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Characteristics of seed size and its relationship to germination in American ginseng (*Panax quinquefolius* L.)

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Abstract
The aims of this work were to investigate the variation of seed size related parameters and their relationship as well as whether seed size impact on germination in American ginseng. A total of 8596 American ginseng seeds from eight different regions were included in this study. There was a normal distribution for the frequency of seed weight, length, width and thickness. A great portion of seeds (more than 90%) varied within 2 mm for dimension parameters. Significant positive liner relationship was observed between seed weight and width, length and weight, and width and length. Seed size significantly affected kernel area, plumpness, split percentage and germination. Seed split and germination percentage is more depended on plumpness but not seed size. Medium seeds had the highest germination percentage, followed by large and small seeds with no significant difference between them. Both extremely large and small seed performed poorly for germination, and extremely large seeds exhibited higher germination percentage than extremely small seeds. The results of the study do not agree with the commonly accepted conception that the larger the better for ginseng seeds.

Keywords: American ginseng, seed size, dimension traits, germination

1. Introduction

American ginseng (*Panax quinquefolius* L.) is extensively used as a traditional medicine to cure many kinds of diseases around the world. In recent decades, a variety of pharmacological activities of American ginseng, such as anti-oxidative, anti-diabetic, anti-inflammatory, anticancer, neuroprotective, and immunomodulatory effects, have been justified by in vitro cell and in vivo animal experiments (Duda *et al.*, 1999; Li *et al.*, 1999; Kitts *et al.*, 2000; Park *et al.*, 2003; Darshan and Doreswamy, 2004; Predy *et al.*, 2006; Wang *et al.*, 2015) [1-7]. The high value and demand of American ginseng resulted in the overharvesting of wild resources, causing *P. quinquefolius* to be listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) in 1973 (Xie *et al.*, 2004) [8]. Therefore, artificial cultivation of American ginseng was thrived in northern U.S., Canada and northern China since 1990's.

Seed size is one of the central features of a plant species' life history as well as a trait of importance for crop seed quality. Seed size is highly related to seed fate, germination, dormancy, seedling establishment and stress resistance (Zhu *et al.*, 2004; Gorian *et al.*, 2007; Arellano and Peco, 2012) [9-11]. Generally, larger size seeds have the advantages of higher seed germination and seedling establishment in closed communities (Hwang *et al.*, 2014) [12], such as forests where *P. quinquefolius* grown in. In addition, since a large seed reserves greater energy, the larger seeds normally produce bigger seedlings with a larger area of green leaves capable of photosynthesis (Akinyele and Adegeye, 2011) [13]. It is also reported that smaller size seeds have the advantages of escaping animal and bird predation, forming soil seed banks and vegetation renewal (Zhao *et al.*, 2015) [14]. However, these advantages are not considered for agronomic perspectives. See size is mainly determined by four parameters: seed weight, length, width and thickness. American ginseng propagates from seed, thus seed features have a tie relationship with ginseng production. Previous studies about American ginseng seed have focused on seed dormancy (Li *et al.*, 1994; Zhao *et al.*, 2001) [15, 16], yet few studies have been done on seed size and its related factors. The objective of this research was to investigate the variation of seed weight and dimension traits and the relationship between seed size and germination.

2. Materials and methods

2.1 Plant materials

A total of 8596 American ginseng seeds were included in this experiment. They were harvested from four year old ginseng plants in Fu Song, Ji An, Jing Yu, An Tu, Yan Bian, Hun Chun, He Long and Wang Qing, China. Each seed lot contributed 1000 seeds at least. After receipt, all seeds were stored at 20 °C and 50% RH for seven days to gain consistent moisture content.

2.2 Determination of seed weight and dimension traits

All seeds collected from eight different regions were tested for weight and dimension parameters. Single seed weight, length, width and thickness were measured using analytical balance and vernier calipers respectively.

2.3 Seed grading

American ginseng seeds were separated into six different size classes according to seed width: less than 4.0 mm, 4.0~4.5 mm, 4.5~5.0mm, 5.0~5.5mm, 5.5~6, higher than 6.0 mm.

