

# A large-scale analysis of seed natural aging revealed stronger aging resistance in weedy rice than coexisting cultivated rice for continuous 4 years across China

weimin dai (✉ [daiweimin4@njau.edu.cn](mailto:daiweimin4@njau.edu.cn))

Nanjing Agricultural University <https://orcid.org/0000-0002-8575-4279>

**Yuan Wang**

Nanjing agricultural university

**Yu-Jie Zhang**

Nanjing Agricultural University

**Xi-Xi Sun**

Nanjing Agricultural University

**Jin-Ling Yang**

Nanjing Agricultural University

**Xiao-Feng Chen**

Nanjing Agricultural University

**Zhi-Hua Shi**

Nanjing Agricultural University

**Xiao-Ling Song**

Nanjing Agricultural University

**Sheng Qiang**

Nanjing Agricultural University

---

## Research article

**Keywords:** Weedy rice, Co-existing rice cultivar, Seed aging, indica/japonica cultivar, Seed dormancy

**Posted Date:** November 27th, 2019

**DOI:** <https://doi.org/10.21203/rs.2.17773/v1>

**License:**  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

Increasing resistance to aging is conducive to seed storage and germination rate of crop. Meanwhile, the resistance to aging is one of the important adaptive mechanisms of weed to thrive in farmland. Weedy rice (*Oryza sativa* f. *spontanea*) and cultivated rice (*Oryza sativa* L.) provide a unique pair demonstrating a weed and conspecific model crop that can be used to study the aging resistance of plants across a diverse geographical range. Chinese weedy rice derived from de-domestication of cultivated rice has rapidly risen to malignant weeds, though the hazard has only been reported for about 20 years. Whether weedy rice rapidly evolves higher seed aging resistance than cultivated rice during the process of dedomestication, which is conducive to its persistence in rice fields, is still unclear. In this experiment, the seeds of weed rice populations and their co-existing rice varieties were collected from 61 regions of China and germinated under normal and high temperatures for consecutive four years (2013–2016). Our study found that the aging resistance of weedy rice was higher than that of the co-existing rice cultivars, and weedy rice may have evolved a different aging resistance mechanism than rice cultivars and could be used as a germplasm resource to cultivate aging-resistant rice. The indica-type has strong aging resistance and no dormancy, while the japonica type has weak aging resistance and a little weak dormancy. Thus, by introducing indica-type aging-resistant alleles into japonica rice, cultivation of aging-resistant japonica rice could be possible.

## Introduction

Seed aging not only decreases the storage ability of seeds but also decreases seed vigor and affect the germination in the field (Sasaki et al., 2005; Sano et al., 2016; Hay et al., 2019). Rice belongs to a medium-longevity (2-4 years) seed type (Cai et al., 2011; Hu 2014; Zhang et al., 2019). After harvesting, seeds usually need to be stored for a certain period of time, ranging from 3–5 months to more than 2 years. Under normal storage conditions, the quality of rice seeds begins to deteriorate after one year and strongly affects its eating quality. Due to poor storage potentiality, a large quantity of rice seed is often discarded every year, which leads to huge loss (Li et al., 2017). According to the annual yield of 200 billion kg in recent years, the loss of food is nearly 6 billion kg (Xu et al., 2015). In addition, decreased seed vigor of rice during the storage also causes huge economic losses. The construction of cold storage facilities might be an option but involves high cost. Conversely, cultivating age-resistant rice varieties is a convenient and feasible alternative (Sasaki et al., 2005; Zhang et al., 2019).

Aging resistance of seed is considered to be an important adaptive mechanism for the persistence of weeds in the field (Baker, 1974). Weedy rice in paddy fields has become one of the most malignant weeds all over the world (Delouche et al., 2007). According to the genome-wide evolutionary analysis, Chinese weedy rice was more closely related to local cultivated rice and considered to be de-domesticated from cultivated rice (Qiu et al., 2017). In the process of de-domestication, weedy rice might have evolved some new germination mechanisms to adapt the local environment. Xia et al., (2011) found that the critical low temperature of seed germination was related to the local habitat temperature in weedy rice populations. Burgos et al. (2006) found that the nitrogen utilization rate was more efficient in weedy rice (Strawhull) of

Southern United States than the high-yield rice cultivar (Drew). Puteh et al. (2013) reported that weedy rice cultivated under less water had higher drought tolerance capacity in comparison to the indica-type rice cultivars (MR219 and MR 232) as well as weedy rice accessions (Bertam and Ketara). Borjas et al. (2016) found that weedy rice and the rice cultivar got a higher rate of germination and seedling vigor at low temperature than the American weedy rice (PSRR-1) along with rice cultivar (Bengal).

