Survey of overwintering trait in Chinese rice cultivars
(*Oryza sativa* L)

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Abstract Overwintering rice can survive through the natural cold-winter field environment, sprout from rice tillering node in the following spring, tiller, flower, seed, and being harvested in the following autumn, which is a type of an extreme case of cold tolerance of rice. The successful utilization of cold tolerance rice is the most economical strategy for the cold tolerance rice cultivar breeding project. This work aims to identify the OW rice for the future development of cold tolerance cultivars. Altogether 1034 Chinese existing rice cultivars including 735 (71.08%) conventional Japonica rice cultivars and 299 (28.92%) conventional Indica rice cultivars were collected and evaluated for their responses to low temperatures under the natural field cold-winter environment. Among them, altogether 262 (25.34%) conventional *Japonica* rice cultivars could withstand cold tolerance to 4 °C of the daily minimum temperatures in December 2019 throughout the cold-winter season and distributed in 13 provinces of China, survive through the natural cold-winter field environment, and sprout from rice tillering node in March 2020. Only 24 (2.32%) conventional japonica rice cultivars could withstand cold tolerance to 0 °C of the daily minimum temperatures in January 2021 throughout the cold-winter season, which could also

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sprout from rice tillering node in March 2021 and distributed in seven provinces of China. The present cold tolerance rice cultivars will provide beneficial breeding germplasm for the future cold tolerance rice breeding project and new strategies involved in elucidating the molecular mechanism of the cold tolerance of rice.

**Keyword**  *Oryza sativa* L. · Overwintering rice cultivar · Cold tolerance to 4 °C · Cold tolerance to 0 °C

**Abbreviations**

- OW  Overwintering
- QTL  Quantitative trait locus
- CV  Coefficient variation
- Min T  Minimum temperatures
- Max T  Maximum temperatures
- CQNU  Chongqing Normal University
- CNRRI  China National Rice Research Institute

**Introduction**

Rice (*Oryza sativa* L.) is one of the most important crops in China and provides stable food for approximately half of the global population (Fairhurst and Dobermann 2002). The global rice production is 759.6 million tons, where China ranks first in terms of the area and total production (Cheng 2010). For a long time, the innovative utilization of rice germplasm resource has promoted the development of the rice genetics and breeding project (Yuan 1966). The successful breeding of each new rice variety with good quality and high yield and the publication of each world-class paper of rice are indispensable to the innovative utilization of excellent rice germplasm (Qin et al. 2021). In addition, rice germplasm plays an important role in the rice genetic and breeding project for ever and lays a good material foundation on the study of basic theory and application of rice.

Compared with wheat (*Triticum aestivum* L.) and perennial bamboo (*Phyllostachys*) of Poaceae, rice is more sensitive to low temperature and can easily be damaged from cold throughout the world (Zhang et al. 2017). Particularly in China, the low-temperature disaster leads to the loss of 300–500 million tons of grain per year (Zhu et al. 2015; Zhang et al. 2017). Breeding a cold tolerance rice variety is the best economic strategy involved in reducing the loss of grain cold damage. However, the identification of cold tolerance rice germplasm is a crucial step toward the cold tolerance rice breeding project. In rice production, cold damage usually occurs at several different stages of germination, seedling, booting, and mature growth of rice (Dai et al. 2004; Zhou et al. 2010; Shinada et al. 2013; Pan et al. 2015; Wang et al. 2016). Therefore, a series of excellent cold tolerance rice germplasms including Silewah, Koshihikari, M202, Norin-PL8, Dongxiang wild rice, Kunmingxiaobaigu, and Lijiangxintuanhei have been selected as the donor parent for the identification of QTL underlying cold tolerance or even the cold tolerance rice breeding project. Currently, more than 250 QTLs controlling cold tolerance have been roughly located on 12 chromosomes of rice and functionally identified for the cold tolerance of rice using phenotypic data on cold tolerance at several different stages of germination, seedling, booting, and mature growth of rice (Andaya and Mackill 2003; Liu et al. 2003; Xu et al. 2008; Kuroki et al. 2009; Mori et al. 2011; Shirasawa et al. 2012; Ma et al. 2015; Biswas et al. 2017; Zhao et al. 2017). The previous studies focused on the identification of cold tolerance rice germplasm throughout the whole reproductive period and have promoted the development of stress biology in rice. However, the specific cold tolerance rice germplasm that can survive through the natural cold-winter field environment will not be given more emphasis even if China exhibited abundant cold-tolerant rice germplasm resources.

Currently, knowledge about the cold tolerance of Chinese existing rice cultivars and theirs backbone parents is still limited. In particularly, the overwintering (OW) rice germplasm is an extreme case of cold tolerance of rice, which can survive through the cold winter season and sprout from rice tillering node in the following spring, tiller, flower, seed, and being harvested in the following autumn even if the rice stubble is being exposed to the natural cold-winter field environment. The field overwintering phenomenon of OW rice will be regarded as the highest state of cold tolerance in rice production. However, the widely planted ratooning rice is a type of special *Indica* variety, which can be planted once a year but harvested twice in rice production within single year, which sprouted from the rice pile axillary after being harvested before the winter coming (Xu et al. 2015; Zhang et al. 2017).
However in this study, the OW rice germplasm exhibited significant different from the widely planted ratooning rice cultivars that couldn’t survive through the cold winter season after being exposed to the natural cold-winter field environment and couldn’t sprout from rice tillering node in the following spring even if about 0.4 million hm² ratooning rice with 4.5 t hm⁻² have been widely planted in South China (Xu et al. 2015). Recently, the genetic of OW trait in Chinese perennial Dongxiang wild rice and OW rice cultivars has been preliminary reported (Liang et al. 2018). The perennial rice variety has been successfully developed by selecting Oryza longistaminate as gene donors and commercially released to farmers (Hu et al. 2003; Zhang et al. 2014; Huang et al. 2018). However, the OW trait of Chinese rice cultivars is still unknown and has not been given more attention although approximately 2124 rice cultivars have been successfully developed and commercially released to farmers (www.ricedata.com). Currently, there is a little about the OW characteristic of Chinese existing rice cultivars has been publicly reported (Liang et al. 2021). However, the OW trait of Chinese existing rice cultivars and their backbone parents including maintainer lines, restorer lines, and Indica/Japonica conventional rice cultivars is still unknown. Therefore, it is necessary for the study on OW trait of Chinese rice cultivars even though the OW rice is a type of novel genetic and breeding germplasm. This study aims to evaluate the OW trait of Chinese existing rice cultivars for the future cold tolerance rice breeding project or even understanding the genetic mechanism of OW characteristics of rice.

