tree Star Inc., Ashland, OR, USA) software. Three to six individuals were tested for calculating GS of each landrace.

2.5. Contribution of Ecogeographical Variables to Changes in Morphology and GS

For 22 landrace collection sites, values of nineteen biologically relevant climatic variables with approximately 1 km^2 spatial resolutions were extracted using a statistical computing environment [33] from the WORLDCLIM dataset. For assessment of the relationship among seed characteristics and environmental parameters, Pearson correlation analyses (using SPSS) were performed.

2.6. Statistical Analysis

Differences in GS, LL, ABM, TD, UBM, SS, and SW was evaluated in one way analysis of variance (ANOVA) at a significant level of 1% (α = 0.01) and Tukey's honest significant difference test was used to evaluate differences among groups of landraces in SPSS 15.0 (IBM Corporation, Somers, NY, USA). PCA was performed in R statistical computing platform.

3. Results

3.1. Variation in Morphological Characteristics

Significant differences in LL, ABM, and TD were observed among landraces (LL: $F_{21,102}$ = 8.74 p = 0.00; ABM: $F_{21,102}$ = 4.08, p = 0.00 and TD: $F_{21,102}$ = 1.89 p = 0.02). No significant difference was observed for UGB ($F_{21,102}$ = 1.38, p = 0.154). The average values of LL, ABM, TD, and UGB of landraces from the Hengduanshanwere found to be significantly ($p < 0.01$) higher ((LL = 75.39 $\pm$ 3.32 cm, $F_{2,102}$ = 9.37 p = 0.00), (ABM =0.42 $\pm$ 0.05 kg, $F_{2,102}$ = 10.87, p = 0.00), (TD = 107.27 $\pm$ 7.68 mm $F_{2,102}$ = 3.60, p = 0.03) and (UGB = 0.61 $\pm$ 0.01, $F_{2,102}$ = 4.48 p = 0.00)) compared to other environments. The lowest average value of ABM, TD, and UGB was observed from the QTP environment (ABM = 0.18 $\pm$ 0.03 kg, TD = 80.31 $\pm$ 5.03 mm and UGB = 0.26 $\pm$0.05 kg). The lowest average LL was observed from the Himalaya environment (LL = 58.74 $\pm$ 2.40 cm). From these analyses, it was evident that morphological differences existed among landraces adapted in three different environments under consideration. Furthermore, turnips of the Hengduanshan were healthier in above-ground and below-ground phenotypic traits compared to races adapted to the Himalayas and the QTP environments (Figure 2a–d).
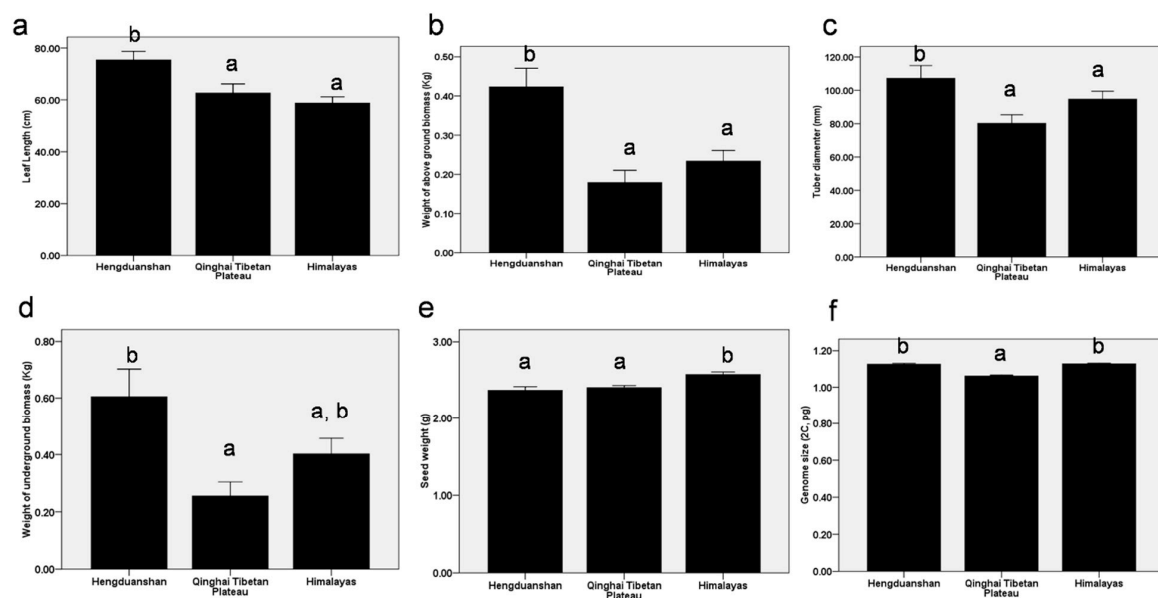

Figure 2. Variation in morphological traits and genome size among the three environments selected in this investigation. The bar diagrams show (**a**) average leaf length, (**b**) average weight of above ground biomass, (**c**) average tuber diameter, (**d**) average weight of underground biomass, (**e**) average seed weight, and (**f**) average genome size investigated from three environments. The histograms with same letters are statistically similar estimates ($p < 0.01$).

