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Research Article

Germination Percentage of Seeds and Genetic Diversity on Wild *Allium Tuberosum* from the Tibet

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Abstract

Wild *Allium tuberosum* from Tibet as a stylish food is attracting wide attention, especially in China. Here, we selected 11 wild *Allium tuberosum* samples germplasms. We examined the seeds morphology, seeds response to NaCl and PEG stress and analyzed genetic diversity by Sequence Related Amplified Polymorphism (SRAP) method. These results showed that the seeds appeared shield shape and brown color. Wild *Allium tuberosum* belonging to alpine plant species showed higher germination percentage for NaCl and PEG stress, which suggested that wild *Allium tuberosum* had fine regulation of tolerance under high altitudes conditions. Accordingly, wild *Allium tuberosum* develops many genetic variation characteristics to adapt to complicated environments.

Keywords: Genetic Diversity; Germination Percentage; Seeds; Tibe; Wild *Allium Tuberosum*

Introduction

Wild *Allium tuberosum* from the Tibet is disturbed widely in Tibetan area [1]. Wild *Allium tuberosum* as a stylish food is attracting wide attention, especially in China, because of its rich and nutritional value, capability of removing free radicals and inhibiting the activity of pathogen [2-5]. In addition, for a long process of wild growth, wild *Allium tuberosum* has developed excellent characteristics including to strong resistance to root knot nematodes and easy cultivation [6]. Thus, wild *Allium tuberosum* can be used as the primary gene pool in modern cultivation and breeding programs for cultivated *Allium tuberosum*. Using these genes, they need genetic improvement for adaption to harsh environments and insect pest. Generally, the understanding of genetic information is the basic of genetic engineering. However, a wide range of genetic diversity on wild *Allium tuberosum* from Tibet has not been studied.

Here, we selected 11 wild *Allium tuberosum* samples germplasms. We examined the seeds morphology, seeds response to NaCl and PEG stress and analyzed genetic diversity by Sequence Related Amplified Polymorphism (SRAP) method. SRAP is cost-effective and reliable genetic marker to estimate genetic diversity and relationships in various plant species [7,8].

Materials and Methods

Plant Materials

All wild *Allium tuberosum* samples were collected from Langkazi Kare Country of SHANNAN site [(29˚13’48”-29˚14’05” (Longitude, E) and 90˚23’56”-90˚24’04” (Latitude, N)] from Tibet. All the detailed information of 11 samples was listed in (Table 1).

Samples code	The number of samples	Altitude (m)	Length of seeds (μm)	Width of seeds (μm)	Figures
A1	2	4057	2878.33 ± 256.66 c	1798.43 ± 321.48 a	Figure 1-A
A2			2760.05 ± 249.15 c	1790.51 ± 200.66 a	
B1	3	4022	2742.73 ± 146.85 c	1739.04 ±156.23 a	Figure 1-B
B2			3015.50 ± 310.01 b	1926.70 ± 185.21 a	
B3			3693.62 ± 123.89 a	1794.89 ± 231.06 a	
C1	3	3929	2784.22 ± 123.56 c	1758.10 ± 213.05 a	Figure 1-C
C2			2841.46 ± 311.75 c	1860.37 ± 195.91 a	
C3			2403.55 ± 309.21 d	1664.80 ± 195.91 b	
D1	3	3900	2287.75 ± 199.89 d	1654.75 ± 123.65 b	Figure 1-D
D2			2865.61 ± 224.49 c	1928.26 ± 184.04 a	
D3			3042.88 ± 206.87 b	1899.66 ± 14.388 a	

Table 1: The information and parameters of seeds of *Allium hookeri* Thwaites.

Seeds Morphology

Surface texture of seeds randomly collected from 30 individuals were observed and imaged using a digital camera (Olympus SZX7, lympus Corporation, Japan). Two exomorphic parameters including the length and width of seeds, the length and width of wings were measured (Figure1).