Materials and methods

Rice cultivars

The 1034 Chinese rice cultivars in this study were partially obtained from the National Center for Rice Improvement, China National Rice Research Institute (CNRRI), Zhejiang Province, China, and are widely planted or applied in the hybrid rice breeding project. The 735 (71.08%) rice cultivars are conventional Japonica rice cultivars and the remaining 299 (28.92%) conventional Indica rice cultivars were backbone parents of modern commercial hybrid rice variety, including 32 (3.09%) maintainer lines, 242 (23.40%) restorer lines, and 25 (2.42%) conventional indica rice cultivars. Most of the rice seeds were provided by Professor Gong (CNRRI).

Description of field screening experiments

Rice field experiments between March 2019 and March 2021 were conducted at the Rice Biotechnology Testing Station of Chongqing Normal University (CQNU), Shapingba district, Chongqing (29°32′N, 106°32′E), P. R. China. In March 2019, the seeds of 1034 rice cultivars were sown on 13 March 2019. The 40-day-old seedlings of all tested rice were transplanted into a single row with five plants, having a 20-cm gap between plants within each row and a 25-cm gap between rows (Fig. 2a). Similarly, a parallel test was performed repeatedly in March 2020, and the seeds of 1034 rice cultivars were also sown. The 40-day-old seedlings of 1034 rice cultivars were also transplanted into a single row with five plants, having a 20-cm gap between plants within each row and a 25-cm gap between rows (Fig. 4a). The rice stubble of 1034 rice cultivars was retained in crop field after being harvested in August 2019 and 2020 and exposed to the natural field cold-winter environment for OW rice cultivar evaluated (Fig. 2b; Fig. 4b). No fertilizer was applied after being harvested, but insecticide must be applied only once to control pest throughout the cold-winter season.

A rice compound fertilizer (375 kg ha⁻¹) occupied more than 40% of the total nutrients, was made up of 18% N, 8% P₂O₅, and 14% K₂O, and was used as base fertilizer for rice. Nitrogen fertilizer (60 kg ha⁻¹, 46% N) and seedling herbicide were applied 2 weeks after transplanting. The water management strategy was adapted to shallow water at the transplanting stage and flooding mid-season with drainage-reflooding-moist intermittent irrigation. Disease, pest, and weed management were carried out at different growth stages.

Monitoring the temperature condition throughout experiment period

Winter in Chongqing including November, December, January, and February exhibited the daily minimum (Min T, °C) temperature throughout the whole year. For a long time, the daily minimum (Min T,
°C) temperature of the rice experimental field at the Rice Biotechnology Testing Station of CQNU usually occurs in December and January per year in Chongqing. Consequently, the daily minimum (Min T, °C) temperatures in both December 2019 and January 2021 were applied to the referable index on field experiment climatic conditions for OW rice germplasm evaluated. At the present field experiment, meteorological data on the daily minimum (Min T, °C) and maximum (Max T, °C) temperatures from November 2019 and 2020 to February 2020 and 2021 for OW rice cultivar evaluated were collected from the Shapingba district weather station nearby the Rice Biotechnology Testing Station of CQNU, Chongqing. The mean daily temperature and its standard derivation (SD) were calculated with the statistical software GraphPadPrism5.0, respectively. The broken line chart was drawn using Microsoft Excel 2010.