3.2. Variation in Seed Characteristics

When significant variation in above-ground and below-ground morphological traits was observed, we were interested in knowing the variation in reproductive characters from three agroecological environments. To elucidate this, we measured SS and SW of landraces grown in the same environment (Ninglang, China) all in the same time. Significant difference in SS ($F_{21,2178}$ = 99.28, p = 0.00) and SW ($F_{21,308}$ = 100.62, p = 0.00) were observed among landraces. To our surprise, the highest average SS and SW were not from the Hengduanshan environment, which we expected from their morphological observation of LL, ABM, TD, and UGB. Instead, the highest average SS was observed from the QTP environment (SS = 1.68 $\pm$ 0.00 mm) and the highest average SW was observed from the Himalaya environment (SW = 2.77 $\pm$ 0.03 gm). The lowest average SS (1.60 $\pm$ 0.00 mm) and SW (2.36 $\pm$ 0.05 gm) were found in landraces from the Hengduanshan environment. Thus it was clear that

the morphological and reproductive characters among the landraces were different and an evolutionary study for this pattern from the three environments was needed (Figure 2e, Supplementary Table S1).

3.3. Variation in GS

GS is one of the important criteria for evolutionary study at the intraspecific scale. When a conflicting pattern between morphological and reproductive character was established, we were interested in elucidation of genomic plasticity of turnip landraces adapted to cultivation from three environments. Aneuploidy is one of the characteristics features of Brassicavegetables. In our previous study, we systematically analyzed the cytological features of the Yunnan and QTP environments which showed the absence of aneuploidy among the studied landraces [24]. We followed well-established protocol to achieve precision in our flow cytometric examination of GS. The coefficient of variation of the peak of nuclei of standard and test species were <2% which helped us in obtaining reliable data. The symmetric histogram of turnip and soybean from two environments (QTP and Himalaya) was illustrated (Figure 3a,b, Supplementary Figure S1). Another double peak was represented in Figure 3c which showed true intraspecific GS differentiation between the two environments (the QTP and the Himalaya). The step-by-step gating procedure of flow cytometry data was shown in Supplementary Figure S1. To our surprise, average GS of the QTP turnip was smaller than average GS of the Himalaya and the Hengduanshan turnip landraces.

Significant difference in GS was observed among 22 turnip landraces ($F_{21,623}$ = 22.25, p = 0.00). The highest average GS was reported from the Himalayan environment (2C = 1.13 ± 0.00 pg) with the highest intraspecific GS variation (10.28%; 2C = 1.07 pg (min) – 1.18 pg (max)). The lowest average GS (2C = 1.06 ± 0.00 pg) was observed from the QTP environment with 7.76% intraspecific variation. Intraspecific GS variation of turnip landraces from the Hengduanshan environment was found to be 4.63% with average 2C GS of 1.12 ± 0.00 pg (Figure 2f, Supplementary Table S1).
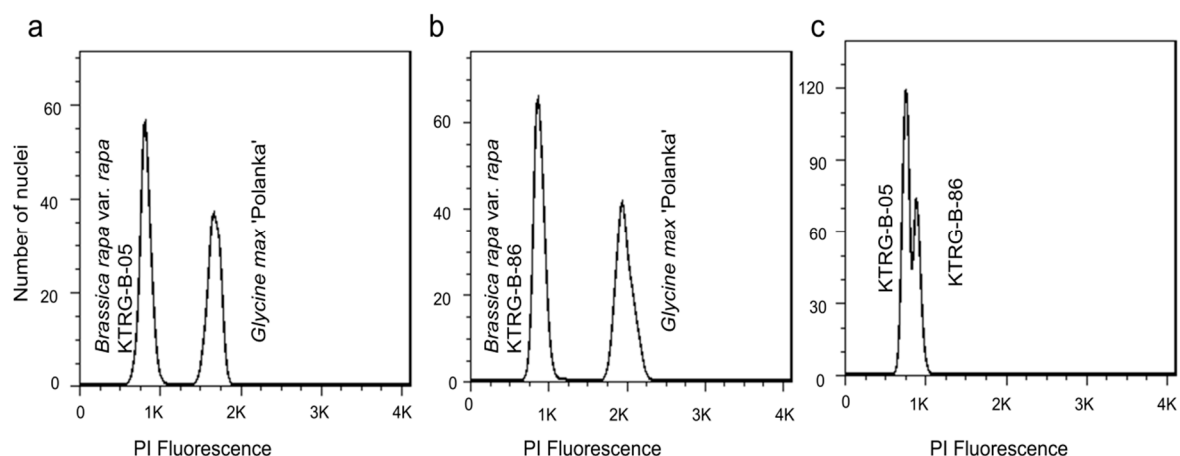

Figure 3. Histograms of flow cytometric data represent genome size estimation of landrace from KTRG-B-05 (**a**) and KTRG-B-86 (**b**) in internal standardization with Glycine max 'Polanka'. Co-chopping of landraces showed differences in genome size (**c**).

