Application of vigor indexes to evaluate the cold tolerance in rice seeds germination conditioned in plant extract

Sheila Bigolin Teixeira¹, Stefânia Nunes Pires², Gabriele Espinel Ávila², Bruna Evelyn Paschoal Silva², Victoria Novo Schmitz², Cristiane Deuner¹, Rodrigo da Silva Armesto³, Diogo da Silva Moura⁴ & Sidnei Deuner²*¹

Rice is a crop that presents sensitivity to cold, especially in the germination phase, which leads to high economic losses. Alternative management forms are essential to increase tolerance to low temperatures, and seed priming represents a promising tool. The objective of this study was to investigate the priming effect of the aqueous extract of carrot roots on rice seeds to increase tolerance to low temperatures during germination. Seeds from cultivars BRS Querência (cold-susceptible) and Brilhante (cold-tolerant) were soaked for 24 h in concentrations of 0, 25, 50, and 100% carrot extract, sown on germitest paper and conditioned in BOD for 21 days at 15 °C. As a control, the seeds soaked in water were also germinated at 25 °C. They were evaluated for germination, first germination count, and germination speed index to calculate the stress indices: tolerance index, susceptibility index, and harmonic mean. They were also evaluated for the length and dry mass of shoot and root. The results showed that the rice seeds conditioning in carrot extract effectively reduces the damage caused by cold, significantly increasing the germination speed and the percentage of final germination and the growth evaluations, more expressive at 100% concentration. The stress indexes are efficient in estimating the tolerance of the cultivars and the effect of the different conditions in low-temperature conditions, highlighting the superiority of the Brilhante cultivar.

Plants can suffer limitations in development and growth due to environmental changes, including extreme events such as droughts, floods, high or low temperatures. Such adverse conditions cannot be controlled, so crop losses are considered inevitable. Studies suggest that more than 60% of the fluctuations in the annual yield of essential crops are attributed to climatic variability. Food security and biodiversity conservation are essential issues discussed to assess decisions to increase agricultural production through sustainable intensification. Studies indicate that the world population will exceed nine billion by 2050, and from this scenario, it is necessary to increase food production, quantity, and quality, to meet the nutritional needs of the world population.

Rice is one of the essential foods since it is grown in more than 100 countries and often consumed by more than 3.5 billion people worldwide, a number that represents almost half of the world population. This culture can be grown in different environments depending upon water availability and temperature conditions. However, low temperature is a significant factor limiting rice growth and yield, and the seedling is one of the developmental stages at which sensitivity to cold stress is higher, being a recurrent problem in several regions of the world, including Brazil, mainly in the southern area of Rio Grande do Sul (RS), state responsible for more than 70% of national production. Still, globally, more than 15 million ha of rice is damaged by the low temperature at the seedling and reproductive stages. Because of low temperature, nearly 7 million ha of potential rice land in South and Southeast Asia remain unplanted, dominated by cultivars of the indica subspecies that possess tropical origin being very sensitive to low temperature.

¹Postgraduate Program in Seed Science and Technology, Eliseu Maciel Faculty of Agronomy, Federal University of Pelotas, Pelotas, Rio Grande do Sul 96010-900, Brazil. ²Postgraduate Program in Plant Physiology, Department of Botany, Institute of Biology, Federal University of Pelotas, Pelotas, Rio Grande do Sul 96010-900, Brazil. ³Eliseu Maciel Faculty of Agronomy, Federal University of Pelotas, Pelotas, Rio Grande do Sul 96010-900, Brazil. ⁴Riograndense Higher Education Center, Marau, RS 99150-000, Brazil. *email: sdeuner@yahoo.com.br
As reported by, plants are classified by the intrinsic tolerance level to low temperatures. Rice cultivars of the subspecies japonica present more tolerance to cold than those of the subspecies indica, due to their origin in places with different climatic characteristics, being the cold tolerance the immediate solution to damage. The great majority of rice cultivars used to come from tropical origin, so the optimum temperature of cultivation is between 20 and 35°C, varying accordingly to the stage of crop development. For this reason, temperatures below 20°C are a limiting factor in a large part of the crop cycle, mainly in the germination, seedling development, and reproductive stages were depending on the stress duration, the damage can be very severe for the culture.