The aging resistance of seeds is usually studied using the natural aging and artificial aging (Hay et al., 2019). Relative to natural aging, artificial aging is to accelerate seed aging using high temperature and humidity (Zeng et al., 2002; Zeng et al., 2006; Xu et al., 2013; Xu et al., 2015). By comparing the results of artificial aging with natural aging, some researchers have found that artificial aging does not fully reflect all the processes that occur during true natural aging (Zhang and Hu, 2015; Xu et al., 2013). The vitality of natural aging seeds can persist to affect the whole growth process and ultimately affect the rice yield. On the contrary, the vigor of artificial aging seeds only affects the early production performance of the rice plant and ultimately has little effect on the production performance of later stage (Zhang and Hu, 2015). Xu et al. (2013) found that the results were not completely consistent through comparing seed germination rate of rice varieties through quantitative trait loci (QTL) analysis of indica-japonica recombination inbred line population under natural aging (2 years) and artificial aging (high temperature and high humidity), respectively. Hang et al. (2015) found that *qSSH-2-1* and *qSSH-2-2* could be detected under natural and artificial aging, while the other 4 QTLs (*qSSH-4*, *qSSs-5-1*, *qSSs-5-2* and *qSSH-12*) and 7 QTLs (*qSSH-1*, *qSSH-3-1*, *qSSH-3-2*, *qSSH-3-3*, *qSSH-7-1*, *qSSH-7-2* and *qSSH-11*) were only detected under natural aging and artificial aging, respectively.

In this experiment, 61 weedy rice populations (each population included 8 to 33 accessions) and coexisting rice cultivars across China were collected and germinated under normal and high-temperature dormancy-breaking treatment for consecutive four years (2013–2016). The research aimed to solve the following issues: 1) whether the weedy rice populations have stronger aging resistance than coexisting rice cultivars; 2) to reveal the relationship between indica-japonica type and aging resistance; 3) to explore the relationship between seed dormancy and aging resistance.

# 1 Material And Methods

## 1.1 Plant materials

Based on the geographical distribution of rice planting areas in China, 61 weedy rice populations and coexisting rice cultivars were collected from 2009 to 2012 (Supplemental Fig. 1; Wang et al., 2019). Latitude, Altitude and Sowing method of sampling sites were recorded in Table S1. Mean Annual precipitation, Mean Annual temperature, Mean temperature in the sowing month, and Mean precipitation in the sowing month were collected from <http://www.worldclim.org> (Table S1).

Each population included 8 to 33 accessions sampled from the same field and each population was separated from others by a distance of more than 10 km. When weedy rice was collected at each sample site, co-existing rice cultivar was also collected from the field which was more than one hectare. These rice varieties are recommended to cultivate by local government in China. Most of farmers buy rice seeds every year, especially for those farmers who own more than one hectare of land in China. In order to eliminate maternal, environmental, varying seed age, and storage effects, all weedy rice populations and coexisting rice cultivars were planted at Jiangpu Experimental Station, Nanjing Agricultural University, Nanjing, Jiangsu, China, in 2013. At 30 days after sowing, 50 seedlings from each population were transplanted in a random block design with three repetitions. The experimental field was managed with conventional rice cultivation methods. Mature seeds from each population were harvested 50 days later after the heading date. The harvested seeds dried under well-ventilated conditions to a constant weight (about 12 % seed moisture content) and were stored at room temperature (< 30 °C) and 40–60 % relative humidity in Nanjing from 2013 to 2016. At November each year seeds were germinated at 28°C constant temperature after normal temperature (25 °C, 48 h) and high temperature (50 °C, 48 h) treatment, respectively (Hang et al., 2015).

## 1.2 Normal and high temperature treatments

A group of 180 plump rice seeds representing each sampled population were divided into six samples and placed on two layers of filter paper in Petri dishes. Three samples under normal temperature treatment (25 °C, 48 h) were germinated with 6 mL distilled water while three samples under high temperature treatment (50 °C, 48 h) to break dormancy were germinated with 6 mL distilled water. Then, the dishes were wrapped in plastic bags and placed into incubators (SPX-400-GBH) at a steady temperature of 28°C with a light: dark cycle of 16:8 h. The number of germinated seeds was recorded each day at 7th days. Germination was evaluated visually by protrusion of the radicle from the hull by  $\geq 3$  mm (Baskin & Baskin, 1998).  $GR = (\text{number of seeds germinated by Day 7} / \text{total seed number}) \times 100\%$ .

## 1.3 Identification of *indica-japonica* type of weedy rice

Above 10 individuals in each population were randomly selected to identify *indica-japonica* type by means of Cheng index (Cheng et al., 1984; Liu & Yang 1991; Chen et al., 2015). Cheng index contains 6 characters, that is, hull color at heading stage, epidermal hair on leave at heading stage, epidermal hair on hull at heading stage, length between 1<sup>st</sup>-2<sup>st</sup> panicle node at mature stage, phenol reaction of seed at mature stage, and length-width ratio of seed at mature stage.

## 1.4 Data analysis

Analysis of Variance (ANOVA) was performed using SPSS 18.0 (IBM, New York, New York, USA). The geographic information map was drawn using ArcGIS 10.0 (ESRI, Redlands, California, USA). Other figures were drawn using Excel 2010 (Microsoft Corporation, Redmond, Washington state,) and Origin 8.0 (Originlab, Northampton, Massachusetts, USA).

## 2. Results

### *2.1 Comparing seed aging resistance between weedy rice populations and coexisting rice cultivars*

One-way ANOVA showed that there was no significant difference in the germination rate between weedy and cultivated rice in 2013 and 2014 under normal temperature treatment condition, while the germination rate of weedy rice was significantly higher than that of cultivated rice in 2015–2016 (Fig.1; Table 1).