3.4. Correlation between Plant Characteristics

Correlation experiments reveal the link between two variables in which one variable is either increased or decreased with the subsequent changes in the other variable. Thus, we checked the correlation of morphological, reproductive, and genomic characters for turnip landraces from three environments. The correlation analyses within morphological traits (LL, ABM, TD, and UGB) and reproductive traits (SS and SW) revealed strong correlation (Supplementary Table S2). Nevertheless, GS was neither correlated with morphological (LL, ABD, TD, and UGB) characters nor with reproductive (SS and SW) characters illustrating GS was not affected by subsequent morphological variation in turnip landraces (Figure 4, Supplementary Table S2).
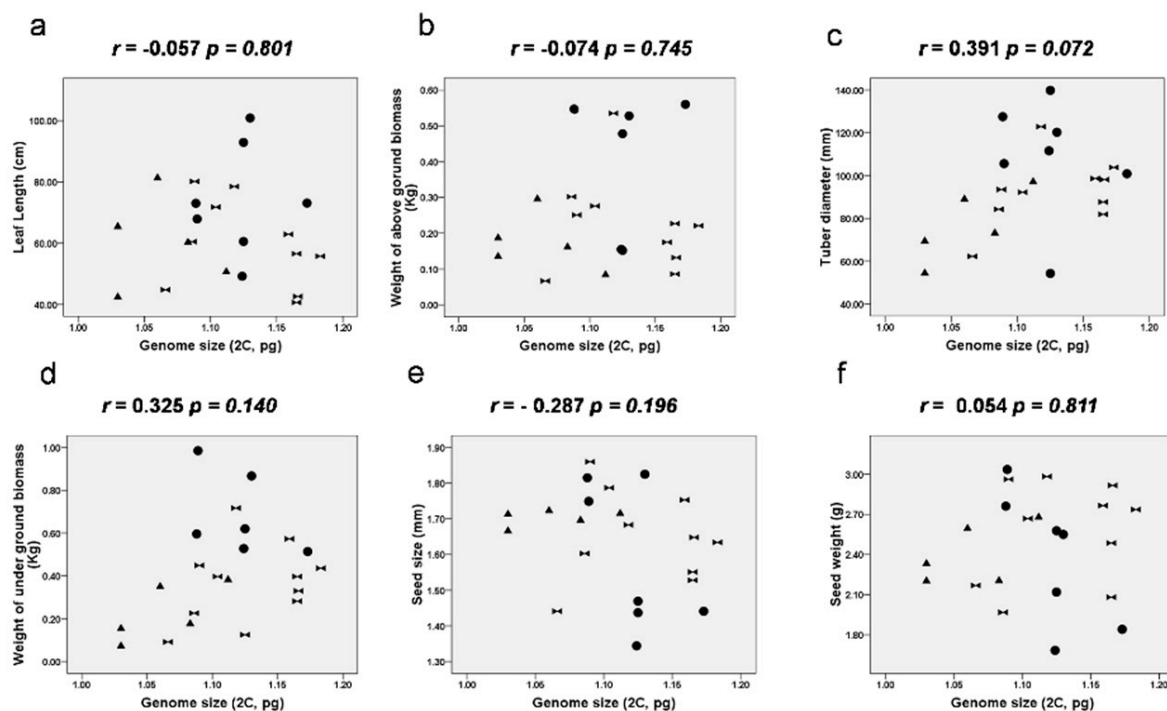
Figure 4. Correlation of genome size with morphological and reproductive characters showing genome size isunaffected by morphological traits. The scatter plot are showing correlation of (**a**) genome size-leaf length, (**b**) genome size–above-ground biomass, (**c**) genome size–tuber diameter, (**d**) genome size–weight of below-ground biomass, (**e**) genome size-seed size, (**f**) genome size-seed weight. The filled circles represent landraces from Hengduanshan, filled triangles represent landraces from Qinghai Tibetan Plateau, and the filled dumbbellshapes represent landraces from the Himalayas.

3.5. Correlation between Environments and Plant Characteristics

Effects of environmental factors on the turnip's vegetative, reproductive traits, and genomic plasticity were assessed through correlation analyses. Among morphological parameters, TD was strongly correlated with 13 environmental variables. Along with this, UGB was strongly correlated with four environmental variables. Thus, the underground biomass of different landraces was affected strongly by environmental factors. Among the reproductive characters, no correlation of SS and SW with environmental factors was observed depicting SS and SW changes were controlled by other factors apart from environment and GS (Supplementary Table S3). It was surprising to us that apart from latitude and elevation (significant negative correlation) GS was not correlated with other environmental factors of temperature and precipitation (Figure 5, Supplementary Table S3).
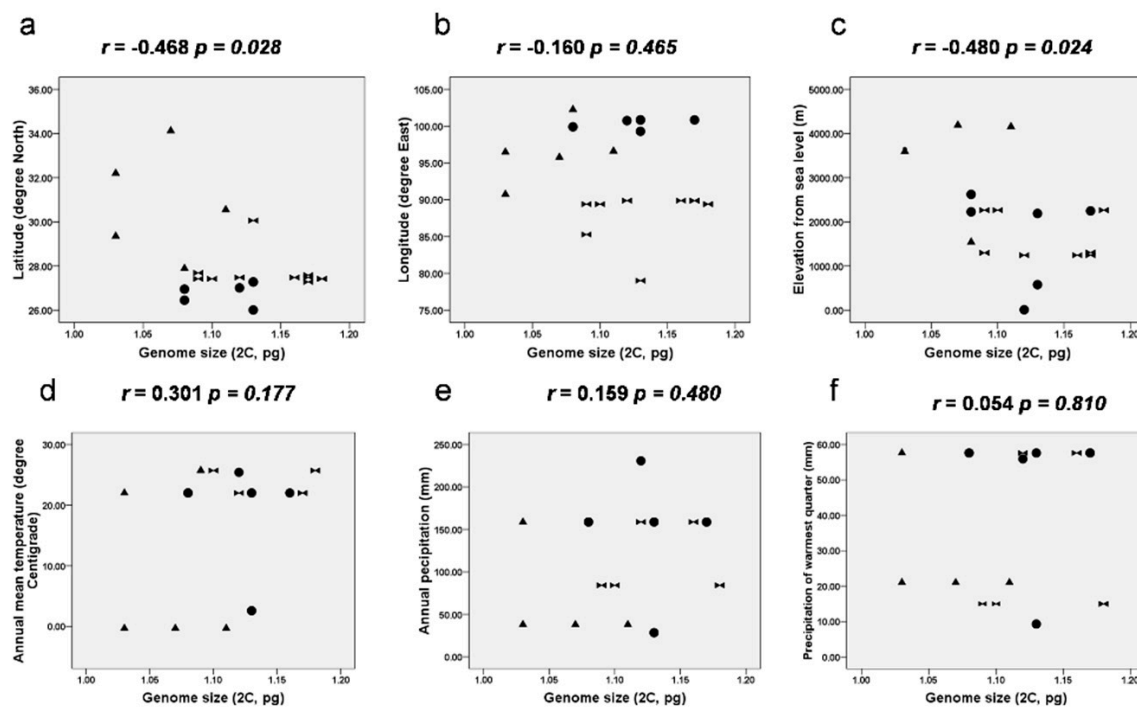
Figure 5. Correlation of genome size with environmental variables showing genome size is unaffected by environmental variables apart from latitude and altitude. The scatter plot shows correlations of (**a**) genome size with latitude, (**b**) genome size with longitude, (**c**) genome size with altitude, (**d**) genome size with annual mean temperature, (**e**) genome size with annual precipitation, and (**f**) genome size with precipitation of warmest quarter. The filled circles represent landraces from Hengduanshan, filled triangles represent landraces from Qinghai Tibetan Plateau, and the filled dumbbell shapes represent landraces from the Himalayas.