In the State of Rio Grande do Sul (RS), low winter temperatures often extend into the spring during September and October, timing in which many producers carry out sowing so that the reproductive stage coincides with the highest solar radiation months, December and mainly January. Consequently, seed germination and seedling’s emergence can be delayed by more than 20 days, especially in the most sensitive cultivars. The irrigation water temperature is another factor that affects the irrigated rice from the initial irrigation phase until the beginning of the panicle formation. The crop is more affected by the water temperature than the air.

In order to obtain a higher percentage of germination and plant establishment speed, particularly under adverse climatic conditions, has been used the technique of seed priming. This technique has been well studied, mainly using water or solutions with known osmotic potentials. However, there is little study related to using a substance that aggregates desired characteristics to seeds, such as plant extracts. Many plants are rich in bioactive compounds with high antioxidant potential, which minimizes the effects of stress on plants by preventing oxidative damage caused by reactive oxygen species. Among the plants with high antioxidant capacity, carrots are stand out, mainly due to the significant amount of carotenoids and phenolic compounds and the presence of tocopherols, nitrogen compounds, and vitamins, among others. These facts seem to suggest that extracts from many different kinds of plant materials contain typical growth-promoting substances, which may be involved in the mechanism of induction of growth in various kinds of plant tissue.

Therefore, the objective of this study was to investigate the priming effect of the aqueous extract of carrot roots on rice seeds in response to tolerance to low temperatures during germination. Vigor indexes were analyzed in order to obtain this data.

### Material and methods

The experiment was conducted in a completely randomized design, in a factorial arrangement with four replications; factor A included two rice cultivars: BRS Querência (ssp. Indica: cold-susceptible) and Brilhante (ssp. Japonica: cold-tolerant) and factor B, included four treatments for seed conditioning: 0, 25, 50, and 100% aqueous carrot extract. All methods were carried out in accordance with relevant regulations and guidelines.

Seeds of the rice were disinfected with sodium hypochlorite (2% active chlorine) for 5 min and then placed in beakers containing solutions of 25%, 50%, and 100% aqueous carrot extract, and kept under soaking for 24 h at 15°C. Two hundred and fifty grams of carrot roots of the cultivar Kuronan, which has cylindrical roots with a uniform dark-orange coloration, were processed in a centrifuge (model Mondial Premium 700 W) at room temperature, obtaining a volume of 100 mL of crude extract. The extract was prepared on the day of its use, and the concentration of carrot extract was diluted with ultra-pure water (v/v). The resulting extract was diluted in ultra-pure water to obtain the different established percentages, being characterized by pH, osmotic potential (Ψs), electrical conductivity (EC), and β-carotene content (Table 1).

After soaking, the seeds were sown on “germist” paper moistened with water to a volume of 2.5 times their weight and were kept in a germinating chamber regulated at 15°C with a photoperiod of 12 h. As a control, seeds were soaked in distilled water (0%), germinated at 15°C and 25°C, forming the negative and positive control, respectively.

The germination test was conducted with 200 seeds (four subsamples of 50 seeds), as specified by the Rules for Seed Analysis. The first germination count (FGC) was carried out together with the germination test and evaluated on the tenth day after sowing. The germination speed index (GSI) was also carried out simultaneously with the germination test by counting the number of normal seedlings with seeds with a root extension equal to or greater than 2 mm until the number of normal seedlings remained constant. However, the root and shoot length (RL and SL) were determined after 21 days from the beginning of the test because a low temperature was applied (15°C), and the development of rice at low temperatures occurs more slowly than in suitable temperature such as 25°C. Thus, RL and SL were determined in 10 seedlings of each sub-sample, randomly assessed for each treatment using a graduated ruler. These seedlings were then separated and transferred to an oven at 70 ± 2°C until a constant mass was obtained to determine the dry mass of the shoots and roots.

The seedling vigor index was calculated according to the equation, where: $SI = (SL + RL) \times G\%$. With the data obtained from GSI, the following indices were also calculated: Cold stress tolerance index (STI = (Yp × Ys)/Xp²); cold stress susceptibility index (SSI = (1 – (Ys/Yp))/((1 – (Xs/Xp)))/2); proposed by and harmonic mean.