Under high-temperature dormancy-breaking treatment condition, the germination rate of weedy and cultivated rice was not significantly different in 2013, while the germination rate of weedy rice was significantly higher than that of cultivated rice in 2014–2016 (Fig. 1; Table 1). The germination rate of weedy and cultivated rice under high-temperature dormancy-breaking treatment was significantly higher than that of normal temperature treatment in 2013, which showed that seeds harvested in the first year (2013) had a weak dormancy, so breaking dormancy could increase its germination rate. In 2014, the germination rate of weedy and cultivated rice under high-temperature dormancy-breaking treatment was significantly lower, which indicated that high temperature accelerates seed aging. Two-way ANOVA between temperature treatments (normal temperature and high temperature treatment) and genotypes (weedy and cultivated rice) showed that there were significant differences in genotypes in 2013–2016 except for 2013 (Table 2). Furthermore, with the increase of aging, the germination rate of weedy rice was significantly higher than that of cultivated rice (Fig 1; Table 1).

### *2.2 Relationship between indica-japonica type and aging resistance of weedy rice and coexisting rice varieties*

The germination rate along with the latitude gradient was plotted under normal temperature and high-temperature treatment, respectively (Fig.2 A, B). The germination rate of weedy and cultivated rice decreased with latitude, which suggests that the indica-japonica type may affect the aging resistance. One-way ANOVA of germination rates showed that under normal temperature treatment, germination rates of indica-type weedy and cultivated rice were significantly higher than japonica-type weedy and cultivated rice in 2013–2016 (Fig. 4). In the third year (2015), the germination rate of japonica-type weedy and cultivated rice was decreased to about 40% and 10%, respectively, while the germination rate of indica-type weedy and cultivated rice remained at about 90%. Correspondingly, under the high-

temperature dormancy-breaking treatment, there was no significant difference in the germination rate of indica-japonica type weedy and cultivated rice in the first year (2013). In 2014–2016, the germination rate of indica type was significantly higher than that of japonica type for both weedy rice and cultivated rice, and the germination rate of japonica-type weedy rice was significantly higher than that of japonica-type cultivated rice (Fig. 3). During the comparison of normal temperature treatment with high-temperature treatment, the weedy and cultivated rice showed significantly increased germination rate in 2013, but the high-temperature dormancy-breaking treatment significantly reduced the germination rate in 2014–2016 (Fig. 4). Besides, two-way ANOVA between subspecies (indica and japonica) and genotypes (weedy and cultivated rice) under normal temperature treatment revealed that there was a significant difference in the subspecies but no significant difference was there in genotypes in 2013–2014 (Table 3). In 2015–2016, there were significant differences in the subspecies and genotypes. Correspondingly, under high-temperature dormancy-breaking treatment, the subspecies in 2013 and 2016 had significant differences, while the genotypes did not have significant differences. In 2014–2015, there were significant differences in both the subspecies and genotypes (Table 3).

In summary, the aging resistance of indica-type weedy and cultivated rice was both significantly higher than that of japonica-type weedy and cultivated rice (Fig. 3). In addition, indica-type weedy and cultivated rice showed no dormancy, while japonica-type weedy and cultivated rice showed weak dormancy (Fig. 3).

### 3. Discussions

#### *3.1 The aging tolerance of weedy rice is higher than that of cultivated rice and the aging resistance of indica type is higher than that of japonica type*

Our results indicated that the germination rate of weedy rice was significantly higher than that of coexisting rice varieties during the natural aging (Table 1, 2; Figs. 1, 2). Weedy rice may develop a new adaptation mechanism in the farmland environment during the process of domestication (Xia et al., 2011; Kuester et al., 2016; Qiu et al., 2017). Li et al. (2010) found that under artificial aging treatment, the storage resistance of weedy rice was higher than that of cultivated rice, while comparing the storage resistance of 13 weedy rice accessions with 6 rice cultivars. Ma et al (2016) found that under natural aging treatment, the storage resistance of weedy rice was better than that of cultivated rice through comparing 27 weedy rice accessions with 5 rice varieties.

During the comparative analysis of seed germination rate with latitude in 2013–2016 (Fig. 2), it was noticed that the germination rate of weedy rice and cultivated rice was decreased with the increase in latitude. Indica-type rice varieties are normally adapted to warm, low-latitude or high-altitude tropical environments, while japonica-type rice varieties are adapted to temperate environments with relatively high latitudes or high altitudes with relatively cool climatic conditions (Glaszmann, 1987). By analyzing the whole genome sequencing of 155 weedy rice accessions and 76 locally rice varieties in four representative regions of China, Qiu et al. (2017) found that the genetic distance between weedy rice and local varieties was lesser than other groups. Therefore, the variation in germination rate may be related to

the distribution of indica-japonica type in weedy and cultivated rice. Additionally, by analyzing the relationship between indica-japonica type and aging resistance of weedy and cultivated rice (Fig. 2, 3), it was noticed that the germination of indica-type weedy and cultivated rice was significantly higher than that of japonica-type weedy and cultivated rice in 2013–2016. Under artificial aging treatment in 410 rice varieties (40° C, relative humidity 95%, ten days), Zeng et al. (2002) found that indica rice was having more storage resistance than japonica rice. Under artificial aging treatment, Wu et al. (2010) measured the germination rate of Chinese rice micro-core germplasm (193 rice varieties) and found that the germination rate in japonica-type rice was 58.9% while that in indica-type rice was only 29.1%. Sasaki et al. (2005) studied the QTL of seed aging resistance using Milyang 23 (Indica-type) and Akihikari (Japonica-type) recombinant inbred lines and found that indica-type Milyang 23 alleles of all QTLs promoted germination and normal seedling growth. Recently, by introducing qSS–9 gene of indica rice (Kasalath) into Ningjing 4 by backcrossing and selfing with Ningjing 4, Zhang et al. (2019) bred a new rice variety, which was not only resistant to storage but also agronomically close to japonica type rice Ningjing 4.