3.6. PCA of Bioclimatic Variables and Seed Characteristics

To elucidate the role of other factors controlling genome plasticity of turnip landraces, PCA was conducted to group landraces on the basis of the complete data set—for instance GS information plus 28 predictor variables. The approach to cluster landraces through PCA enables researchers to illustrate the interrelationship of variables as vectors of defined magnitude and direction in the climatic space created by principal components. The first principal component (PC1) explained 60.62% of total variance and the second component (PC2) explained 14.33% of the variance exhibiting robust distribution of landraces (Figure 5). Together, these two principal components grouped particular subset of landraces. For example, landraces belonging to the Hengduanshan environment formed cluster I, landraces of the Himalayas formed cluster II, and landraces from the QTP formed cluster III. Moreover the relationship of 29 variables in our case was reduced to two components as clusters helping us to comment on landrace usage [20]. For instance, it was possible to infer that variables related to temperature and precipitation (BIO1, BIO3, BIO6, BIO8, BIO9, BIO10, BIO11, BIO13, BIO16, BIO18, and BIO19) have a correlated effect on the groupings of landraces due to similar direction of their associated vectors. It was surprising to observe that 19 out of 28 vectors were directed towards landraces from cluster I, i.e., landraces from the Hengduanshan region which signify the highest landrace usage compared to other environments. Five vectors were pointed towards cluster II (Himalaya environments) and cluster II (the QTP), showing the least landrace usage from the QTP and the Himalayas (Figure 6).
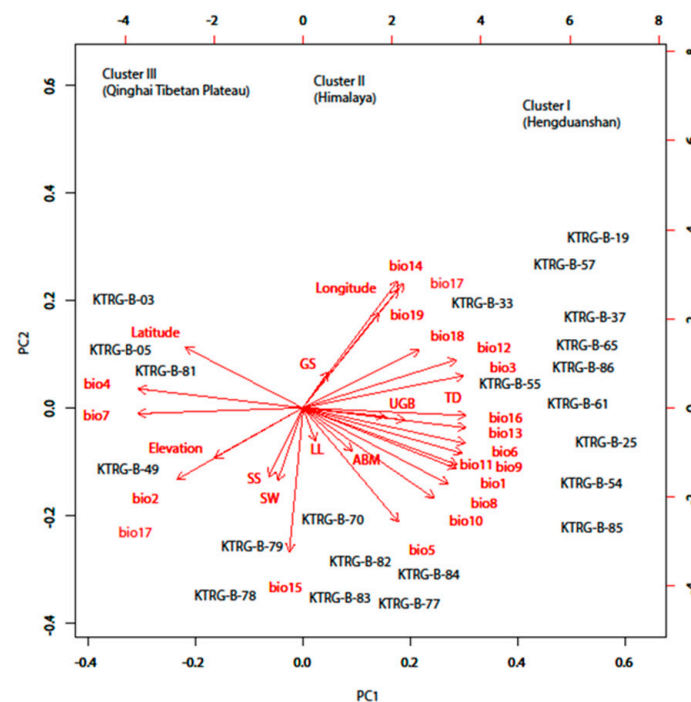
Figure 6. Principal component analysis (PCA) of environmental variables, GS, morphological and reproductive traits among the 22 turnip landraces under investigation. Principal components PC1 and PC2 explain 60.62% and 14.33% of variance, respectively. The landraces are represented by their accession numbers according to Supplementary Table S1. This figure shows the difference in landrace usage from three environments.