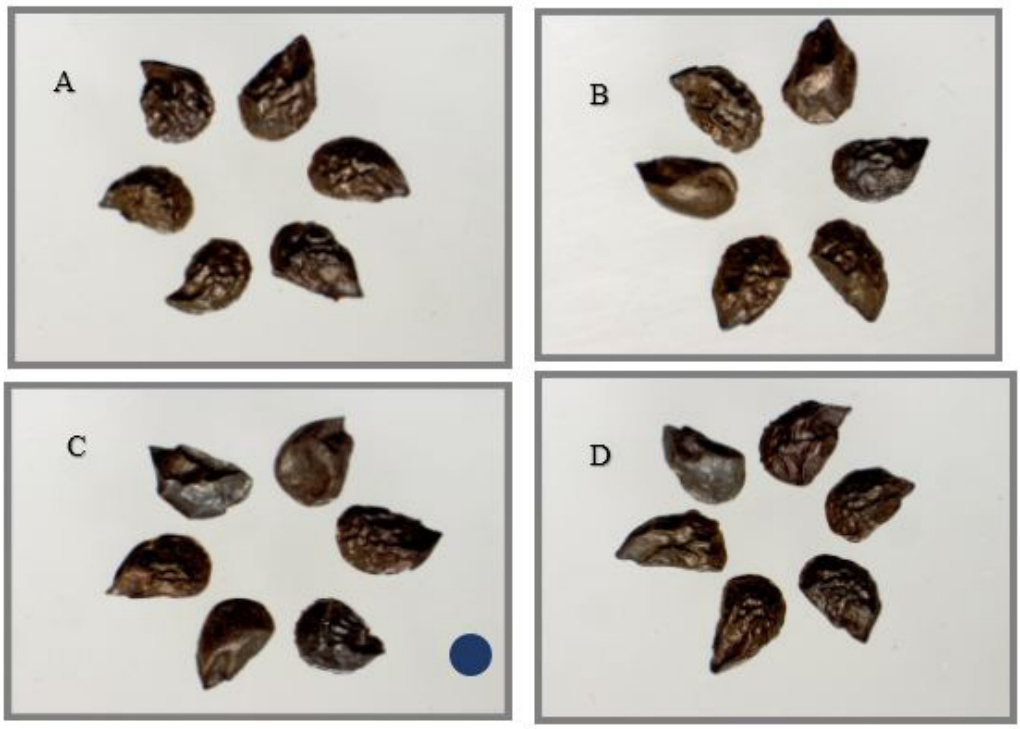

Figure 1: The morphous of seeds on *Allium hookeri* Thwaites from different altitude (**A**, 4057 m; **B**, 4022 m; **C**, 3929 m; **D**, 3900 m. Bar: 1cm).

Measurement of Germination Percentage Under NaCl and PEG

Germination percentage was tested under NaCl and PEG. Five NaCl concentration (0 mM, 50 mM, 100 mM, 150 mM and 200 mM), five PEG concentrations (0%, 5%, 10%, 15% and 20%) and the combination of NaCl and PEG (NaCl (0 mM) + PEG (0%); NaCl (50 mM + PEG (5%); NaCl (100 mM + PEG (10%); NaCl (150 mM + PEG (15%);) was selected to calculate germination percentage. For all tests, three replicates of 30 seeds per treatment were sown on the surface of 2% agar in water in Petri dishes and incubated with complete darkness.

SRAP Analysis

DNA Extraction

Total genomic DNA of seeds was isolated according to the modified CTAB method of Doyle (1990). DNA concentration was measured by a spectrophotometer (Eppendorf, Eppendorf China Limited, China).

Ten SRAP primers were used for amplification. The information on all primers was listed in (Table 2).

Primer pairs	Sequences (3′-5′)	Total number of fragments(TN)	The number of polymorphic fragments(NPF)	The percentage ofpolymorphic fragments (PPF, %)
ME3-EM3	TGAGTCCAAACCGGAAT-GACTGCG-TACGAATTGAC	61	60	98.36
ME3-EM6	TGAGTCCAAACCGGAAT- GACTGCG-TACGAATTGCA	79	77	97.47
ME4-EM3	TGAGTCCAAACCGGACC- GACTGCG-TACGAATTGAC	62	59	95.16
ME4-EM4	TGAGTCCAAACCGGACC-GACTGCG-TACGAATTTGA	80	80	100
ME5-EM4	TGAGTCCAAACCGGAAG- GACT-GCGTACGAATTTGA	49	39	79.59
ME6-EM8	TGAGTCCAAACCGGTAA-GACTGCG-TACGAATTAGC	120	117	97.5
ME7-EM4	GACTGCGTACGAATTCAA-GACT-GCGTACGAATTTGA	127	127	100
ME7-EM6	GACTGCGTACGAATTCAA-GACT-GCGTACGAATTGCA	97	95	97.94
ME8-EM4	TGAGTCCAAACCGGTGT-GACTGCG-TACGAATTTGA	97	93	95.88
ME8-EM6	TGAGTCCAAACCGGTGT-GACTGCG-TACGAATTGCA	81	79	97.53
Mean		**85.3**	**82.6**	**96.83**
Total		**853**	**826**	

Table 2: Information on each primer pairs among all samples in this study.