### *3.2 Relationship between seed dormancy and aging resistance*

Seed dormancy and aging resistance are two traits of seed adaptation to the environment (Baskin and Baskin 1998; Sasaki et al., 2005; Nguyen et al., 2012). Regarding the relationship between seed dormancy and aging resistance, different results were reported in different plants (Roberts 1963; Siddique et al., 1988; Nguyen et al., 2012). Roberts (1963) found no significant relationship between seed dormancy and aging while studying the seed germination rate of six rice cultivars. Siddique et al. (1988) found that the higher-dormancy varieties also had higher aging resistance while analyzing 30 rice varieties under artificial aging treatment. In order to distinguish seed dormancy and aging resistance, normal treatment, as well as high temperature dormancy-breaking treatment, was set in this experiment. In 2013, the germination rate under high-temperature dormancy-breaking treatment was significantly higher than that of normal temperature, indicating that the seeds harvested in the first year had weak dormancy. Further analysis of the indica-japonica type of weedy and cultivated rice showed that the japonica type had weak dormancy, while the indica type had no significant dormancy (Table 3). This is consistent with previous report of Xia et al. (2011) that Chinese weedy rice has weak dormancy or no primary dormancy. In 2014–2016, the germination rate of japonica type under high-temperature dormancy-breaking treatment was significantly lower than that of normal temperature treatment, indicating that high temperature became new stress, which accelerated aging. The outcome of this study clearly shows that the aging resistance, but not dormancy, in the weedy and cultivated rice is related to the indica-japonica type. The aging resistance of the indica type was high but with no dormancy, while the aging resistance of the japonica type was low with comparatively higher dormancy. Similarly, Nguyen et al (2012) found that the high storage ability was related to weak seed dormancy, and the low storage ability is associated with high seed dormancy in *Arabidopsis*.

## 4. Conclusion

The present study analyzed the germination rate of 61 weedy rice populations and coexisting rice varieties in consecutive four years (2013–2016), and found that the aging resistance of weedy rice was stronger than that of cultivated rice. Thus, weedy rice might be used as germplasm resources to cultivate aging resistant rice cultivars. The aging resistance in the weedy and cultivated rice is closely related to indica-japonica type but not to the dormancy. The introduction of aging-resistant alleles from indica type into japonica rice may be utilized to breed aging-resistant japonica rice.

## Declarations

### Ethics approval and consent to participate

Not applicable

### Consent for publication:

Not applicable.

### Availability of data and material

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

### Funding

This research was financially supported by the National Special Transgenic Project of China (2016ZX08012005), the China Transgenic Organism Research and Commercialization Project (Grant Nos. 2016ZX08011-001, 2011ZX08011-001, and 2009ZX08012-020B), the Key Technology for Biological Security (Grant No. 2016YFC1201202), Special Fund for Agro-scientific Research in the Public Interest (Grant No. 201303022), the National Natural Science Foundation of China (Grant No. 30800604).

## Authors' contributions

Wei-Min Dai and Yuan Wang performed the statistical analysis and drafted the manuscript. Yuan Wang, Jin-Ling Yang, Xiao-Feng Chen, Xi-Xi Sun, Yu-Jie Zhang participated in the data collection. Wei-Min Dai, Xiao-Ling Song and Sheng Qiang discussed and designed the study. Zhi-Hua Shi helped to draft the manuscript. All authors read and approved the final manuscript.

## Acknowledgements

Not applicable

## References

1. Baker HG. The evolution of weeds. *Annual of Review of Ecological System*. 1974. 5: 1-24.
2. Baskin JM, Baskin CC. *Seeds*. New York,Academic Press. 1998.
3. Borjas AH, De-Leon TB, Subudhi PK. Genetic analysis of germinating ability and seedling vigor under cold stress in US weedy rice. *Euphytica*. 2016. 208: 251–264.
4. Burgos NR, Norman RJ, Gealy DR, and Black H. Competitive N uptake between rice and weedy rice. *Field Crops Research*. 2006. 99: 96–105.
5. Cai QH, Wu FX, Zhu YS, Xie HG, Zhang JF, and Xie HA. Screening of rice germplasms with storability and analysis of their genetic diversity. *Fujian Journal of Agricultural Sciences*. 2011. 26(4): 562-566. (In Chinese with English abstract)
6. Chen XF, Qiang S, Yang JL, Zhang BH, Zhang Z, Song XL, and Dai WM. Hierarchical clustering and indica-japonica classification: uncover mutual spread and indica-japonica differentiation for weedy rice in Jiangsu Province. *Chinese Journal of Rice Science*. 2015. 29(1):82-90. (In Chinese with English abstract)
7. Cheng KS, Wang XK, Zhou JW, Lu YX, Lou J, and Huang HW. Studies on indigenous rices in Yunnan and their utilization. II. A revised classification of Asian cultivated rice. *Acta Agronomica Sinica*.10: 271-280. (In Chinese with English abstract)
8. Delouche JC, Burgos NR, Gealy DR, Zorilla-San Martin G, Labrada R, Larinde M, and Rosell C. Weedy rices-origin, biology, ecology and control. *Food and Agriculture Organization (FAO) of the United Nations*, Rome, Italy, 2007. p129.
9. Glaszmann JC. Isozymes and classification of Asian rice varieties. *Theoretical and Applied Genetics*. 74:21-30.
10. Hang NT, Lin QY, Liu LL, Liu X, Liu SJ, Wang WY, Li LF, He NQ, Liu Z, Jiang L, and Wan JM. Mapping QTLs related to rice seed storability under natural and artificial aging storage conditions. *Euphytica*.