4. Discussion

The climate of south Asia is affected significantly by the extent and altitude of the QTP and the Himalaya. The QTP was formed by tectonic uplift that began approximately 50 million years ago (mya) and continued to occur whereas the Himalaya's uplift started to occur 15 mya [34]. Climatic disturbance (temperature and rainfall) in the QTP may have caused difference in the phenotypic characteristics; LL, ABM, TD, and UGB were significantly lesser (the significance test is shown in the results section) for turnip landraces from the QTP and the Himalayas compared to the Hengduanshan. Nevertheless, the reproductive part of landraces from the QTP and the Himalaya were found to be significantly higher (SS {one sample t (2199) = 432.43, p = 0.00} and SW {one sample t (328) = 110.14, p = 0.00} compared to the similar feature from the Hengduanshan environment. This observation was similar to the observation by other researchers who investigated positive intraspecific and negative interspecific elevation–SW relationship stating the requirement for its advantage during seedling establishment in high elevation areas (stress tolerance mechanisms) [35]. Thus, stress tolerance mechanism of SS and SW may play a role in turnip landraces if we found correlation of SS/SW with environmental variables. Unfortunately, no strong correlation of SS/SW with any environmental variables was elucidated. Thus, stress tolerance mechanism on seed traits may not be acting on the turnip landraces.

Along with the phenotypic and reproductive traits, climate is also major factor influencing the distribution of GS in angiosperms of the QTP and the Himalayas; the heritable increase/decrease in GS is a central feature of plant diversification in this region [34]. The climatic influence of the QTP/Himalayas on the local landraces is not clear and continues to daunt us about crop adaptation of this region [27]. A previous report on the natural population highlighted that polyploidy is not driving the evolution of flowering plants in Nepal, Himalaya [36], and the QTP [34]. The intraspecific variation of GS is one of the evolutionary signatures for becoming younger taxa [37]. The highest intraspecific variation of GS was observed for landraces belonging to Himalayas (10.28%) followed by QTP (7.76%), whereas least intraspecific variation in GS was observed for landraces belonging to the

Hengduanshan (4.63%); reflecting the fact that turnip from the Himalayas are in continuously evolving stage. Since altitude of samples collected from turnip landraces of the Himalayas (average elevation 1758 m) were not of similar altitude as the QTP (3896 m), we were unable to predict that differences in GS between the two plateau environments (the QTP and Himalaya) was due to difference of altitude or something else (the QTP and Himalayas). Thus, we chose turnip landraces from a third environment (average elevation of 1715 m from the Hengduanshan). GS investigation showed that average GS of the Himalaya environment (2C = 1.13 ± 0.00 pg) and the Hengduanshan (2C = 1.12 ± 0.00 pg) were of comparable magnitude whereas average GS of the QTP was on the lower side (2C = 1.06 ± 0.00 pg). A previous report [38] on GS investigation of the family Brassicaceae showed that the 1C genome size of *Brassica rapa* is 0.539 ± 0.018 pg, whereas the average 1C value of turnip reported in our report is 0.555 ± 0.000. The smallest GS of turnip landrace was reported in our study is from Qinghai province from altitudinal environment above 3600 m. Thus, we showed that turnip landraces adapted in the QTP are morphologically and genetically different compared to the landraces adapted in the Himalaya and the Hengduanshan region which we hypothesize due to the adaptation in the extreme climate [29]. This might be due to the fact that ultraviolet spectrum of the QTP is much stronger compared to the Himalayas and Hengduanshan resulting in a significant decrease in GS [39]. Then, we interrogated the question whether morphological and genomic plasticity of turnip landraces across the roof of the world is interlinked.

To confirm our speculation, we did correlation analyses between GS and morphological traits. Investigation revealed GS variation was neither associated with morphological traits (LL, ABM, TD, and UGB) nor with reproductive traits (SS and SW). Three separate groups (cluster I to III) were observed in PCA, landraces were intermixed from one cluster to another. For instance, some landraces from the Himalaya environment (cluster II) got mixed with turnip landraces from the Hengduanshan (cluster I:KTRG-B-77, KTRG-84, KTRG-86, and KTRG-B-85) and QTP (cluster III: KTGR-B-81) (Figure 6). Some differences in landrace usage were also observed among the landraces from the south Asian agroclimates. For instance, the least number of vectors were pointing towards landraces from the extreme climate of Tibet (KTRG-B-03, KTRG-B-05, and KTRG-B-49) and Uttarakhand (KTRG-B-81) demonstrating their least usage. On the other hand, landraces from Tibet (KTRG-B-57), Sichuan (KTRG-B-19), Hengduanshan (KTRG-B-33, KTRG-B-37, KTRG-B-54, KTRG-B-55, KTRG-B-61, and KTRG-B-65), and Paro (KTRG-B-77, KTRG-B-84, KTRG-B-85, and KTRG-B-86) received higher landrace usage as demonstrated by PCA. This skewed distribution of landrace usage happened due to the fact that China enjoys a monopoly in turnip production and most of the Brassica members are under strong selection pressure for breeding. Moreover, the nonsignificant correlation of GS with morphological parameters and environmental factors (apart from latitude and altitude) might happen on account of adaptation to farmer's selection [40]. It might be due to lack of undertaking of phylogenetic correction in our correlation analyses, which would have better resolving power for predicting selection pressure on intraspecific scale. The variability in oil and protein content in the seed reserve may encounter for this kind of nonsignificant correlation which was not tested in our investigation. It is envisaged that plant growth form, GS, precipitation, and temperature are factors controlling SW [12]. GS increase causes gradual increase in cell size of seed parts (seed coat, embryo, and endosperm) resulting in increased mass of the seed [12]. Although GS is correlated with several phenotypes in plants [12], GS and SS/SW correlation in turnip was not significant in our investigation. Similar observations were also reported by other scholars too in wild and cultivated plant materials [5,8,13]. Literature supports this result as it is not imperative that GS variation should always be accompanied by changes in phenotype [41]. The results presented here support the generalization made by Beaulieu et al. [12] that the maximum SW for any given GS may be controlled by other factors. Since SS/SW was not also influenced by the precipitation and temperature, we may conclude that plant growth form (annual vs. biennial, generation time, and growth habit) may be the factor controlling the SS/SW in different regions.