### Table 1. Characterization of the carrot extract as pH, osmotic potential (Ψs), electrical conductivity (EC), and β-carotene content.

| Carrot extract (%) | pH   | Ψs (MPa) | EC (mS cm⁻¹) | β-Carotene (mg mL⁻¹) |
|-------------------|------|----------|--------------|----------------------|
| 25                | 6.04 | −0.13    | 1.19         | 0.0109               |
| 50                | 6.04 | −0.31    | 1.98         | 0.0171               |
| 100               | 6.04 | −0.61    | 3.63         | 0.0352               |
Table 2. First germination count (FGC), germination test (G), and germination speed index (GSI) of rice seeds, cv. BRS Querência and cv. Brilhante in response to conditioning on carrot extract and low temperature. Means followed by the same letter, upper case in the column and lower case in the row, do not differ by Tukey’s test (P ≤ 0.05). CV coefficient of variation, ±SD standard deviation.

Data analysis showed a significant interaction between cultivars and seed conditioning treatments. The low temperature (15 °C) resulted in a significant reduction in the first germination count (FGC), the percentage of germinated seeds (G), and the germination speed index (GSI) in both cultivars however, cv. Brilhante was superior to BRS Querência in all parameters evaluated (Table 2).

Comparing the germination test at 25 °C with 15 °C for the seeds conditioned in water (positive and negative control), there was a reduction of more than 70% in FGC and 90% in GSI, for both cultivars, showing the negative effect of low temperature on the speed of germination, even in cv. Brilhante, considered tolerant. In the germination test, cv. Brilhante suffered a reduction of only 30.6%, compared to 59.4% for cv. BRS Querência.

However, analyzing the effect of conditioning treatments on rice seeds, carrot extract significantly responded to the evaluated parameters, resulting in the maintenance of seed vigor under low temperature in germination (Table 2). Although the tests of FGC, G, and GSI for conditioning in carrot extract, in general, had shown superiority to that observed in water conditioning, when germination at 15 °C, the best response was observed for treating 100% of the extract, reaching a percentage of 91% of germinated seeds for cv. Brilhante, very close to the 91.5% observed in seeds germinated at 25 °C, sets the optimum temperature for germination of rice.

The effect of temperature variation on rice can also be observed in the evaluation of seedling growth parameters. When germinated at 25 °C, shoot length (SL) and root length (RL) showed no significant difference between the two cultivars studied (Fig. 1A,C). However, under 15 °C, there was a significant reduction for these variables, more expressive in cv. BRS Querência. In response to carrot extract, the SL increased with the extract on germination at 15 °C, reaching the maximum point at 80% and 94% of extract for the cv. BRS Querência and the cv. Brilhante, respectively (Fig. 1B). For the RL, the cv. Brilhante linearly increased with the use of the extract, while the cv. BRS Querência reached the maximum point with 72% extract (Fig. 1D).

The shoot dry mass (SDM) and root dry mass (RDM) showed a significant difference between the germination treatments at 25 °C, 15 °C and between the cultivars for the same germination condition (Fig. 2A,C). At 15 °C was observed less dry mass of the seedlings, but seedlings from conditioned seeds in carrot extract showed an increase in SDM, reaching the maximum point with 58% and 77% of extract in BRS Querência and Brilhante, respectively (Fig. 2B). For RDM, cv. BRS Querência presented positive linear behavior with the extract use, but it was not very expressive, increasing only 0.1 mg of the treatment with 0% to 100% of the extract and the cv. Brilhante reached the maximum point with 82% of extract (Fig. 2D).

The tolerance indexes (STI) were used to determine the level of tolerance to each cultivar’s cold stress and test how much the carrot extract can increase this factor. The higher the index (closer to 1) and the harmonic mean (HM) (closer to the non-stress GSI), implies that the treatment is closer to the maximum response of the cultivar (control at 25 °C), and the opposite occurs with the stress susceptibility index (SSI).

Based on the calculations of STI, SSI, and HM, it was observed that cv. Brilhante is more tolerant to cold stress than cv. BRS Querência (Table 3) and the use of conditioning in carrot extract improve the cold tolerance in germination, as it increases the STI, together with the HM, and decreases the SSI. The SVI also showed a similar result, indicating the cv. Brilhante, with greater vigor and being an index that considers both the germination and the total length of the seedlings, can differentiate better the extracts treatments, standing out the treatment

\[(HM = 2 \times (Yp \times Ys))/((Yp + Ys))\], according to26, where: Ys: corresponds to GSI of each treatment with stress environment (15 °C); Yp: corresponds to GSI of cultivar in the non-stress environment (25 °C); Xs: corresponds to the mean of the GSI of the control of both cultivars in a stress environment (15 °C); Xp: corresponds to the mean of the GSI of the control of both cultivars in the non-stress environment (25 °C).