2015. 203:673–681.
11. Hay FR., Valdez R, Lee JS, and Cruz PCS. Seed longevity phenotyping: recommendations on research methodology. *Journal of Experimental Botany*, 2019. 70 (2): 425–434.
  12. Hu J. Seed science [M]. China Agriculture Press in Beijing, China. 2014. p110-111.
  13. Kuester A, Wilson A, Chang SM, and Baucom RS. A resurrection experiment finds evidence of both reduced genetic diversity and potential adaptive evolution in the agricultural weed *Ipomoea purpurea*. *Molecular Ecology*. 2016. 25: 4508-4520.
  14. Li CS, Shao GS, Wang L, Wang ZF, Mao YJ, Wang XQ, Zhang XH, Liu ST, and Zhang HS. QTL identification and fine mapping for seed storability in rice (*Oryza sativa* L.). *Euphytica*. 2017. 213:127.
  15. Li MB, Wang H, Piao ZZ, Shen GH, and Wen GY. Primary study on artificial aging and storage characteristics in weedy rice. *Crops*. 2010. 5: 30-33. (In Chinese with English abstract)
  16. Liu WY, Yang ZY. Preliminary report on the classification of F1 from cross between indica and japonica rices. *Journal of Shenyang Agricultural University*. 22: 82-86. (In Chinese with English abstract)
  17. Ma J, Sun JC, Wu B, and Wang XS. Research of habits and characteristics about survival and spread of weedy rice in Ningxia III - dormancy and viability of weedy rice seed. *Seed*. 2016. 9: 75-77. (In Chinese with English abstract)
  18. Nguyen TP, Keizer P, Eeuwijk F, Smeekens S and Bentsink L. Natural variation for seed longevity and seed dormancy are negatively correlated in *Arabidopsis*. *Plant Physiology*. 2012.160:2083–2092,
  19. Puteh AB, Saragih AA, Ismail MR, and Monda MMA. Chlorophyll fluorescence parameters of cultivated (*Oryza sativa* L. ssp. indica) and weedy rice (*Oryza sativa* L. var. nivara) genotypes under water stress. *Australian Journal of Crop Science*. 2013. 7: 1277-1283.
  20. Qiu J, Zhou YJ, Mao LF, Ye CY, Wang WD, Zhang JP, Yu YY, Fu F, Wang YF, Qian FJ, Qi T, Wu SL, Sultana MH, Cao YN, Wang Y, Timko MP, Ge S, Fan LJ, and Lu YL. Genomic variation associated with local adaptation of weedy rice during de-domestication. *Nature Communications*. 8: 15323.
  21. Roberts EH. An investigation of inter-varietal differences in dormancy and viability of rice seed. *Annual Botany*. 1963. 27, 365-369.
  22. Sasaki K, Fukuta Y and Sato T. Mapping of quantitative trait loci controlling seed longevity of rice (*Oryza sativa* L.) after various periods of seed storage. *Plant Breeding*. 2005. 124: 361-366.
  23. Sano N, Rajjou L, North HM, Debeaujon I, Marion-Poll A, and Seo M. Staying alive: molecular aspects of seed longevity. *Plant Cell Physiology*. 2016. 57(4): 660–674.
  24. Siddique SB, Seshu DV, and Pardee WD. Rice cultivar variability in tolerance for accelerated aging of seed. *IRRI Research Paper Series*. 1988. 131: 2-7.
  25. Wu FX, Zhu YS, Xie HG, Zhang JF, and Xie HA. Preliminary study on storability of chinese micro-core collections of rice. *Journal of the Chinese Cereals and Oils Association*. 2010. 25(10):124-128. (In Chinese with English abstract)

26. Wang Y, Mo SD, Kong MY, Chao J, Chen XF, Yang JL, Yan YJ, Shi ZH, Qiang S, Song XL, and Dai WM. Better performances of germination in hyperosmotic solutions in conspecific weedy rice than cultivated rice. *Journal of Systematics and Evolution*. 2019. in press.
27. Xia HB, Xia H, Ellstrand NC, Yang C, and Lu BR. 2011. Rapid evolutionary divergence and ecotypic diversification of germination behavior in weedy rice populations. *New Phytologist*. 191: 1119–1127.
28. Xu HB, Wei YD, Zhu YS, Lian L, Xie HG, Cai QH, Chen QS, Lin ZP, Wang ZH, Xie HA and Zhang JF. Antisense suppression of LOX3 gene expression in rice endosperm enhances seed longevity. *Plant Biotechnology Journal*. 2015. 13: 526–539.
29. Xu HB, Wei YD, Lian L, Zhu YS, Xie HA, Wang ZH, and Zhang JF. Comparative analysis of artificial aging and natural aging with rice seeds. *Molecular Plant Breeding*. 2013, 11(5): 552-556.
30. Zeng DL, Guo LB, Xu YB, Yasukumi K, Zhu LH and Qian Q. QTL analysis of seed storability in rice. *Plant Breeding*. 2006, 125: 57-60.
31. Zeng DL, Qian Q, Yasukumi K, Teng S, and Fujimoto H. Study on storability and morphological index in rice (*Oryza sativa* L. ) under artificial ageing. *Acta Agronomica Sinica*. 2002, 28(4): 551-551. (In Chinese with English abstract)
32. Zhang HS, Hu J. Seed science [M]. Science Press in Beijing, China. 2015, p176.
33. Zhang P, Jiang YM, Cao PH, Zhang FL, Wu HM, Cai MY, Liu SJ, Tian YL, Jiang L, and Wan JM. Introducing qSS-9<sup>Kas</sup> into Ningjing 4 by molecular marker-assisted selection to improve its seed storage ability. *Acta Agronomica Sinica*. 2019. 45(3): 335-343. (In Chinese with English abstract)