Evaluation of OW rice cultivars through the natural cold-winter environment

The rice stubbles of 1034 Chinese rice cultivars were retained in crop field after being harvested in August 2019 and 2020. And then, all tested rice stubble would be exposed to the natural cold-winter field environment from November 2019 and 2020 to March 2020 and 2021. The winter in Chongqing turns to spring in mid-February, the daily minimum (Min T, °C) temperature of Chongqing began to rise in mid-to-late February 2020 and 2021. From November 2019 and 2020 to February 2020 and 2021, the average daily minimum temperatures were 6.61 °C (December 2019) and 3.87 °C (January 2021), respectively. A significant difference exists on average the daily minimum temperatures between December 2019 and January 2021 (Fig. 1, Table 1). The average daily minimum temperature exhibited the lowest temperature of 6.61 °C in December 2019 throughout the cold-winter season. The daily minimum temperature with 4 °C occurred on December 6 and 28, 2019 and exhibited a narrow range from 4 °C on December 6 and 28 to 10 °C on December 14 and 15. Particularly in December 2019, the daily minimum temperature of 0 °C could not occur. However, the average daily minimum temperature was 3.87 °C in January 2021. Particularly in January 2021, altogether 1034 Chinese rice cultivars suffered from the cold tolerance of daily minimum temperature at 0 °C, 1 °C, and 2 °C throughout cold-winter season, the daily minimum temperature at 0 °C occurred on January 11, 12, and 17 and exhibited a wide range from 0 °C on January 11, 12 and 17 to 7 °C on January 28–31. The daily minimum temperature of 1 °C occurred on January 14,
| Dates       | November (°C) | December (°C) | January (°C) | February (°C) |
|-------------|---------------|---------------|--------------|---------------|
|             | Max T | Min T | Max T | Min T | Max T | Min T | Max T | Min T | Max T | Min T | Max T | Min T | Max T | Min T | Max T | Min T |
| 11.1        | 20    | 14    | 15    | 14    | 12.1 | 11    | 5     | 12    | 8     | 1.1  | 11    | 8     | 9     | 2.1  | 9     | 7     | 7     |
| 11.2        | 19    | 13    | 15    | 14    | 12.2 | 12    | 5     | 11    | 9     | 1.2  | 12    | 8     | 9     | 4.2  | 10    | 7     | 12    |
| 11.3        | 22    | 14    | 17    | 13    | 12.3 | 12    | 6     | 9     | 8     | 1.3  | 13    | 8     | 9     | 6.3  | 11    | 5     | 13    |
| 11.4        | 21    | 15    | 17    | 14    | 12.4 | 11    | 6     | 9     | 7     | 1.4  | 13    | 9     | 9     | 6.4  | 13    | 7     | 13    |
| 11.5        | 19    | 13    | 17    | 13    | 12.5 | 13    | 5     | 12    | 8     | 1.5  | 12    | 9     | 8     | 6.5  | 11    | 7     | 14    |
| 11.6        | 16    | 11    | 19    | 13    | 12.6 | 14    | 4     | 10    | 8     | 1.6  | 10    | 8     | 7     | 4.6  | 13    | 8     | 15    |
| 11.7        | 19    | 13    | 22    | 12    | 12.7 | 15    | 5     | 9     | 7     | 1.7  | 9     | 6     | 4     | 7.7  | 12    | 8     | 18    |
| 11.8        | 18    | 14    | 21    | 14    | 12.8 | 13    | 7     | 12    | 8     | 1.8  | 13    | 7     | 5     | 2.8  | 12    | 9     | 16    |
| 11.9        | 18    | 13    | 23    | 11    | 12.9 | 14    | 7     | 10    | 8     | 1.9  | 10    | 8     | 6     | 3.9  | 13    | 9     | 17    |
| 11.10       | 18    | 14    | 25    | 13    | 12.10| 15    | 7     | 11    | 9     | 1.10 | 12    | 8     | 5     | 2.10 | 13    | 8     | 19    |
| 11.11       | 18    | 15    | 25    | 12    | 12.11| 12    | 8     | 10    | 8     | 1.11 | 10    | 7     | 8     | 0.11 | 17    | 9     | 20    |
| 11.12       | 17    | 12    | 24    | 11    | 12.12| 14    | 8     | 11    | 9     | 1.12 | 14    | 4     | 10    | 0.12 | 21    | 11    |
| 11.13       | 15    | 11    | 24    | 12    | 12.13| 14    | 8     | 9     | 4     | 1.13 | 14    | 7     | 11    | 2.13 | 19    | 11    |
| 11.14       | 19    | 9     | 24    | 13    | 12.14| 12    | 10    | 7     | 6     | 1.14 | 11    | 9     | 13    | 1.14 | 19    | 3     | 20    |
| 11.15       | 18    | 12    | 24    | 13    | 12.15| 13    | 10    | 6     | 5     | 1.15 | 9     | 7     | 13    | 2.15 | 7     | 13    |
| 11.16       | 17    | 10    | 24    | 15    | 12.16| 14    | 8     | 8     | 6     | 1.16 | 9     | 7     | 9     | 2.16 | 13    | 3     | 17    |
| 11.17       | 15    | 6     | 24    | 14    | 12.17| 13    | 7     | 8     | 5     | 1.17 | 9     | 7     | 7     | 0.17 | 13    | 6     | 19    |
| 11.18       | 14    | 7     | 21    | 14    | 12.18| 8     | 6     | 8     | 5     | 1.18 | 11    | 6     | 10    | 1.18 | 10    | 7     | 17    |
| 11.19       | 11    | 8     | 23    | 14    | 12.19| 10    | 7     | 7     | 3     | 1.19 | 10    | 6     | 12    | 1.19 | 13    | 7     | 20    |
| 11.20       | 12    | 7     | 19    | 10    | 12.20| 9     | 7     | 10    | 4     | 1.20 | 12    | 8     | 10    | 4.20 | 10    | 8     | 22    |
| 11.21       | 15    | 8     | 15    | 7     | 12.21| 9     | 6     | 11    | 4     | 1.21 | 11    | 7     | 9     | 6.21 | 14    | 8     | 24    |
| 11.22       | 14    | 11    | 8     | 7     | 12.22| 12    | 6     | 8     | 6     | 1.22 | 11    | 7     | 14    | 5.22 | 13    | 11    |
| 11.23       | 15    | 11    | 9     | 8     | 12.23| 12    | 7     | 10    | 5     | 1.23 | 14    | 9     | 15    | 5.23 | 11    | 10    | 24    |
| 11.24       | 12    | 6     | 9     | 9     | 12.24| 12    | 7     | 8     | 5     | 1.24 | 13    | 9     | 12    | 8.24 | 12    | 10    | 27    |
| 11.25       | 11    | 6     | 10    | 9     | 12.25| 11    | 7     | 8     | 6     | 1.25 | 9     | 7     | 11    | 6.25 | 17    | 12    | 18    |
| 11.26       | 10    | 8     | 13    | 10    | 12.26| 9     | 5     | 8     | 4     | 1.26 | 8     | 6     | 10    | 1.26 | 17    | 11    |
| 11.27       | 12    | 8     | 12    | 9     | 12.27| 7     | 5     | 11    | 7     | 1.27 | 11    | 6     | 10    | 1.27 | 13    | 11    |
| 11.28       | 12    | 9     | 11    | 7     | 12.28| 7     | 4     | 9     | 5     | 1.28 | 11    | 6     | 12    | 1.28 | 20    | 7     | 11    |
| 11.29       | 12    | 8     | 12    | 8     | 12.29| 13    | 7     | 7     | 4     | 1.29 | 12    | 5     | 11    | 7.29 | 19    | 10    |
| 11.30       | 11    | 6     | 11    | 9     | 12.30| 10    | 8     | 6     | 5     | 1.30 | 14    | 4     | 12    | 1.30 | 19    |
17, and 18, and the daily minimum temperature of 2 °C occurred on January 1, 7, 8, 10, 13, 15, and 16. The daily minimum temperature in January 2021 exhibited the biggest coefficient variation (CV) value of 63.80%. Screening pressure on the daily minimum temperature in January 2021 is significant higher than that of December 2019. Consequently, the data on OW rice germplasm screened under low-temperature climate in January 2021 throughout the cold-winter season was more reliable than that in December 2019.