Data were subjected to analysis of variance (P ≤ 0.05), and the means were compared by Tukey’s test, at 5% probability. When a significant effect was found, these were tested by polynomial regression models using the SigmaPlot program, version 12.5, and the means compared using the R software27.
with 50% of extract for the cv. BRS Querência and 100% for cv. Brilhante. The conditioning with carrot extract presented more significant results in the susceptible cultivar (BRS Querência) since the indexes evaluated showed a higher percentage of increase in this cultivar.

With the data of Pearson linear correlation was possible to verify that the STI, SSI, HM, and SVI had a high correlation with all data measured, not only viability but also growth parameters (Table 4). Only the root dry mass did not show a high correlation for cv. BRS Querência, but was significant by t test. The HM, despite the high correlation, was not significant with the extract, in both cultivars, by t-test.

Discussion
Seed germination and seedling emergence are the initial steps for establishing a culture, influenced by several environmental factors, such as temperature, water, light, and seed burial depth. Temperature is one of the primary factors affecting the percentage and speed of germination, which directly works via seed imbibition and the biochemical reactions that regulate the metabolism involved in the germination process.

Thus, the low-temperature stress at the seedling stage is a major constraint to rice production. As can be observed in this study, there were sharp reductions in the germination and growth of the rice seedlings of the cultivars BRS Querência and Brilhante when subjected to low temperature, 15 °C (Table 2 and Fig. 1). Similar results were observed by22,31, studying rice genotypes in which the cold negatively affected germination, root length, and coleoptile length. According to23, the temperature has a significant effect on the length of the coleoptile and radicle, on the percentage of damage to the seed and germination rate in a germination period under

Figure 1. Shoot length of controls (A), shoot length of treatments germinated at 15 °C (B), root length of controls (C), and root length of treatments germinated at 15 °C (D) of rice seedlings of cultivars BRS Querência (filled circle) and Brilhante (filled triangle). Means followed by the same letter, upper case in the comparison of the cultivars and lower case in temperatures, do not differ by Tukey’s test (P ≤ 0.05). Bars represent the standard error of the mean of four replicates.
Figure 2. Shoot dry mass of controls (A), shoot dry mass of treatments germinated at 15 °C (B), root dry mass of controls (C) and root dry mass of treatments germinated at 15 °C (D) of rice seedlings of cultivars BRS Querência (filled circle) and Brilhante (filled triangle). Means followed by the same letter, upper case in the comparison of the cultivars and lower case in temperatures, do not differ by Tukey’s test (P ≤ 0.05). Bars represent the standard error of the mean of four replicates.