## Tables

Table 1 Germination rate of 61 weedy rice populations and coexisting rice cultivars in normal temperature treatment and high temperature treatment for consecutive 4 years (2013-2016)

Treatment	Years	Maximum value (%)		Minimum value (%)		Mean value (%)	
		weedy rice	Rice cultivars	weedy rice	Rice cultivars	weedy rice	Rice cultivars
Normal temperature	2013	100.00	100.00	73.89	72.22	93.97	93.85
	2014	100.00	100.00	61.11	42.22	91.06	84.61
	2015	100.00	98.89	0.00	0.00	66.82	33.87
	2016	99.44	95.56	0.00	0.00	46.24	21.58
High temperature	2013	100.00	100.00	91.67	91.11	96.82	96.88
	2014	100.00	100.00	9.44	0.00	79.10	43.65
	2015	100.00	100.00	0.00	0.00	62.16	28.65
	2016	99.44	97.78	0.00	0.00	38.25	18.58

**Table 2** Two-way ANOVA between temperature treatments (normal and high temperature) and genotypes (weedy and cultivated rice) on the germination rate for consecutive 4 years (2013-2016)

Years		Mean Square	F	Sig.
2013	Temperature treatment	525.423	23.312 **	0.000
	Genotype	0.048	0.002	0.963
2014	Temperature treatment	42704.353	68.104 **	0.000
	Genotype	26776.009	42.702 **	0.000
2015	Temperature treatment	1488.188	1.120	0.291
	Genotype	67370.983	50.711 **	0.000
2016	Temperature treatment	1845.861	1.466	0.227
	Genotype	29967.281	23.797 **	0.000

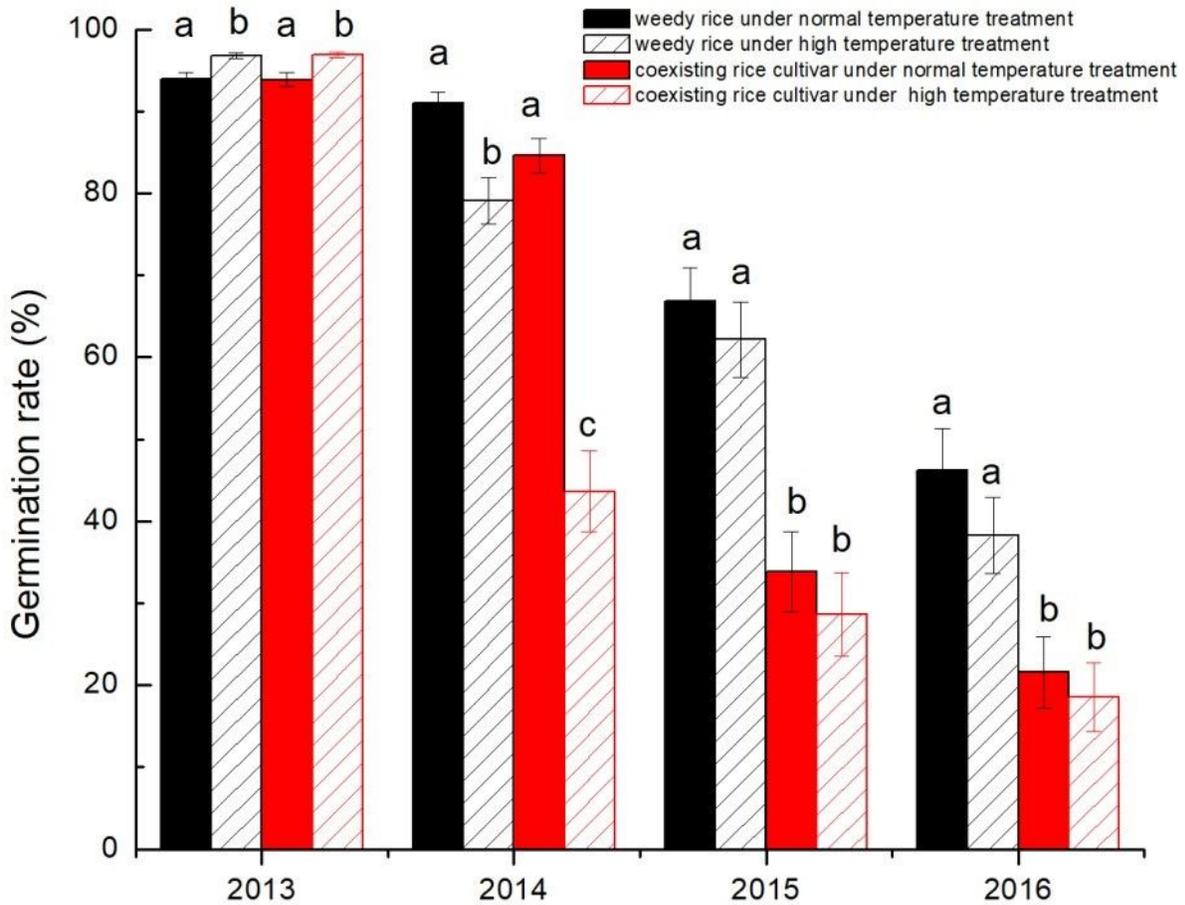
Note: Bootstrap value is 1000. Significance levels are indicated by \*\*  $p < 0.01$ . F means F test. Sig. means significant level.