Among the morphological features investigated, UGB was strongly affected by environmental variation but not SS/SW. The environment (latitude, altitude, temperature, and drought) seems to play a pivotal role in shaping GS by imposing selection pressure on physiology and life history traits that are correlated with GS [42]. Although the pattern of correlation of GS and environmental variables (such as altitude) is ambiguous for natural populations in different publications (positive [43], negative [44], and no correlation [45]), crops exhibit a negative correlation with altitude (such as maize [20]). The need for shorter generation time in cooler climate is the reason for negative correlation of GS with altitude for landraces [46]. Although, we found significant negative correlation of GS with altitude and latitude in our current investigation, GS variation could not be explained by environmental factors of temperature and precipitation. Previous report on the GS correlation with latitude suggested that temperature and precipitation gradient may serve as proxy for significant correlation of GS with latitude and altitude [47]. Unfortunately, our correlation analyses did not support this claim. Thus, we predict that the GS–environmental correlation of turnip landrace showed a complex pattern. Furthermore, we agree with the previous observation [42] that association of GS with environmental factors is stronger for species with larger GS but not in case of turnip with very small GS. In future, investigation of GS under phylogenetic comparative approach for large number of samples from these three environments is required to understand the role of selection.

5. Conclusions

Since prehistoric times introduction of landraces from highlands to agricultural site situated at lowlands was practiced to produce broadly adapted, improved varieties to increase production [48]. To achieve this goal, plant breeders cross-pollinate adapted highland and high yielding lowland landraces [49]. The pattern of GS variation with morphological traits seemed an extremely important factor in determining the range of environment in which a landrace can be grown without compromising its yield. The significance of this research was to provide knowledge of GS variation from diverse agroclimates with difference in morphological characteristics which may provide new insights into the domestication history of turnip and other fodder crops of the QTP. Statistically significant variation in GS, LL, ABM, TD, SS, and SW was observed among landraces from the QTP and its adjacent region. Correlation analyses illustrated that the GS plasticity was neither linked to plasticity of vegetative and reproductive traits nor with precipitation and temperature. Thus, these results revealed GS variation might be caused by the farmer's selection during cultivation.

Author Contributions: Conceptualization Y.Y.; Methodology, Y.Y., X.S., S.B. and G.W.; Software, S.B.; Validation, Y.Y., X.S., S.B. and G.W.; Formal analysis, S.B. and G.W.; Investigation, S.B. and G.W.; Resources, Y.Y.; Data Curation, S.B.; Writing—Review and Editing, S.B.; Visualization, Supervision, X.S. and Y.Y.; Project Administration, X.S. and Y.Y.; Funding Acquisition, Y.Y.

References

1. Yan, H.; Martin, S.L.; Bekele, W.A.; Latta, R.G.; Diederichsen, A.; Peng, Y.; Tinker, N.A. Genome size variation in the genus *Avena*. *Genome* **2016**, *59*, 209–220. [CrossRef] [PubMed]

2. Pellicer, J.; Hidalgo, O.; Dodsworth, S.; Leitch, I.J. Genome size diversity and its impact on the evolution of land plants. *Genes* **2018**, *9*, 88. [CrossRef] [PubMed]

3. Benor, S.; Fuchs, J.; Blattner, F.R. Genome size variation in *Corchorusolitorius* (Malvaceae s.l.) and its correlationwith elevation and phenotypic traits. *Genome* **2011**, *54*, 575–585. [CrossRef] [PubMed]

4. Du, Y.P.; Bi, Y.; Zhang, M.F.; Yang, F.P.; Jia, G.X.; Zhang, X.H. Genome size diversity in *Lilium* (Liliaceae) is correlated with karyotype and environmental traits. *Front. Plant Sci.* **2017**, *8*, 1303. [CrossRef] [PubMed]

5. Gallagher, R.V.; Leishman, M.R.; Miller, J.T.; Hui, C.; Richardson, D.M.; Suda, J.; Trávníček, P. Invasiveness in introduced Australian acacias: The role of species traits and genome size. *Divers. Distrib.* **2011**, *17*, 884–897. [CrossRef]

6. Khorami, S.S.; Arzani, K.; Karimzadeh, G.; Shojaeiyan, A.; Ligterink, W. Genome size: A novel predictor of nut weight and nut size of walnut trees. *HortScience* **2018**, *53*, 275–282. [CrossRef]

7. Krahulcová, A.; Trávníček, P.; Krahulec, F.; Rejmánek, M. Small genomes and large seeds: Chromosome numbers, genome size and seed mass in diploid Aesculus species (Sapindaceae). *Ann. Bot.* **2017**, *119*, 957–964. [CrossRef]

8. Realini, M.F.; Poggio, L.; Cámara-Hernández, J.; González, G.E. Intra-specific variation in genome size in maize: Cytological and phenotypic correlates. *AoB Plants* **2016**, *8*. [CrossRef]