2.4 Determination of kernel projective area

One hundred seeds for each grade were used for kernel area and plumpness measurement, and each grade with three replications. The projective area of seed kernel and coat was measured using the *WinFOLIA* analyses system (Regent Instruments Inc. Canada). Then, the plumpness was calculated from the equation, plumpness = seed kernel area / seed coat area × 100%.

2.5 Determination of splitting and germination percentage

Five thousands of American ginseng seeds for each grade were included in splitting and germination percentage testing. To break dormancy, the seeds were mixed with three times the volume of wet sand for stratification treatment. All seeds were stratified at room temperature (18-25 °C) for 100 days and then stratified at 4 °C for 70 days. The moisture content of the sand was kept at about 60% throughout. After that, five replicates of 100 seeds from each size were selected randomly for split percentage and germination test. American ginseng seeds were placed in 120 mm-diameter Petri dishes lined with germination paper and 10 ml distilled water added. All dishes were placed in an incubator with a constant temperature of 25 °C without light. Germinated seeds were counted after 10 days of incubation.

2.6 Statistical analysis

The relationships between seed parameters were tested using Pearson correlation analysis (SPSS 20.0). Data of kernel area, plumpness, splitting and germination percentage were subjected to one-way ANOVA using SPSS 20.0. Means were compared using LSD's test at $P<0.05$.

3. Result and discussion

3.1 Distribution of seed size parameters

There was a normal distribution for the frequency of seed size parameters (weight, length, width and thickness) in American ginseng (figure 1). American ginseng seed length ranged from 3.5 to 8.4 mm and the majority of seeds had a length of 5 to 7 mm, accounting for 94.4% of the samples. Seed width ranged from 3.0 to 6.6 mm, and most seeds had a seed width between 4 and 6 mm, corresponding to 95.8% of samples. Most (97.5%) values of seed thickness varied between 2 and 4 mm, while the values of seed thickness for all samples ranged from 1.0 to 5.4 mm. As indicated by the above results, a great portion of seeds (more than 90%) varied within 2 mm for dimension parameters. Seed weight ranged from 11 to 65 mg, and the main distribution range (20 ~50 mg) covered 96.5% of the distributional data.

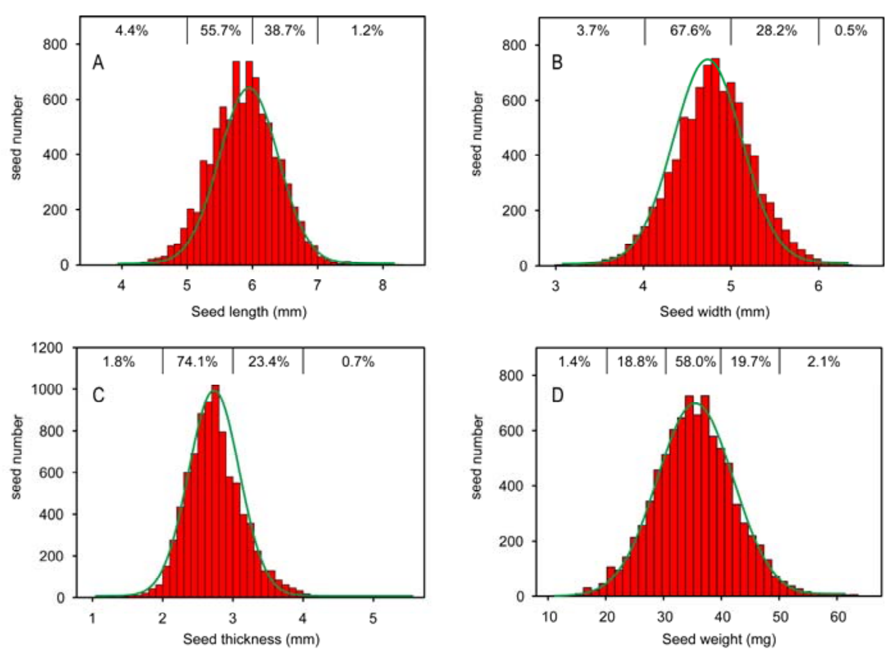

Fig 1: Distributions of seed weight, length, width and thickness in a combined American ginseng population from 8 regions.