Evaluation the capacity cold tolerance of OW rice cultivars in winter season 2019

Altogether 1034 rice cultivars have been exposed to the natural field cold-winter environment after being harvested from November 2019 to February 2020 (Fig. 2b). Among them, 262 (25.34%) Japonica rice cultivars could withstand cold damage of 4 °C of the daily minimum temperature on December 6 and 28, 2019 (Fig. 1; Table 2), which could survive through the natural cold-winter field environment, stay strong activity of stems and leaves, and sprout from rice tillering node in March 2020 (Fig. 2b–d). The remaining 772 rice cultivars with the withered root system could not sprout from rice tillering node in March 2020 and might not carry the gene or QTLs controlling the OW trait. Among of them, there no Indica rice cultivars were found to withstand cold tolerance to 4 °C.

A total of 262 OW rice cultivars are distributed in 13 different provinces or cities of China and range from 2 (0.76%) in Heilongjiang province to 65 (24.81%) in Liaoning province (Fig. 3, Table 3). Of these, the cultivated rice from Liaoning had 65 (24.81%) rice cultivars and exhibited the highest percentage of OW rice cultivars with tolerance to 4 °C. Rice cultivars in provinces of Jiangsu, Jilin, Tianjin, and Yunnan exhibiting 41 (15.65%), 29 (11.07%), 28 (10.68%), and 24 (9.16%), respectively, provide a relative higher percentage of OW rice cultivars with tolerance to 4 °C. Rice cultivars in the provinces of Beijing, Hubei, and Heilongjiang provided a relatively lower percentage of rice cultivars with tolerance to 4 °C and exhibited 4 (1.53%), 4 (1.53%), and 2 (0.76%), respectively. In particular, the province of Heilongjiang lies in the northeast of China.
Fig. 1 Daily maximum (MaxT, °C) and minimum temperature (MinT, °C) in December 2019 and January 2021

Fig. 2 OW rice cultivar evaluated from spring 2019 to spring 2020. a Field performance of 1034 rice cultivars transplanted in May 2019; b Field performance of 1034 rice cultivars in December 2019; c, d OW rice cultivars germinated in March and April 2020
Table 2  OW rice cultivar with cold tolerance to -4 °C of the daily minimum temperature in December 2019