Table 3. Stress tolerance index (STI), stress susceptibility index (SSI), harmonic mean (HM), and seedling vigor index (SVI) of two rice cultivars, BRS Querência and Brilhante, in response to the conditioning of the seeds in the extract of carrot and the low germination temperature. Means followed by the same letter in the column do not differ from each other by the Tukey’s test (P ≤ 0.05). ± SD standard deviation.
| Extract | GSI | FGC | G | SL | RL | SDM | RDM | STI | SSI | HM | SVI |
|---------|-----|-----|---|----|----|-----|-----|-----|-----|----|-----|
| Extract | 1   | 0.70** | 0.65** | 0.67** | 0.74** | 0.60* | 0.58* | 0.45** | 0.70** | −0.71** | 0.70** | 0.68** |
| GSI     | 0.77** | 1   | 0.79** | 0.96** | 0.75** | 0.54* | 0.83** | 0.49w | 0.99** | −1.00** | 1.00** | 0.96** |
| FGC     | 0.73** | 0.98** | 1   | 0.70** | 0.42w | 0.17** | 0.60* | 0.54* | 0.78** | −0.78** | 0.79** | 0.69** |
| G       | 0.71** | 0.97** | 0.95** | 1   | 0.77** | 0.61* | 0.84** | 0.44w | 0.96** | −0.96** | 0.97** | 1.00** |
| SL      | 0.76** | 0.58* | 0.63** | 0.53* | 1   | 0.76** | 0.75** | 0.51* | 0.74** | −0.76** | 0.76** | 0.79** |
| RL      | 0.72** | 0.66** | 0.62** | 0.63** | 0.46** | 1   | 0.66** | 0.52* | 0.52* | −0.55** | 0.54* | 0.64** |
| SDM     | 0.81** | 0.89** | 0.91** | 0.88** | 0.81** | 0.61* | 1   | 0.71** | 0.82** | −0.84** | 0.83** | 0.85** |
| RDM     | 0.83** | 0.93** | 0.91** | 0.89** | 0.73** | 0.61* | 0.93** | 1   | 0.46w | −0.49** | 0.48w | 0.44w |
| STI     | 0.76** | 1.00** | 0.98** | 0.97** | 0.58* | 0.66** | 0.89** | 0.92** | 1   | −0.99** | 0.99** | 0.96** |
| SSI     | −0.77** | −1.00** | −0.98** | −0.97** | −0.60** | −0.65** | −0.89** | −0.93** | −1.00** | 1   | −1.00** | −0.96** |
| HM      | 0.76** | 1.00** | 0.98** | 0.97** | 0.58* | 0.65** | 0.89** | 0.93** | 1.00** | −1.00** | 1   | 0.96** |
| SVI     | 0.79** | 0.97** | 0.96** | 0.99** | 0.64** | 0.70** | 0.93** | 0.92** | 0.97** | −0.97** | 0.98** | 1   |

### Table 4. Pearson correlation for 12 variables, evaluated in the germination season in two rice cultivars, BRS Querência (above diagonal) and Brilhante (below diagonal), in response to the conditioning of the seeds in carrot extract and the low temperature in germination. ** Significant at 1% probability of error; * significant at 5% probability of error; ns not significant.

stress conditions. Thus, characteristics such as germination rate, the root, and coleoptile length have been used in cold tolerance studies on germination\(^{11,33}\).

Also reported by other authors\(^{34}\), low temperature at the seedling stage may result in additional poor germination in stunted seedlings, yellowing or withering, and reduced tillering in rice. With this study, it was possible to verify that the highest losses were observed for cv. BRS Querência, probably related to its origin.

The adverse effects of several abiotic stress factors are often mitigated by the application of natural and artificial growth promoters, or compounds with antioxidant activity\(^{16,35}\), which can be applied in different ways, such as spraying or priming of seeds by presoaking in these compounds. Seed priming is a cheap, quick, and straightforward methodology accessible to farmers in order to achieve higher germination rates and may be taken to counteract the adverse effects of abiotic stresses\(^{37,38,40}\).

According to\(^{38}\), carrot root extract can be used to stimulate plant growth because it is known to contain a variety of plant growth-stimulating compounds. Still, according to these authors, the analysis of carrot roots by HPLC showed that its extract includes a high content of vitamin A as β-carotene, protein, carbohydrates, fat, and vitamins B1, B2, B6, C, D, and E with notable antioxidant that reduce free radicals and cell injury.

In the present study, the positive effect of rice seed conditioning on carrot extract on germination and vigor tests in low temperature can be observed (Table 2). The results show that the best responses were obtained when the seeds were soaked (seed priming) in the pure extract (100%), where the cv. Brilhante maintains a percentage of germination equal to the control (germination at 25 °C). This behavior may be associated with the carrot extract’s β-carotene content (0.0352 mg mL\(^{-1}\)) (Table 1). Several studies have reported the effect of the use of aqueous extracts on seed germination in response to saline stress\(^{38,41,42}\), drought stress\(^{38}\), and cold\(^{43}\).

The growth parameters evaluated also positively affected the extract, which practically doubled with 100% extract compared with the negative control, mainly observed in cv. Brilhante (Figs. 1 and 2). The tolerance of plants to cold stress depends mainly on the ability of their membranes to prevent injury and maintain their integrity\(^{44}\). In this sense, studies report that carrot root extract can reduce cell damage caused by free radicals, protecting the cell membranes mainly due to the presence of antioxidant compounds\(^{38,40}\).