3.2 Correlation relationship between seed size parameters

Of the 8596 seeds tested, there was a significant positive liner relationship between the seed weight and width (r=0.7024, $p<0.01$), length and weight (r=0.6481, $p<0.01$), as well as width and length (r=0.5834, $p<0.01$; figure 2 A, B, C). However, we did not find liner relationship between seed thickness and the other parameters (figure 2 D, E, F). When American ginseng seeds were divided into seven groups according to seed width (~3.5 mm, 3.5~4.0 mm, 4.0~4.5 mm, 4.5~5.0 mm, 5.0~5.5 mm, 5.5~6.0 mm, 6.0 mm ~), the average seed width had positive liner relationships with average seed weight, length and thickness, and the correlation coefficients (0.9874, 0.9769 and 0.9576 for average seed width with weight, length and thickness respectively) were higher than that of using single seed data (figure 2 G, H, I). The above results indicated that seed width had better correlation with other seed size parameters, thus we considered seed width as a good indicator of seed size. Further, using seed width as a grading criterion will also facilitate commercial production.

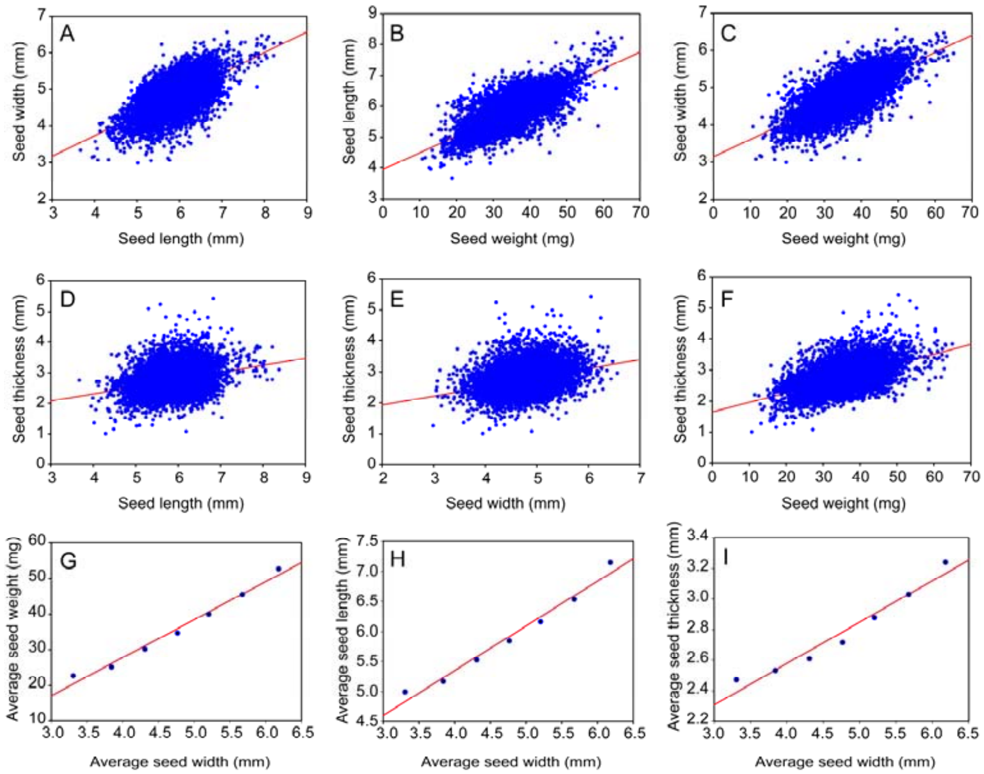

Fig 2: Relationships between seed weight, length, width and seed thickness. Linear regression model was used to test the significance of the relationships.