PCR condition was followed was carried out in 25 μl volume containing 50 ng DNA templates, 1U *Taq* polymerase (TakaRa, China) and 20 ng of forward and reverse primers and 2.5 μL 10×PCR buffer. PCR condition was followed: 95 ˚C denaturation for 5 min, 5 cycles for 1 min denaturation at 94 ˚C, 1 min at annealing at 35 ˚C, and then 72˚C elongation for 1 min, the next 30 cycles including 10 min annealing at 48 ˚C, 72 ˚C final extension. PCR products were detected on 6% polyacrylamide sequencing gel (Figure 2).

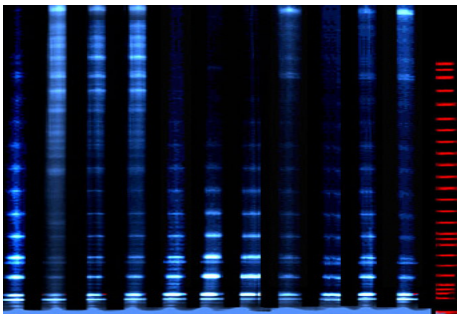

Figure 2: Fingerprint patterns generated by primers ME7-EM6 from the genomic DNA of the 11 genotypes of *Allium hookeri* Thwaites.

The distinct and reproducible SRAP bands were scored as absent (0) or present (1). The dendrogram was constructed according to the Jaccard's similarity coefficients by the NTSYS-pc Version 2.10e [9]. Some basic parameters including total number of fragments (TN), the Number of Polymorphic Fragments (NPF) and the percentage of polymorphic fragments (PPF, %) were calculated. Genetic diversity between samples was estimated based on the method of Analysis of Molecular Variance (AMOVA) Version 1.55 [10].

Results

Observation of Seeds

The parameters of seeds measurement are listed in (Table 1). The characteristics of seeds were showed in (Figure. 1). Although there is little difference, the seed morphous of wild *Allium tuberosum* from four altitudes showed similar features. The seeds showed shield shape, had brown color. There is wrinkle or smooth on sexine ornamentation. The length of seeds ranges from 2287.75 μm to 3693.62 μm. The width of seeds ranges from 1654.75 μm to 1928.26 μm.

Assays of Germination Percentage Under NaCl and PEG

Under NaCl stress, there were significant treatment effects on germination percentage for cultivated and wild samples (Figure 3). NaCl stress resulted in the decrease of germination percentage for cultivated samples, while there were no treatment differences under 50 mM and 100 mM NaCl. 200 mM NaCl was found to be inhibitorier to germination percentage (0%) of cultivated samples, compared with wild samples (5%).

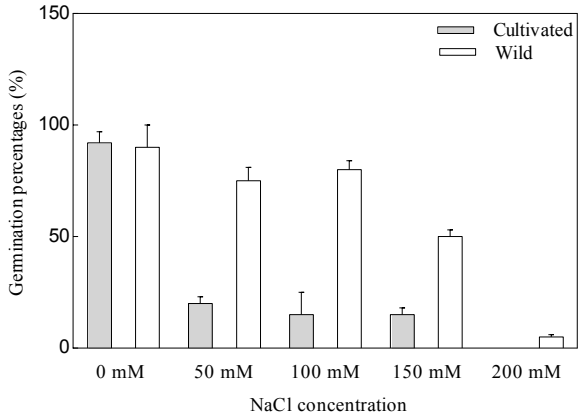

Figure 3: Effects of different NaCl concentration on the germination percentage of *Allium hookeri* Thwaites.

Under no PEG stress, we observed no obvious effects for cultivated and wild samples. However, there are strong effects of PEG on germination percentage on cultivated samples (Figure 4). In contrast, wild samples had significantly higher germination percentage value under PEG stress compared to cultivated samples (Figure 4).