The results of the stress tolerance indexes provided information that allowed to select the most tolerant to cold, but they were not efficient in the selection of the best percentage of carrot extract to increase the rice tolerance under cold stress, showing that these indexes do not work with minor differences in vigor (Table 3). Other studies have also found superior performance of the cultivar Brilhante, with greater growth in relation to the other cultivars tested\(^{39,40}\). However, the use of seedling vigor index provided sufficient data for the selection of the best treatment of carrot extract for each cultivar, as well as an efficient selection of the best kelp extract in the growth of tomato seedlings\(^{47}\), because it is a particular index, taking into account both germination and seedling length, variables which are very responsive to cold stress and conditioning in the extract.

### Conclusion

Carrot roots extract mitigates the cold effects on germination and initial growth of rice plants, both sensitive (BRS Querência) and tolerant (Brilhante) cultivars. The germination followed by growth evaluations provided sufficient information for the differentiation of the cultivars and the treatments with carrot extract, concerning the greater cold tolerance, through the indexes of tolerance to stress and vigor of seedlings.

Received: 12 October 2020; Accepted: 11 May 2021
Published online: 26 May 2021
References

1. Matiu, M., Ankerst, D. P. & Menzel, A. Interactions between temperature and drought in global and regional crop yield variability during 1961–2014. Plos One. 12(5), e0178339 (2017).
2. Baker, J. S., Havlik, P., Beach, R., Leclere, D., Schmid, E., Valin, H. & McFarland, J. Evaluating the effects of climate change on US agricultural systems: Sensitivity to regional impact and trade expansion scenarios. Environ. Res. Lett. 13, 064019 (2018).
3. Tschirntke, T., Clough, Y., Wang, T. C., Jackson, L., Motzke, I., Perfecto, I., Vandermeer, J. & Whitbread, A. Global food security, biodiversity conservation and the future of agricultural intensification. Biol. Conserv. 151(1), 53–59 (2012).
4. UNITED NATIONS. Department of Economic and Social Affairs, Population Division. World Population Prospects 2019: Highlights(ST/ESA/SER.A/423) (2019).
5. IRRI. Rice Science for a Better World. http://irri.org/ (Institute International Rice Research, 2019).
6. Buti, M. et al. Transcriptome profiling of short-term response to chilling stress in tolerant and sensitive Oryza sativa ssp. Japonica seedlings. Funct. Integr. Genomics. 18, 627–644 (2018).
7. Dahi, T. & Khanna, V. K. Effect of climate change on rice agrotechnology. Agrotechnology 7(181), 1001818 (2018).
8. CONAB. Companhia Nacional de Abastecimento. Acompanhamento da Safra Brasileira de Grãos. Monitoramento agrícola-Safra 2018/2019. 5º Levantamento 1–125. ISSN 2318-6852 (2019).
9. Suh, J. P. & Sacks, E. J. Comparison of methods to evaluate rice (Oryza sativa L.) germplasm for tolerance to low temperature at the seedling stage. Agronomy 11(385), (2021).
10. SOSBAI. Arroz irrigado: Recomendações técnicas da pesquisa para o sul do Brasil. XXXII Reunião técnica da cultura do arroz (2020).
11. Jeller, H. & Perez, S. C. J. G. A. Priming in cássia-do-nordeste seeds under water, thermic and salt stress. J. Seed Sci. 42, e202042031 (2020).
12. Hassimotto, N. M. A., Genovese, M. I. & Lajolo, F. M. Antioxidant capacity of Brazilian fruit, vegetables and commercially-frozen fruit pulps. J. Food Compos. Anal. 22(5), 394–396 (2009).
13. Kasote, D. M., Katyare, S. S., Hegde, M. V. & Bae, H. Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int. J. Biol. Sci. 11(8), 982–991 (2015).
14. Jini, V. M., Guevara, E., Herrera, J. & Bangerth, F. Evolution of endogenous hormone concentration in embryogenic cultures of carrot during early development of embryogenesis. Plant Cell Rep. 23(8), 567–572 (2005).