3.3 Effect of seed size on plumpness, splitting and germination

Seed size significantly affected kernel projective area, plumpness, splitting rate and germination percentage (table 1). Kernel area increased with seed size increasing and statistically significant differences were found between each size classes. However, the highest percentage of plumpness was found for three seed size classes (small: 4.0~4.5 mm, medium: 4.5~5.0 mm and 5.0~5.5 mm), and the values did not significantly (P>0.05) differ from each other within these three size classes. As expected, the extremely small (<4.0 mm) seeds had the lowest plumpness, but on the contrary, the extremely large seeds (>6.0 mm) had the second lowest plumpness. Large seeds (5.5~6.0 mm) exhibited a moderate plumpness, which was significant (P<0.05) higher than that of extremely large and small seeds. Similar trends were found for split and germination percentage, the maximum split and germination percentage were observed for medium seed size classes (4.5~5.0 mm and 5.0~5.5 mm), followed by large and small seeds, extremely large seeds, and extremely small seeds in descending order. It seems that split and germination percentage is more in accordance with plumpness but not seed size and kernel area. Moreover, we speculated that when the plumpness was on the equal level, larger seeds gave greater split and germination percentage. For instance, small and medium seeds had the same plumpness, however medium seeds exhibited higher split and germination percentage than small seeds. For the comparison between small and large seed size classes, small classes had higher plumpness but lower split and germination percentage.

Table 1: Effects of seed size on kernel area, plumpness, split percentage and germination in American ginseng.

Seed width (mm)	Kernel area (mm^2)	Plumpness (%)	Split percentage (%)	Germination percentage (%)
<4.0	10.6a	78.3a	55.7a	52.3a
4.0~4.5	13.1b	90.2d	82.8c	81.3c
4.5~5.0	16.2c	93.6d	90.5d	88.6d
5.0~5.5	19.2d	92.4d	92.3d	89.8d
5.5~6	21.7e	87.7c	84.4c	82.5c
>6.0	23.6f	83.9b	66.1b	63.2b

Means followed by the same letters within a column indicate no significant difference between different sizes at P<0.05 by a LSD test.

Kernel size is an important factor related to crop yield and quality and has been under selection since early domestication (Alexander *et al.*, 1984; Kumar *et al.*, 2016) [17, 18]. In American ginseng, kernel is the real seed on the basis of botanical studies, while the seed coat is endocarp. However, in most cases the kernel together with seed coat was recognized as ginseng seed. The main reason is that seed coat is difficult to remove and it benefits seed storage. Fortunately, the wonderful liner relationship (r=0.9941, p<0.01) between seed width and kernel projective area made the two parameters no difference when used for seed grading.

American ginseng seeds need to break both morphological and physiological dormancy before germination. Thus, the germination procedure of ginseng seed is more complex than non-dormancy species. In the present study, the split and germination percentage of different seed sizes consisted with plumpness in trend. This result may be due to seed with higher plumpness is easier to break dormancy, because seed coat should be fully filled with kernel before germination. Many studies have demonstrated that large seeds have greater germination percentage than small seeds (George *et al.*, 2003; Iakovoglou *et al.*, 2007; Kaya, *et al.*, 2008) [19-21]. However, in this study medium seed size classes had the maximum values of germination and related parameters. Similar results that medium seed size class gained better germination than larger and small classes were reported by Srimathi *et al.* (1991) [22]. and Khera *et al.* (2004) [23]. Although large seeds did not perform as excellent as medium seeds for germination, it acted the same as small seeds. Therefore, the findings of this study also did not agree with the findings that small seeds germinated better than large seeds (Saranga *et al.*, 1998) [24]. The extremely large and extremely small seed performed very poorly for germination. Undeveloped embryo may account for this result. A great portion of seeds from these two classes have very small embryo, which can hardly been seen with naked eye. These results did not support the conventional conception that the larger, the better for American ginseng seed.

4. Conclusion

The present study indicates that the values of seed size parameters conform to normal distribution, and most seed varied within a small range. Small, Medium and large seeds have the satisfactory split and germination percentage, while extremely small and large seeds should be removed to improve seed quality.

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