9. Knight, C.A.; Beaulieu, J.M. Genome size scaling through phenotype space. *Ann. Bot.* **2008**, *101*, 759–766. [CrossRef]

10. Herben, T.; Suda, J.; Klimešová, J.; Mihulka, S.; Říha, P.; Šímová, I. Ecological effects of cell-level processes: Genome size, functional traits and regional abundance of herbaceous plant species. *Ann. Bot.* **2012**, *110*, 1357–1367. [CrossRef]

11. Morgan, H.D.; Westoby, M. The relationship between nuclear DNA content and leaf strategy in seed plants. *Ann. Bot.* **2005**, *96*, 1321–1330. [CrossRef]

12. Beaulieu, J.M.; Moles, A.T.; Leitch, I.J.; Bennett, M.D.; Dickie, J.B.; Knight, C.A. Correlated evolution of genome size and seed mass. *New Phytol.* **2007**, *173*, 422–437. [CrossRef] [PubMed]

13. Gunn, B.F.; Baudouin, L.; Beulé, T.; Ilbert, P.; Duperray, C.; Crisp, M.; Issali, A.; Konan, J.L.; Rival, A. Ploidy and domestication are associated with genome size variation in palms. *Am. J. Bot.* **2015**, *102*, 1625–1633. [CrossRef] [PubMed]

14. Stetter, M.G.; Muller, T.; Schmid, K.J. Incomplete domestication of South American grain amaranth (*Amaranthuscaudatus*) from its wild relatives. *bioRxiv* **2015**. [CrossRef]

15. Münzbergová, Z. The effect of genome size on detailed species traits within closely related species of the same habitat. *Bot. J. Linn. Soc.* **2009**, *160*, 290–298. [CrossRef]

16. Kahiluoto, H.; Kaseva, J.; Balek, J.; Olesen, J.E.; Ruiz-Ramos, M.; Gobin, A.; Kersebaum, K.C.; Takáč, J.; Ruget, F.; Ferrise, R.; et al. Decline in climate resilience of European wheat. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 123–128. [CrossRef]

17. Henry, R.J.; Nevo, E. Exploring natural selection to guide breeding for agriculture. *Plant Biotechnol. J.* **2014**, *12*, 655–662. [CrossRef] [PubMed]

18. Jarvis, D.I.; Brown, A.H.; Cuong, P.H.; Collado-Panduro, L.; Latournerie-Moreno, L.; Gyawali, S.; Tanto, T.; Sawadogo, M.; Mar, I.; Sadiki, M.; et al. A global perspective of the richness and evenness of traditional crop-variety diversity maintained by farming communities. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 5326–5331. [CrossRef] [PubMed]

19. Reed, T.E.; Schindler, D.E.; Waples, R.S. Interacting effects of phenotypic plasticity and evolution on population persistence in a changing climate. *Conserv. Biol.* **2011**, *25*, 56–63. [CrossRef] [PubMed]

20. Díez, C.M.; Gaut, B.S.; Meca, E.; Scheinvar, E.; Montes-Hemandez, S.; Eguiarte, L.E.; Tenaillon, M.I. Genome size variation in wild and cultivated maize along altitudinal gradients. *New Phytol.* **2013**, *199*, 264–276. [CrossRef]

21. Pellicer, J.; Estiarte, M.; Garcia, S.; Garnatje, T.; Peñuelas, J.; Sardans, J.; Vallès, J. Genome size unaffected by moderate changes in climate and phosphorus availability in Mediterranean plants. *Afr. J. Biotechnol.* **2010**, *9*, 6070–6077.

22. Leitch, I.J. Genome sizes through the ages. *Heredity* **2007**, *99*, 121–122. [CrossRef] [PubMed]

23. Zeng, X.; Guo, Y.; Xu, Q.; Mascher, M.; Guo, G.; Li, G.; Li, S.; Mao, L.; Liu, Q.; Xia, Z.; et al. Origin and evolution of qingke barley in Tibet. *Nat. Commun.* **2018**, *9*, 5433. [CrossRef] [PubMed]

24. Liu, J.; Milne, R.I.; Cadotte, M.W.; Wu, Z.Y.; Provan, J.; Zhu, G.F.; Gao, L.M.; Li, D.Z. Protect Third Pole's fragile ecosystem. *Science* **2018**, *362*, 6421.

25. Meng, L.H.; Yang, J.; Guo, W.; Tian, B.; Chen, G.J.; Yang, Y.P.; Duan, Y.W. Differentiation in drought tolerance mirrors the geographic distributions of alpine plants on the Qinghai-Tibet Plateau and adjacent highlands. *Sci. Rep.* **2017**, *7*, 42466. [CrossRef] [PubMed]

26. Wei, Y.; Wang, S.; Fang, Y.; Nawaz, Z. Integrated assessment on the vulnerability of animal husbandry to snow disasters under climate change in the Qinghai-Tibetan Plateau. *Glob. Planet. Chang.* **2017**, *157*, 139–152. [CrossRef]

27. D'AlpoimGuedes, J. Rethinking the spread of agriculture to the Tibetan Plateau. *Holocene* **2015**, *25*, 1498–1510. [CrossRef]

28. Facts and Details. Agriculture in Tibet. Available online: http://factsanddetails.com/china/cat6/sub37/item1693.html (accessed on 11 July 2017).