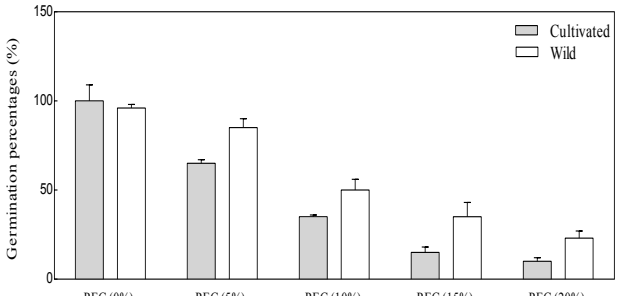

Figure 4: Effects of different PEG concentration on germination percentage of *Allium hookeri* Thwaites.

To investigate the effects of different NaCl and PEG concentration on germination percentage, seeds sensitivity to exogenous NaCl and PEG was investigated. Stress (NaCl and PEG) lead to decrease of germination percentage, however, the decrease in wild samples under NaCl and PEG was less significant in Cultivated (Figure 5).

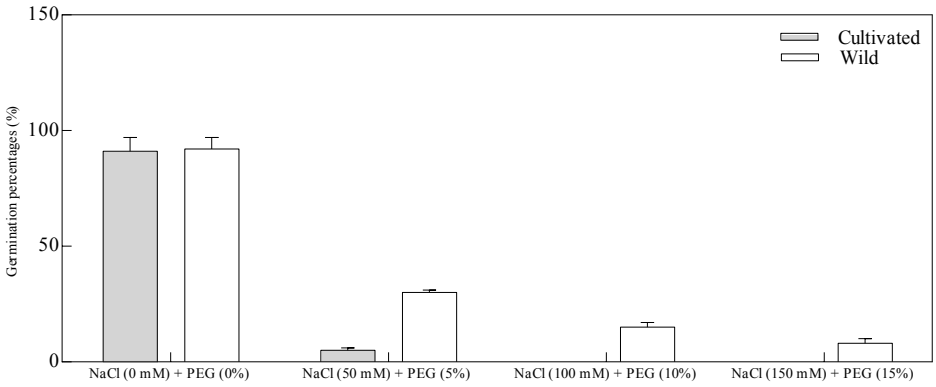

Figure 5: Effects of different NaCl and PEG concentration on germination percentage of *Allium hookeri* Thwaites.

Genetic Diversity Analysis

The genetic diversity on 11 samples was assessed using ten primer pairs (Table 2). 853 fragments were detected, of which 826 fragments were polymorphic. The percentage of polymorphic fragments was ranged from 79.59% (ME5-EM4) to 100% (ME4-EM4 and ME7-EM4) with an average of 96.83. In addition, the mean of similarity coefficients among all samples is found to be 0.463 (Table 3). The Min similarity coefficient (0.355) was found between A-2 and D-3 and the Max similarity (0.575) was found between D-2 and D-3, respectively. The SRAP profile amplified by the primer primers ME7-EM6 is shown in (Figure 2). Cluster analyses were carried out based on the UPGMA method. All samples were clustered into 3 groups (Cluster I, Cluster II and Cluster III) (Figure. 6). Most of these samples clustered appeared scattered distribution.

	A-1	A-2	B-1	B-2	B-3	C-1	C-2	C-3	D-1	D-2	D-3
A-1	1										
A-2	0.421	1									
B-1	0.482	0.465	1								
B-2	0.506	0.457	0.445	1							
B-3	0.447	0.382	0.511	0.432	1						
C-1	0.445	0.381	0.464	0.489	0.554	1					
C-2	0.448	0.37	0.496	0.475	0.53	0.521	1				
C-3	0.414	0.381	0.488	0.422	0.456	0.45	0.449	1			
D-1	0.466	0.364	0.452	0.464	0.489	0.508	0.522	0.49	1		
D-2	0.515	0.417	0.498	0.454	0.447	0.482	0.469	0.534	0.497	1	
D-3	0.46	0.355	0.49	0.442	0.422	0.429	0.431	0.498	0.488	0.575	1

Table 3: The similarity coefficients among all samples based on UPGMA dendrogram.