| No | Cultivars | Provinces | No | Cultivars | Provinces | No | Cultivars | Provinces | No | Cultivars | Provinces |
|----|-----------|-----------|----|-----------|-----------|----|-----------|-----------|----|-----------|-----------|
| 1  | Nao10#    | Anhui     | 67 | Changnong4# | Jiangsu  | 133 | Qianchonglong2# | Liaoning |
| 2  | Zhongjing8612# | Anhui | 68 | Wujing120# | Jiangsu  | 134 | Yaifeng57 | Liaoning |
| 3  | R96-2#    | Anhui     | 69 | Wumo8333 | Jiangsu  | 135 | Hanjing2# | Liaoning |
| 4  | R96-2#    | Anhui     | 70 | Wujing13# | Jiangsu  | 136 | Zhaongfang60-93 | Liaoning |
| 5  | Guanjiao102# | Anhui | 71 | Wujing14# | Jiangsu  | 137 | Xiangfeng180 | Liaoning |
| 6  | Huaping102# | Anhui | 72 | Wujing15# | Jiangsu  | 138 | Shemong9017 | Liaoning |
| 7  | Danyu11# | Anhui     | 73 | Wujing16# | Jiangsu  | 139 | Liangou10# | Liaoning |
| 8  | Wangjing1# | Anhui     | 74 | Wujing17# | Jiangsu  | 140 | Liangou45# | Liaoning |
| 9  | Wangjing1# | Anhui     | 75 | Wujing22# | Jiangsu  | 141 | Liangou9# | Liaoning |
| 10 | Wangjing1# | Anhui     | 76 | Wujing23# | Jiangsu  | 142 | Dongming801 | Liaoning |
| 11 | Wangjing1# | Anhui     | 77 | Wujing24# | Jiangsu  | 143 | Liangou Import | Liaoning |
| 12 | Wangjing1# | Anhui     | 78 | Wujing25# | Jiangsu  | 144 | Liangou207 | Liaoning |
| 13 | Wangjing1# | Anhui     | 79 | Wujing26# | Jiangsu  | 145 | Liangou278 | Liaoning |
| 14 | Wangjing1# | Anhui     | 80 | Wujing28# | Jiangsu  | 146 | Liangou31 | Liaoning |
| 15 | Wangjing1# | Anhui     | 81 | Wujing29# | Jiangsu  | 147 | Liangou362 | Liaoning |
| 16 | Wangjing1# | Anhui     | 82 | Wujing30# | Jiangsu  | 148 | Liangou395 | Liaoning |
| 17 | Wangjing1# | Anhui     | 83 | Wujing31# | Jiangsu  | 149 | Liangou427 | Liaoning |
| 18 | Wangjing1# | Anhui     | 84 | Wujing32# | Jiangsu  | 150 | Liangou453 | Liaoning |
| 19 | Wangjing1# | Anhui     | 85 | Wujing33# | Jiangsu  | 151 | Liangou478 | Liaoning |
| 20 | Wangjing1# | Anhui     | 86 | Wujing34# | Jiangsu  | 152 | Liangou497 | Liaoning |
| 21 | Wangjing1# | Anhui     | 87 | Wujing35# | Jiangsu  | 153 | Liangou502 | Liaoning |
| 22 | Wangjing1# | Anhui     | 88 | Wujing36# | Jiangsu  | 154 | Liangou507 | Liaoning |
| 23 | Wangjing1# | Anhui     | 89 | Wujing37# | Jiangsu  | 155 | Liangou512 | Liaoning |
| 24 | Wangjing1# | Anhui     | 90 | Wujing38# | Jiangsu  | 156 | Liangou517 | Liaoning |
| 25 | Wangjing1# | Anhui     | 91 | Wujing39# | Jiangsu  | 157 | Liangou521 | Liaoning |
| 26 | Wangjing1# | Anhui     | 92 | Wujing40# | Jiangsu  | 158 | Liangou526 | Liaoning |
| 27 | Wangjing1# | Anhui     | 93 | Wujing41# | Jiangsu  | 159 | Liangou531 | Liaoning |
| 28 | Wangjing1# | Anhui     | 94 | Wujing42# | Jiangsu  | 160 | Liangou536 | Liaoning |
| 29 | Wangjing1# | Anhui     | 95 | Wujing43# | Jiangsu  | 161 | Liangou541 | Liaoning |
| 30 | Wangjing1# | Anhui     | 96 | Wujing44# | Jiangsu  | 162 | Liangou546 | Liaoning |
| 31 | Wangjing1# | Anhui     | 97 | Wujing45# | Jiangsu  | 163 | Liangou551 | Liaoning |
| 32 | Wangjing1# | Anhui     | 98 | Wujing46# | Jiangsu  | 164 | Liangou556 | Liaoning |
| 33 | Wangjing1# | Anhui     | 99 | Wujing47# | Jiangsu  | 165 | Liangou561 | Liaoning |
| 34 | Wangjing1# | Anhui     | 100| Wujing48# | Jiangsu  | 166 | Liangou566 | Liaoning |
| No. | Cultivars | Provinces |
|-----|-----------|-----------|
| 35  | E3#       | Hubei     |
| 36  | E4#       | Jiangsu   |
| 37  | Baoting1# | Jiangsu   |
| 38  | Changxu10#| Jilin     |
| 39  | Dongdao50/86# | Jilin     |
| 40  | Jiu53#    | Jilin     |
| 41  | Jiu6755#  | Jilin     |
| 42  | Jiu61410# | Jilin     |
| 43  | Jiu6134#  | Jilin     |
| 44  | Jiu6128#  | Jilin     |
| 45  | Jiu6127#  | Jilin     |
| 46  | Jiu6126#  | Jilin     |
| 47  | Jiu6125#  | Jilin     |
| 48  | Jiu6124#  | Jilin     |
| 49  | Jiu6123#  | Jilin     |
| 50  | Jiu6122#  | Jilin     |
| 51  | Jiu6121#  | Jilin     |
| 52  | Jiu6120#  | Jilin     |
| 53  | Jiu6119#  | Jilin     |
| 54  | Jiu6118#  | Jilin     |
| 55  | Jiu6117#  | Jilin     |
| 56  | Jiu6116#  | Jilin     |
| 57  | Jiu6115#  | Jilin     |
| 58  | Jiu6114#  | Jilin     |
| 59  | Jiu6113#  | Jilin     |
| 60  | Jiu6112#  | Jilin     |
| 61  | Jiu6111#  | Jilin     |
| 62  | Jiu6110#  | Jilin     |
| 63  | Jiu6109#  | Jilin     |
| 64  | Jiu6108#  | Jilin     |
| 65  | Jiu6107#  | Jilin     |
| 66  | Jiu6106#  | Jilin     |

(Continued)
but only provides two (0.76%) OW rice cultivars. However, the province of Jiangsu lies in the south of China but provides the second-highest percentage of rice cultivars with tolerance to 4 °C. In summary, altogether 262 OW rice cultivars exhibited a strong tolerance to 4 °C throughout the growth stage under the natural cold-winter field environment.

**Evaluation the capacity cold tolerance of OW rice cultivars in winter season 2020**

Altogether 1034 rice cultivars have been exposed repeatedly to the natural field cold-winter environment after being harvested from November 2020 to February 2021 (Fig. 4a). Among them, only 24 (2.32%) Japonica rice cultivars could withstand cold damage of 0 °C of the daily minimum temperature on January 11, 12, and 17, 2021 and display withering stems and leaf (Fig. 4b–f). To our surprised, twenty-four OW Japonica rice cultivars could sprout from rice tillering node in March 2021 due to theirs strong root system activity (Fig. 4b–f, Table 4). However, 1010 (97.68%) rice cultivars with the withered root system could not withstand cold damage at 0 °C of the daily minimum temperature in January 2021 and not sprout from rice tillering node in March 2021 (Fig. 4b–f). No Indica rice cultivars were also found to withstand the cold tolerance to 0 °C.