15. Anwar, S., Shafi, M., Bakht, J., Jan, M. T. & Hayat, Y. Response of barley genotypes to salinity stress as alleviated by seed priming. Pak. J. Bot. 57, 45–59 (2017).
16. Lazaridou, M. A., Tzouvelekidou, A., Andridas, K. I., Vlahos, M. T., Vlahos, G. C. & Ganas, S. Application of stress indices for low temperature and deep sowing stress screening of rice genotypes. Pak. J. Biol. Sci. 16(22), 1618–1622 (2013).
17. Orcutt, T. E. & Mitchell, G. J. Cold tolerance at the germination stage of rice: Methods of evaluation and characterization of genotypes. Sci. Agric. 61(1), 1–8 (2004).
18. Shahnab, S., Ahmed, B., Talukder, A. H. M. M. R., Hossain, A., Rashid, T. M., Alam, J. M., Rahman, M., Sultana, M., Akter, R. & Nahar, L. Seasonal low temperatures affect the growth of rice seedlings in Northern Bangladesh. Int. J. Appl. Res. 3(2), 18–23 (2017).
19. Sharma, N., Reinek, R. & Sacks, E. J. Comparison of methods to evaluate rice (Oryza sativa L.) germplasm for tolerance to low temperature at the seedling stage. Agronomy 11(385), (2021).
20. Buti, M. A., Sacks, E. J. & Sacks, E. J. Comparison of methods to evaluate rice (Oryza sativa L.) germplasm for tolerance to low temperature at the seedling stage. Agronomy 11(385), (2021).
21. Casim, W. A., Nesse, A. A. & Gaber, A. Alleviation of drought stress in Vicia faba by seed priming with ascorbic acid or extracts of garlic and carrot. Egypt. J. Bot. 57, 45–59 (2017).
22. Galvizi-Fajardo, Y. C., Bortolin, G. S., Deuner, S., Amarante, L., Reolon, F. & Moraes, D. M. Seed priming with salicylic acid potentiates water restriction-induced effects in tomatod seed germination and early seedling growth. J. Seed Sci. 42, e202402301 (2020).
23. Maguire, J. D. Speed of germination—Aid in selection and evaluation for seedlings emergence and vigor. Crop Sci. 2(2), 176–177 (1962).
24. Orchard, T. J. Estimating the parameters of plant seedling emergence. Seed Sci. Technol. 61–69 (1977).
25. Fischer, R. A. & Maurer, R. Drought resistance in spring wheat cultivars. I. Grain yield responses. Aust. J. Agric. Res. 29(5), 897–907 (1978).
26. Mardeh, A. S. S., Ahmadi, A., Poustiti, K. & Mohammadi, V. Evaluation of drought resistance indices under various environmental conditions. Field Crop Res. 98(2–3), 222–229 (2006).
27. R Development Core Team. R. A Language and Environment for Statistical Computing. (Austria: R Foundation for Statistical Computing, 2019). https://www.R-project.org/.
28. Javid, M. M. & Tanveer, A. Germination ecology of Emex spinosa and Emex australis, invasive weeds of winter crops. Weed Res. 54, 565–574 (2014).
29. Wu, H., Zhang, P., Chen, G., Pan, L., Li, J. & Dong, L. Environmental factors on seed germination and seedling emergence of Phleum paniculatum Huds. Chil. J. Agric. Res. 78(3), (2018).
30. Marcos Filho, J. M. F. Fisiologia de Sementes de Plantas Cultivadas. (Abrares, 2015).
31. Peyman, S. & Hashem, A. Evaluation of eighteen rice genotypes in cold tolerance at germination stage. World Appl. Sci. J. 11(11), 1476–1480 (2010).
32. Soleymani, A. & Shahrajabian, M. H. Study of cold stress on the germination and seedling stage and determination of recovery in rice varieties. Int. J. Biol. 4(1), 23–30 (2012).
33. Sharifi, P. Evaluation on sixty-eight rice germplasms in cold tolerance at germination stage. Rice Sci. 17(1), 77–81 (2010).
34. Tuszeja, N., Gill, S. S., Tuburcio, A. F. & Tuszeja, R. Rice: Improving cold stress tolerance. In Improving Crop Resilience to Abiotic Stress. Vol. 1, 1524. (2012).
35. Lipiec, J., Douscan, C., Nosalewic, A. & Kondracka, K. Effect of drought and heat stresses on plant growth and yield: A review. Int. Agrophys. 27, 463–477 (2013).