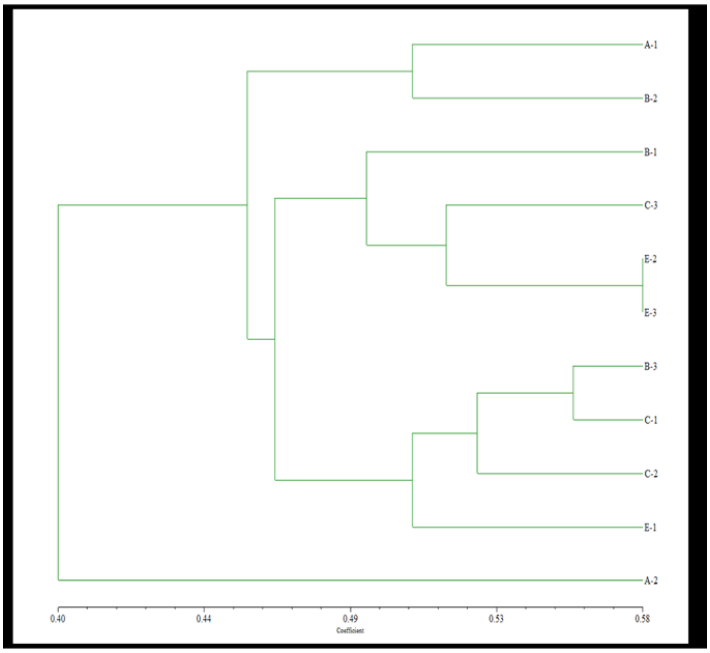

Figure 6: Dendrogram of 11 samples resulting from the UPGMA cluster analysis based on Jaccard's similarity coefficients obtained from SRAP.

Discussion

In our study, the color of the seeds of wild *Allium tuberosum* is brown. This would have indicated that, under special environmental conditions in Tibet, high seed germination could be environmental selection for conditional tolerance. A similar finding was reported for *Sesamum indicum* [11]. Their findings showed sesame genotypes characterized by brown seeds were more tolerant to PEG and NaCl stresses than sesame genotypes characterized by white seeds. Generally, the seed coat is a key tissue that serves as a conduit for nutrients and water [12,13]. It has been reported that environmental stress imposed during seed development can cause changes in seed coat morphology leading to negative effects on seed germination rate, seed quality and seedling vigor [14,15]. Therefore, shrinkle, twinkle and light seed coat of wild *Allium tuberosum* can facilitate the prevention of strong light, low temperature and drought from Tibetan environment.

In this study, we analyzed the seed germination traits of wild *Allium tuberosum* under NaCl and PEG. We found that wild *Allium tuberosum* belonging to alpine plant species generally showed higher germination percentage for NaCl and PEG stress, while cultivated species had lower germination percentage. These results confirmed the trend suggested by Wang (2017), who recorded a higher germination percentage under PEG (5%-15%) and NaCl (0.2%-0.4%). Indeed, these results were also consistent with the observations in other Tibetan plant species, such as *Sophora moorcroftian*, *Hippophae rhamnoides* and *Avena fatua* [16-18] which suggested that wild *Allium tuberosum* from The Tibet had fine regulation of tolerance under high altitudes conditions.

To improve the genetic characteristics, the understanding of genetic diversity on plant species is important. Additionally, the understanding of genetic diversity plays a key role for utilization new gene resources to enlarge genetic variation to plant breeding materials [19]**.** To data, few researches have used molecular markers technology to study the genetic diversity in wild *Allium tuberosum* [20]. In this study, SRAP proved to be a effective, useful and high-resolution technique to the detect the variation among all samples of wild *Allium tuberosum.* Taking no account of the relatively small sample sizes, 10 SRAP primers were sufficient to differentiate all wild *Allium tuberosum* samples from four altitudes.

Additionally, genetic cluster plot showed that all samples from different altitudes are randomly distributed. This scattered distribution suggested that there is high genetic diversity for wild *Allium tuberosum* from Tibet. [20] also reported that wild *Allium tuberosum* from Tibet maintained relatively more genetic diversity. Wild *Allium tuberosum* grows harsh environmental conditions including violent winds, low temperatures, drought, a low oxygen concentration, and strong Ultraviolet (UV) radiation [21]. Accordingly, wild *Allium tuberosum* develops many genetic variation characteristics to adapt to complicated environments. Therefore, these biological characteristics may contribute to maintain a high level of genetic variability of wild *Allium tuberosum.*

Acknowledgement

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