Only 24 OW Japonica rice cultivars are distributed in seven different provinces or cities of China and range from 1 (4.16%) in Jilin province to 5 (20.83%) in Zhejiang province (Fig. 5, Table 4). Of these, the rice germplasm from Zhejiang province had 5 (20.83%) OW rice cultivars and exhibited the first highest percentage of OW rice germplasm with cold tolerance to 0 °C for three days. Rice germplasm in provinces of Anhui, Jiangsu, and Tianjin provides a relatively higher tolerance to 0 °C and displayed 4 (16.67%) OW rice cultivars in each province. Compared with the 65 (24.81%) OW rice cultivars in Liaoning province with cold tolerance to 4 °C in 2020, only 3 (12.5%) OW rice cultivars could withstand cold damage at 0 °C for three days in 2021. This result indicates that OW rice cultivars could withstand the cold damage at 0 °C for three days in January 2021 due to the strong root system activity throughout the growth stage under the natural cold-winter field environment. OW rice cultivars are novel rice germplasm for future cold tolerance rice breeding and might provide a new strategy involved in elucidating the molecular mechanism of cold tolerance rice.

**Discussion**

Ratooning rice is well known as a special rice cultivation mode, once planted and harvested twice, is of great significance for increasing grain yield, ensuring
national food security and improving rice field utilization efficiency (Hhilleris Lamber 1988; Song et al. 2020). In China, there has a 1600 years of history that Chinese farm have planted ratooning rice for high grain yield based on the rice regeneration characteristics in crop production (Xiong et al. 2000; Lin et al. 2015). Currently, the study on the genetics and breeding of ratoon rice and its cultivation technology has been reported. The genetic of ratooning ability in inter-subspecies crosses of rice was quite independent in inheritance (Yan et al. 1992). Altogether fourteen QTLs affecting ratooning ability were detected on chromosomes 1, 3, 4, 5, 6, 7, 8, and 11 based on the field phenotypic data on regenerative character before the winter coming (Tan et al. 1997; Zheng et al. 2004; Yang et al. 2012; Li et al. 2016). The yield formation of ratooning rice has been determined by the germination ability of axillary buds of first cropping rice (Pu et al. 2018; Xu et al. 2019). The germination ability of axillary buds in ratooning rice was affected by multi-factors including different rice varieties (Duan et al. 2018; Lin et al. 2019), irrigation pattern (Lin et al. 2015), Nutrient (Chen et al. 2014, 2017), rice stubble height (Yi et al. 2009; Lian et al. 2017), temperature and light conditions (Lin et al. 2015), and hormones (Jia 2015; Huang et al. 2017). These previous studies have promoted the development of ratooning rice. However, the widely planted ratooning rice is a type of special hybrid rice variety, which sprouted from the rice pile axillary after the rice being harvested in crop field and planted once a year but harvested twice in rice production within single year (Xu et al. 2015). The widely planted ratooning rice couldn’t survive through the cold-winter season under being exposed to the natural cold-winter field environment; exhibit withering roots, stems, and leaves before the winter coming.

The overall aim of this study was to evaluate the OW characteristic of the existing rice cultivars throughout China for future studies on the genetic mechanism of OW rice or even OW rice variety breeding. In rice production, cold damage is one of the most important abiotic stress factors that restrict the production and development of rice. Twenty-four countries throughout the globe, including China, Japan, and Korea, have suffered from the loss of cold damage in rice production (Zhang et al. 2017). Consequently, breeding a cold tolerance rice variety will be the future important direction of rice genetics and

| Provinces | Anhui | Beijing | Henan | Heilongjiang | Hubei | Jilin | Jiangsu | Liaoning | Ningxia | Shanxi | Tianjin | Yunnan | Zhejiang | Total |
|-----------|-------|---------|-------|-------------|-------|------|---------|---------|--------|--------|--------|--------|---------|-------|
| Numbers   |       |         |       |             |       |      |         |         |        |        |        |        |         |       |
| %         |       |         |       |             |       |      |         |         |        |        |        |        |         |       |
| No        | 15    | 4       | 11    | 2           | 4     | 2    | 4       | 2       | 6      | 15     | 11     | 24     | 48     | 262   |
| 5.73      | 1.53  | 4.20    | 0.76  | 1.53        | 1.53  | 1.53 | 0.76    | 1.53    | 1.53   | 1.53   | 1.53   | 1.53   | 100    |
breeding. However, identifying precisely the cold tolerance rice germplasm is one of the most important steps for the cold tolerance rice variety breeding (Glaszmann et al. 2010; Zhao et al. 2011).

Nowadays, several genes underlying cold tolerance rice have been cloned and functionally identified based on the phenotypic data of cold tolerance at the individual growth stage of rice (Liu et al. 2018). However, whether the presently cloned genes underlying the cold tolerance trait according to the cold tolerance phenotypic data at the individual growth period of rice are involved in regulating the OW performance in OW rice germplasm is still a debatable topic. Particularly, the \textit{COLD1} has been cloned and functionally identified using the phenotypic data on cold tolerance at the seedling stage of Japonica rice variety Nipponbare (Ma et al. 2015). However, the \textit{COLD1} could not provide a perfect explanation of OW field performance of rice because the Nipponbare variety cannot survive through the natural cold-winter field environment at Chongqing, Southwest China. Our knowledge about the phenotypic relationship between the OW field phenomenon of rice and the cold tolerance of rice at several different stages of germination, seedling, booting, and mature growth is still limited. Currently, we still understand little about the cold tolerance at several different stages of germination, seedling, booting, and mature growth of all rice cultivars throughout China, particularly for the OW trait of the evaluated rice cultivars. In this study, we examined the OW trait of Chinese rice cultivars under the natural cold-winter field environment. Only 24 (2.32%) Japonica rice cultivars could withstand cold damage at 0 °C for three days in January 2021, could sprout from rice tillering node in March 2021, and

\textbf{Fig. 4} OW rice cultivar evaluated from spring 2020 to spring 2021. \textit{a} Field performance of 1034 rice cultivars transplanted in April 2020; \textit{b–f} OW rice sprouted from tillering node March and April 2021.
exhibit a strong root system activity throughout the natural cold-winter season. No OW germplasm with indica type was investigated to sprout from rice tillering node March 2021, and the present study indicated that no gene or QTLs are distributed on 12 chromosomes of indica rice cultivars throughout China. The cold tolerance of rice and OW phenomenon of rice might be determined by the strong root system activity. Consequently, future studies on the cold tolerance of rice should put more emphasis on the genetic root system activity (He et al. 1996; Zhang et al. 2001; Liang et al. 2013). In summary, the present cold tolerance rice cultivars will provide some beneficial rice germplasm for future cold tolerance breeding and propose new strategies in elucidating the molecular mechanism of the cold tolerance in rice.