**Table 3** Two-way ANOVA between subspecies (indica and japonica) and genotypes (weedy and cultivated rice) on the germination rate for consecutive 4 years (2103-2016)

Treatment	Years		Mean Square	F	Sig.
Normal temperature	2013	subspecies	936.475	29.870**	0.000
		Genotype	48.516	1.547	0.216
	2014	subspecies	7355.962	59.308**	0.000
		Genotype	175.330	1.414	0.237
	2015	subspecies	104258.122	236.921**	0.000
		Genotype	9257.343	21.037**	0.000
2016	subspecies	112135.319	261.578**	0.000	
	Genotype	2388.434	5.572*	0.020	
High temperature	2013	subspecies	81.270	14.165**	0.000
		Genotype	6.558	1.143	0.287
	2014	subspecies	69685.572	178.370**	0.000
		Genotype	15422.467	39.476**	0.000
	2015	subspecies	103627.749	205.805**	0.000
		Genotype	9889.526	19.641**	0.000
2016	subspecies	88313.011	203.481**	0.000	
	Genotype	993.443	2.289	0.133	

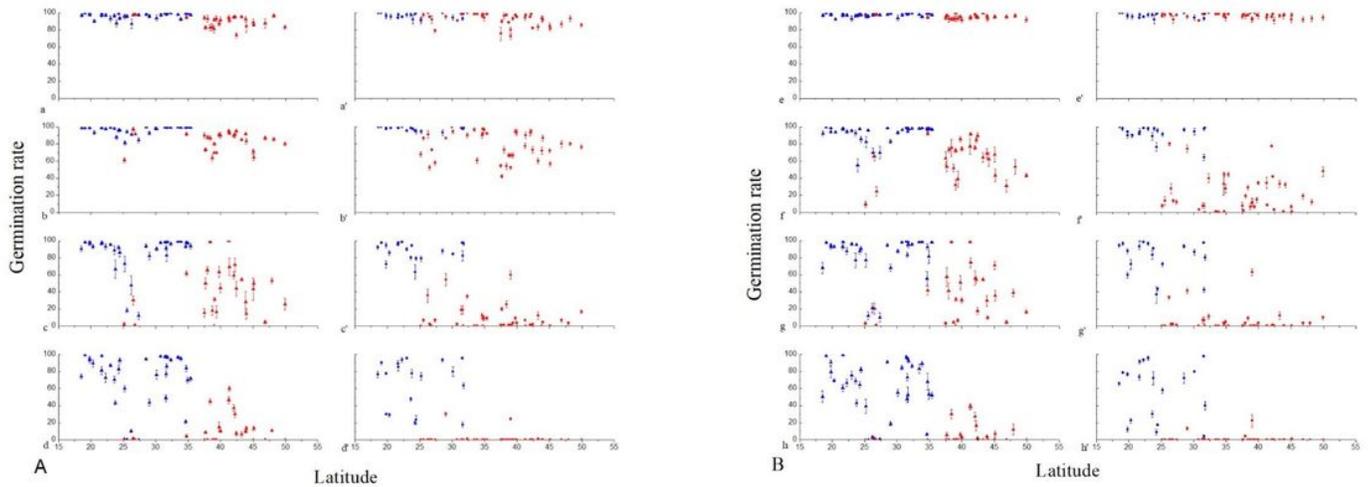
Note: Bootstrap value is 1000. Significance levels are indicated by \* and \*\* for  $p < 0.05$  and  $p < 0.01$ , respectively. F means F test. Sig. means significant level.