29. Basak, S.; Wang, G.Y.; Sun, X.; Yang, Y.P. Variations in genome size of turnip landraces from two high altitude environments. *J. Am. Soc. Hortic. Sci.* **2018**, *143*, 136–143. [CrossRef]

30. Li, X.; Yin, X.; Yang, S.; Yang, Y.Q.; Qian, M.; Zhou, Y.; Zhang, C.; Du, Y.; Yang, Y.P. Variations in seed characteristics among and within Stipapurpurea populations on the Qinghai–Tibet Plateau. *Botany* **2015**, *93*, 651–662. [CrossRef]

31. Doležel, J.; Doleželova, M.; Novak, F.J. Flow cytometric estimation of nuclear DNA amount in diploid bananas (*Musa acuminate* and *M. balbisiana*). *Biol. Plant.* **1994**, *36*, 351–357. [CrossRef]

32. Loureiro, J.; Rodriguez, E.; Doležel, J.; Santos, C. Two new nuclear isolation buffers for plant DNA flow cytometry: A test with 37 species. *Ann. Bot.* **2007**, *100*, 875–888. [CrossRef]

33. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria. Available online: https://www.R-project.org/ (accessed on 26 March 2018).

34. Wang, G.Y.; Basak, S.; Grumbine, R.E.; Yang, Y.P. Polyploidy and aneuploidy of seed plants from the Qinghai Tibetan Plateau and their biological implications. *Plant Syst. Evol.* **2017**, *303*, 565–571. [CrossRef]

35. Qi, W.; Bu, H.; Cornelissen, J.H.C.; Zhang, C.; Guo, S.; Wang, J.; Zhou, X.; Li, W.; Du, G. Untangling interacting mechanisms of seed mass variation with elevation: Insights from the comparison of inter-specific and intra-specific studies on eastern Tibetan angiosperm species. *Plant Ecol.* **2015**, *216*, 283–292. [CrossRef]

36. Adhikari, B.; Pendry, C.A.; Moller, M. New chromosome counts of *Berberis* L. (Berberidaceae) suggest that polyploidy does not play significant role in the diversification of the genus in the Nepal Himalaya. *Edinb. J. Bot.* **2014**, *71*, 297–308. [CrossRef]

37. Šmarda, P.; Bureš, P. Understanding intraspecific variation in genome size in plants. *Preslia* **2010**, *82*, 41–61.

38. Johnston, J.S.; Pepper, A.E.; Hall, A.E.; Chen, Z.J.; Hodnett, G.; Drabek, J.; Lopez, R.; Price, H.J. Evolution of genome size in Brassicaceae. *Ann. Bot.* **2005**, *95*, 229–235. [CrossRef]

39. Sun, H.; Niu, Y.; Chen, Y.S.; Song, B.; Liu, C.Q.; Peng, D.L.; Chen, J.G.; Yang, Y. Survival and reproduction of plant species in the Qinghai–Tibet Plateau. *J. Syst. Evol.* **2014**, *52*, 378–396. [CrossRef]

40. Murray, B.G. When does intraspecific C-value variation become taxonomically significant? *Ann. Bot.* **2005**, *95*, 119–125. [CrossRef] [PubMed]

41. Oliver, M.J.; Petrov, D.; Ackerly, D.D.; Falkowski, P.; Schofield, O.M. The mode and tempo of genome size evolution in eukaryotes. *Genome Res.* **2007**, *17*, 594–601. [CrossRef]

42. Knight, C.A.; Molinari, N.A.; Petrov, D.A. The large genome constraint hypothesis: Evolution, ecology and phenotype. *Ann. Bot.* **2005**, *95*, 177–190. [CrossRef] [PubMed]

43. Caceres, M.E.; De Pace, C.; Scarascia-Mugnozza, G.T.; Kotsonis, P.; Ceccarelli, M.; Cionini, P.G. Genome size variations within *Dasypyrumvillosum*: Correlations with chromosomal traits, environmental factors and plant phenotypic characteristics and behaviour in reproduction. *Theor. Appl. Genet.* **1998**, *96*, 559–567. [CrossRef]

44. Creber, H.M.C.; Davies, M.S.; Francis, D.; Walker, H.D. Variation in DNA C value in natural populations of *Dactylisglomerata* L. *New Phytol.* **1994**, *128*, 555–561. [CrossRef]

45. Lysak, M.; Rostkova, A.; Dixon, J.; Rossi, G.; Doležel, J. Limited genome size variation in *Sesleriaalbicans*. *Ann. Bot.* **2000**, *86*, 399–403. [CrossRef]

46. Poggio, L.; Rosato, M.; Chiavarino, A.M.; Naranjo, C.A. Genome size and environmental correlations in maize (*Zea mays* ssp. *mays*, Poaceae). *Ann. Bot.* **1998**, *82*, 107–115. [CrossRef]

47. De Frenne, P.; Graae, B.; Rodríguez-Sánchez, F.; Kolb, A.; Chabrerie, O.; Decocq, G.; Kort, H.D.; Schrijver, A.D.; Diekmann, M.; Eriksson, O.; et al. Latitudinal gradients as natural laboratories to infer species' responses to temperature. *J. Ecol.* **2013**, *101*, 784–795. [CrossRef]

48. Mercer, K.; Martínez-Vásquez, A.; Perales, H.R. Asymmetrical local adaptation of maize landraces along an altitudinal gradient. *Evol. Appl.* **2008**, *1*, 489–500. [CrossRef]

49. Eagles, H.A.; Lothrop, J.E. Highland maize from central Mexico—Its origin, characteristics, and use in breeding programs. *Crop Sci.* **1994**, *34*, 11–19. [CrossRef]