**Table 4** OW rice cultivar with cold tolerance to 0 °C of the daily minimum temperature in January 2021

| Provinces | Cultivars            | Numbers | %     |
|-----------|----------------------|---------|-------|
|           |                      | No      |       |
| Anhui     | Wandao90             | 4       | 16.67 |
|           | Huangnuo2            |         |       |
|           | Wanda082             |         |       |
|           | WangengM1148         |         |       |
| Hubei     | Ewan15              | 3       | 12.50 |
|           | Ewan17              |         |       |
|           | Ewan13              |         |       |
| Jiangsu   | Changnonggeng3       | 4       | 16.67 |
|           | Suxianggeng2        |         |       |
|           | Sugeng8             |         |       |
|           | Zhendao7            |         |       |
| Jilin     | Changbai7           | 1       | 4.16  |
| Liaoning  | Tiegeng4            | 3       | 12.50 |
|           | laoyan241           |         |       |
|           | Shendao3            |         |       |
| Tianjin   | Jindaol187          | 4       | 16.67 |
|           | Jinyou2006          |         |       |
|           | Jinyuan5            |         |       |
|           | Jingengza4          |         |       |
| Zhejiang  | Zhenuo36            | 5       | 20.83 |
|           | Xiushui42           |         |       |
|           | Xiushui52           |         |       |
|           | Zhegeng23           |         |       |
|           | Jiashao2            |         |       |
| Total     | 24                  | 100     |       |

**Fig. 5** Geographic distribution of OW rice cultivars with cold tolerance of 0 °C in January 2021

**Conclusion**

Altogether 1034 Chinese rice cultivars including 735 (71.08%) conventional Japonica rice cultivars and 299 (28.92%) conventional Indica rice cultivars were collected and evaluated for their responses to low temperatures under the natural field cold-winter environment. Among them, altogether 262 (25.34%) conventional Japonica rice cultivars could withstand cold tolerance to 4 °C of the daily minimum temperatures in December 2019 throughout the cold-winter season and distributed in 13 provinces of China, survive through the natural cold-winter field environment, and sprout from rice tillering node in March 2020. Only 24 (2.32%) conventional japonica rice cultivars with cold tolerance to 0 °C of the daily minimum temperatures in January 2021 throughout the cold-winter season and distributed in seven provinces of China could also sprout from rice tillering node in March 2021, tiller, flower, seed, and being harvested in the following autumn. The field phenomenon of OW trait is a type of an extreme case of cold tolerance of rice. The present cold tolerance rice cultivars will provide beneficial breeding germplasm for the future cold tolerance rice breeding project and new strategies involved
in elucidating the molecular mechanism of the cold tolerance of rice.

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Authors’ contribution YL designed the whole experiment and carried out the study on the evaluation for OW rice cultivars, screened the overwintering cultivated rice, data analysis, and writing the manuscript. Most of rice seeds were provided by Professor Gong (CNRRI). Seven authors of YY, BW, WG, HW, QW, WN, and XQ helped to sow seed and transplant rice seedling over two years and investigate the OW trait of 1034 Chinese existing rice cultivars. HZ was in charge of the direction of research.

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Data availability Data is available, but material is not available.

Declarations

Conflict of interest The authors declare that they have no Conflict of interests.

Consent for publication Written informed consent for publication was obtained from all participants.

Ethical approval All analyses were based on the OW rice cultivars, thus no ethical approval and participant’s consent are required in this manuscript.

References

Andaya V, Mackill D (2003) QTLs conferring cold tolerance at the booting stage of rice using recombinant inbred lines from a japonica×indica cross. Theor Appl Genet 106(6):1084–1090. https://doi.org/10.1007/s00122-002-1126-7

Biswas PS, Khatun H, Das N, Sarkar MM, Anisuzzaman M (2017) Mapping and validation of QTLs for cold tolerance at seedling stage in rice from an indica cultivar Habigangan Boro VI (Hbji.BVI), 3 Biotechnol 7(6):359. https://doi.org/10.1007/s13205-017-0993-1

Chen HF, Zhang ZX, Lin WX (2014) Effects of nitrogen application for bud development on protein expression of ratooning buds of rice. Chin J Eco-Agric 22(12):1405–1413 (In Chinese)

Chen HF, Pang XM, ZhangR ZZX, Xu QH, Fang CX, Li JY, Lin WX (2017) Effects of different water and fertilizer applications on soil enzyme activities and microbial functional diversity in regenerated rice rhizosphere. Acta Agron Sinica 43(10):1507–1517 (In Chinese)

Cheng SH (2010) Chinese super rice breeding. Science Press, Beijing, China (In Chinese)

Dai LY, Lin XH, Ye CR, Ise K, Saito K, Kato A, Xu FR, Yu TQ, Zhang DP (2004) Identification of quantitative trait loci controlling cold tolerance at the reproductive stage in Yunnan landrace of rice, Kunming Xiaobaiyu. Breed Sci 54(3):253–258. https://doi.org/10.1270/jsbs.54.253