## Figures



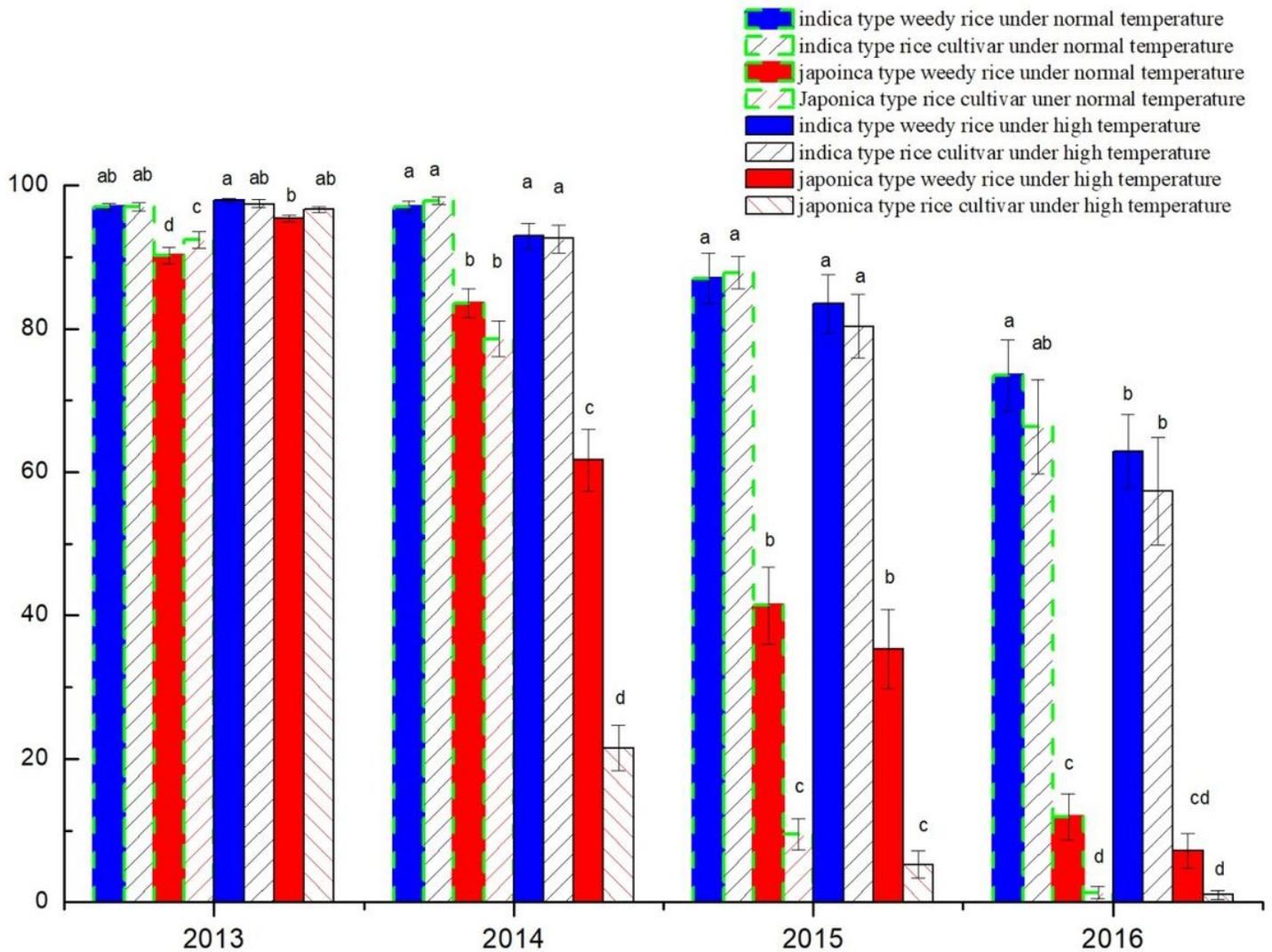
**Figure 1**

One-way ANOVA of 61 weedy rice populations and coexisting rice cultivars under normal and high temperature treatments. Black and red histograms respectively represent weedy rice and rice cultivars in normal treatment. Black diagonal and red diagonal histograms respectively represent weedy rice and rice cultivars in high temperature treatment. Vertical bars indicate  $\pm$  standard error of mean. Significant levels are indicated by letters (a, b, c), respectively.



**Figure 2**

Germination rate of 61 weedy rice populations and coexisting cultivars along latitude for consecutive four years under normal and high temperature treatments. Note. A and B are 61 weedy rice populations and coexisting rice cultivars under normal and high temperature treatments, respectively. Triangle represents weedy rice. Dot represents rice cultivar. Blue and red represents indica and Japonica type, respectively. a and a' represent germination rates of weedy rice populations and coexisting cultivars along latitude in 2013 under normal temperature treatment, respectively; b and b' represent germination rates of weedy rice populations and coexisting cultivars along latitude in 2014 under normal temperature treatment, respectively; c and c' represent germination rates of weedy rice populations and coexisting cultivars along latitude in 2015 under normal temperature treatment, respectively; d and d' represent germination rates of weedy rice populations and coexisting cultivars along latitude in 2016 under normal temperature treatment, respectively; e and e' represent germination rates of weedy rice populations and coexisting cultivars along latitude in 2013 under high temperature treatment, respectively; f and f' represent germination rates of weedy rice populations and coexisting cultivars along latitude in 2014 under high temperature treatment, respectively; g and g' represent germination rates of weedy rice populations and coexisting cultivars along latitude in 2015 under high temperature treatment, respectively; h and h' represent germination rates of weedy rice populations and coexisting cultivars along latitude in 2016 under high temperature treatment, respectively. The vertical bars indicate standard error of the means.



**Figure 3**

One-way ANOVA of germination rate of indica / japonica type weedy rice and coexisting rice cultivars under normal and high temperature treatments for consecutive 4 years in 61 sites across China Note: Blue and red histograms represent indica / japonica type weedy rice, respectively. Blue diagonal and red diagonal histograms represent indica / japonica type rice cultivars. Green dash-line border represents normal temperature treatment. Black solid-line border represents high temperature treatment. Vertical bars indicate  $\pm$  standard error of mean. Significant levels are indicated by letters (a, b, c), respectively.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [0SupplementalFig1Themapof61samplesites.jpg](#)
- [0Supplementaltable1Theinformationof61samplesites.xlsx](#)