36. Abbas, S. M. & Akladious, S. A. Application of carrot root extract induced salinity tolerance in cowpea (Vigna sinensis L.) seedlings. Pak. J. Bot. 45(3), 795–806 (2013).
37. Ahmed, E., Baloch, D. M., Sadiq, S. A., Ahmed, S. S., Hanan, A., Taran, S. A., Ahmed, N. & Hassan, M. J. Plant growth regulators induced drought tolerance in sunflower (Helianthus annuus L.) hybrids. J. Anim. Plant Sci. 24(3), 886–890 (2014).
38. Kasim, W. A., Nesse, A. A. & Gaber, A. Effect of seed priming with aqueous extracts of carrot roots, garlic cloves or ascorbic acid on the yield of Vicia faba grown under drought stress. Pak. J. Bot. 51(6), (2019).
39. Oliveira, J. L. S., Steiner, F., Zuffo, A. M., Zott, T., Alves, C. Z. & Aguilar, V. C. B. Seed priming improves the germination and growth rate of melon seedlings under saline stress. Clêncio Rural. 49(07), (2019).
40. Bortolin, G. S., Teixeira, S. B., Pinheiro, R. M., Ávila, G. E., Carlos, F. S., Pedroso, C. E. S. & Deuner, S. Seed priming with salicylic acid minimizes oxidative effects of aluminum on Trifolium seedlings. J. Soil Sci. Plant Nutr. 20(6), (2020).
41. Latef, A. A. H. A., Mostofa, M. G., Rahman, M., Abdel-Farid, I. B. & Tran, L. S. P. Extracts from yeast and carrot roots enhance maize performance under seawater-induced salt stress by altering physio-biochemical characteristics of stressed plants. J. Plant Growth Regul. 38, 966–979 (2019).
42. Nessem, A. A. & Kasim, W. A. Physiological impact of seed priming with CaCl$_2$ or Carrot root extract on *Lupinus termis* plants fully grown under salinity stress. *Egypt J. Bot.* **59**(3), 763–777 (2019).
43. Afzal, I., Hussain, B., Basra, S. M. A. & Rehman, H. Priming with moringa leaf extract reduces imbibitional chilling injury in spring maize. *Seed Sci. Technol.* **40**(2), 271–276 (2012).
44. Zhang, X. *et al.* Arabidopsis cysteine-rich receptor-like kinase 45 functions in the responses to abscisic acid and abiotic stresses. *Plant Physiol. Biochem.* **67**, 189–198 (2013).
45. Baranska, M., Schulz, H., Baranski, R., Nothnagel, T. & Christensen, L. P. In situ simultaneous analysis of polyacetylenes, carotenoids and polysaccharides in carrot roots. *J. Agric. Food Chem.* **53**(17), 6565–6571 (2005).
46. Rodrigues, H. C. S. *et al.* Application of stress indexes to evaluate cold tolerance of rice cultivars during the initial stage of plant development. *Ciência.* **42**(3), 258–264 (2014).
47. Hernández-Herrera, R. M., Santacruz-Ruvalcaba, F., Ruiz-López, M. A., Norrie, F. & Hernández-Carmona, G. Effect of liquid seaweed extracts on growth of tomato seedlings (*Solanum lycopersicum* L.). *J. Appl. Phycol.* **26**(1), 619–628 (2014).

**Acknowledgements**
The present work was carried out with the support of the Coordination for the Improvement of Higher Education Personnel (CAPES)—financing code 001 for S. B. Teixeira. S. Deuner (grant number 429531/2016-8) are supported by resources from Brazilian National Council for Scientific and Technological Development (CNPq).

**Author contributions**
S.B.T., S.D. conceived and designed the experiment. S.B.T., S.N.P., G.E.Á., B.E.P.S., V.N.S., C.D., R.S.A., D.S.M. carried out the experimental and the laboratory analysis. S.B.T. analyzed the data and prepared the manuscript. All authors discussed the results and contributed to the subsequent improvements and final version. S.D. provided of study materials.

**Competing interests**
The authors declare no competing interests.

**Additional information**

**Correspondence** and requests for materials should be addressed to S.D.

**Reprints and permissions information** is available at [www.nature.com/reprints](http://www.nature.com/reprints).

**Publisher’s note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit [http://creativecommons.org/licenses/by/4.0/](http://creativecommons.org/licenses/by/4.0/).

© The Author(s) 2021