Duan MJ, Wu YZ, Tian YC, Liu YW, Liu ZY, Chen F, Jin T (2018) Comparative study on yield and quality of different varieties of ratooning rice. Crops 2:61–67 (In Chinese)

Fairhurst TH, Dobermann A (2002) Rice in the global food supply. Better Crops Int 16:3–6

Glaszmann JC, Kilian B, Upadhyaya HD, Varshne RK (2010) Accessing genetic diversity for crop improvement. Curr Opin Plant Biol 13:1–7. https://doi.org/10.1016/j.plist.2010.01.004

He GC, Shu LH, Zhou YQ, Liao LJ (1996) The overwintering ability of Dongxiang wild rice (Oryza rufipogon) at Wuhan. J Wuhan Univ 42:252–254 (In Chinese)

Hilleris Lambers D (1988) Varietal improvement for rice rationing: traits, procedures, collaboration rice rationing. Rice Ratooning 8:247–255

Hu FY, Tao DY, Sacks E, Fu BY, Xu P, Li J, Yang Y, McNelly K, Khush GS, Paterson AH (2003) Convergent evolution of perenniality in rice and sorghum. Proc Natl Acad Sci 100(7):4050–4054. https://doi.org/10.1073/pnas.0630531100

Huang XJ, Tu NM, Yi ZX, Zhou WX (2017) Effects of different hormone treatments on yield and related physiological characteristics of ratoon rice. Hybrid Rice 32(4):71–75 (In Chinese)

Huang GF, Qin SW, Zhang SL, Cai XL, Wu SK, Dao JR, Zhang J, Hu FY (2018) Performance, economics and potential impact of perennial rice PR23 relative to annual rice cultivars at multiple locations in Yunnan Province of China. Sustainability 10(4):1086. https://doi.org/10.3390/su10041086

Jia XR (2015) Effects of spraying gibberellin on regeneration rice sprouts and yield in different periods. Modern Agric Sci Technol 14:124–125 (In Chinese)

Kuroki M, Saito K, Matsuba S, Yokogami N, Shimizu H, Ando J, Ise K, Sato Y (2009) Quantitative trait locus analysis for cold tolerance at the booting stage in a rice cultivar, Hatsushiku, Jpn Agric Res Q 43(2):115–121. https://doi.org/10.1073/pnas.063051100

Li XX, Zheng J, Zhou JJ, Yang YQ, Qin XJ, Zhang HM, Liang YS (2016) QTL analysis of rice regenerative ability using recombinant inbred lines from a japonica×indica cross. Mol Plant Breed 14(9):2383–2391 (In Chinese)

Lian H, Zhou HT, Chen WJ, Li GH, Li MJ, Zhou GH, Sheng JH, Li HX, Xiao CX, Xu YL (2017) Effects of sowing date and height of piles on yield and yield components of ratoon rice. Hunan Agric Sci 4:28–31 (In Chinese)

Liang YS, Zhan XD, Wang HM, Gao QZ, Lin ZC, Chen DB, Shen XH, Cao LY, Cheng SH (2013) Locating QTLs controlling several adult root traits in elite Chinese hybrid rice. Gene 526(2):331–335. https://doi.org/10.1016/j.gene.2013.04.010
resistance in rice: comparative mapping within and across species. Theor Appl Genet 103:19–29. https://doi.org/10.1007/s001220000534

Zhang S, Wang WS, Zhang J, Huang WQ, Xu P, Tao D, Hu FY (2014) The progression of perennial rice breeding and genetic research in China. In: Batello G, Wade LJ, Cox TS, Pogna N, Bozzini A, Chopianty J (eds) Perennial crops for food security. FAO, Rome, pp 27–38

Zhang ZY, Li JJ, Pan YH, Li JL, Zhou L, Shi HL, Zeng YW, Guo HF, Yang SM, Zheng WW, Yu JP, Sun XM, Li GL, Ding YL, Ma L, Shen SQ, Dai YL, Zhang HL, Yang SH, Guo Y, Li ZC (2017) Natural variation in CTB4a enhances rice adaptation to cold habitats. Nat Commun 8:14788. https://doi.org/10.1038/ncomms14788

Zhao KK, Tung CW, Eizenga GC, Wright MH, Ali LM, Price AH, Norton GJ, Islam MR, Reynolds A, Mezey J, McClung AM, Bustamante CD, McCouch SR (2011) Genome-wide association mapping reveals a rich genetic architecture of complex traits in Oryza sativa. Nat Commun 2:1–10. https://doi.org/10.1038/ncomms1467

Zhao JL, Zhang SH, Dong JF, Yang TF, Mao XX, Liu Q, Wang XF, Liu B (2017) A novel functional gene associated with cold tolerance at the seedling stage in rice. Plant Biotechnol J 15(9):1141–1148. https://doi.org/10.1111/pbi.12704

Zhang JS, Li YZ, Lin WX (2004) Identification of QTL for ratooning ability and grain yield traits in ratoon rice based on SSR marker. Mol Plant Breed 2(3):342–347 (In Chinese)

Zhou L, Zeng YW, Zheng WW, Tang B, Yang SM, Zhang HL, Li JJ, Li ZC (2010) Fine mapping a QTL qCTB7 for cold tolerance at the booting stage on rice chromosome 7 using a near-isogenic line. Theor Appl Genet 121(5):895–905. https://doi.org/10.1007/s00122-010-1358-x

Zhu YJ, Chen K, Mi XF, Chen TX, Ali J, Ye GY, Xu JL, Li ZK (2015) Identification and fine mapping of a stably expressed QTL for cold tolerance at the booting stage using an interconnected breeding population in rice. PLoS ONE 10(12):e0145704. https://doi.org/10.1371/journal.pone.0145704

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