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Evaluation of the effect of phenolic pigments on rice germination under low temperature conditions

Avaliação do efeito de pigmentos fenólicos na germinação de arroz sob condições de baixa temperatura

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ABSTRACT

In Rio Grande do Sul, the main rice producer State in Brazil, low temperatures can occur during germination and seedling establishment, and in some cases, during the reproductive stage. When low temperatures occur in the early developmental stages cause delay in germination, resulting in a non-homogeneous growing. In reproductive stage, low temperatures cause spikelet sterility, directly interfering with plant yield. Researchers have shown that some phenolic compounds such as proanthocyanidins and anthocyanin are associated with low temperature tolerance in plants due to their antioxidant capacity. The red and black color in the seeds of some rice genotypes is conferred by the phenolic compounds proanthocyanidins and anthocyanin, respectively. Therefore, tis study aimed to verify whether rice genotypes with red or black seeds are more tolerant to low temperatures during germination. In this study, five rice genotypes were tested, two present seeds without pigmentation and with contrasting response to low temperature tolerance (BRS Bojuru - tolerant and BRS Pampeira - sensitive), two genotypes with red seeds (BRS 902, SCS 119 Rubi) and one black seed genotype (SCS 120 Ônix). As expected, the genotypes with pigmented seeds had a greater total phenolic compounds content. However, under low temperature conditions, the genotypes with pigmented seed showed a similar response to the sensitive genotype. Therefore, the presence of proanthocyanidins and anthocyanin in the seed of the studied genotypes does not provide tolerance to low temperatures during germination.

KEYWORDS: antioxidant capacity; black rice; cold stress; flavonoids; red rice.

RESUMO

No Rio Grande do Sul, principal estado produtor de arroz do Brasil, baixas temperaturas podem ocorrer durante a germinação e estabelecimento de plântulas, e em alguns casos, durante o estádio reprodutivo. Quando baixas temperaturas ocorrem no estádio inicial de desenvolvimento causam atraso da germinação, resultando em um crescimento não homogêneo. No estádio reprodutivo, baixas temperaturas podem ocasionar esterilidade das espiguetas, interferindo diretamente na produtividade da planta. Pesquisas têm mostrado que alguns compostos fenólicos como as proantocianidinas e antocianinas estão associadas com tolerância a baixa temperatura em plantas devido sua capacidade antioxidante. A coloração vermelha e preta nas sementes de alguns genótipos de arroz é conferida pelos compostos fenólicos proantocianidinas e antocianinas, respectivamente. Portanto, o objetivo desse estudo foi verificar se os genótipos de arroz com sementes vermelhas ou pretas são mais tolerantes a baixas temperaturas durante a germinação. Neste trabalho, cinco genótipos foram testados, dois com sementes sem pigmentação e com resposta contrastante para tolerância a baixa temperatura (BRS Bojuru - tolerante e BRS Pampeira - sensível), dois genótipos com sementes vermelhas (BRS 902, SCS 119 Rubi) e um genótipo com sementes pretas (SCS 120 Ônix). Como esperado, os genótipos com sementes pigmentadas têm maior conteúdo de compostos fenólicos totais. Entretanto, sob condições de baixa temperatura, os genótipos com semente pigmentada mostraram resposta similar ao genótipo sensível. Dessa forma, a presença de proantocianidinas e antocianinas nas sementes dos genótipos estudados não confere tolerância a baixas temperaturas durante a germinação.

PALAVRAS-CHAVE: arroz preto; arroz vermelho; capacidade antioxidante; estresse por frio; flavonoides.

INTRODUCTION

Rice (*Oryza sativa* L.) originates from tropical or subtropical areas, naturally adapted to heat and sensitive to low temperatures. The optimal temperature range for rice growth is 15 °C to 35 °C, and temperatures below 15 °C affect growth and development and can cause serious physiological damage (ANDAYA & MACKILL 2003, FUJINO et al. 2004, XIE et al. 2020). Yield losses may occur due to damage caused by low temperatures during the germination, vegetative and reproductive growth stages (ADAMSKI et al. 2020). Low temperatures are responsible for the delay in germination and growth of rice seedlings, causing the establishment of less stands and uneven maturity, mainly in no-tillage areas (YANG et al. 2021). In addition, low temperatures can cause changes in architecture, male sterility, delayed maturation, reduced seed fixation rate, and ultimately reduced rice yield (ZHANG et al. 2018).

In addition of crop development stage, the injury caused by low temperatures is influenced by the intensity and duration of this stress (TEIXEIRA et al. 2021). In Rio Grande do Sul (RS), the main rice-producing State in Brazil, low temperatures are common in the germination stage, since sowing is performed soon after winter. This condition can be extended to mid-spring, resulting in delayed germination and seedling emergence for more than 20 days (TEIXEIRA et al. 2021). Low night temperatures may also occur during the reproductive stage. Most cultivars used in RS are of the *indica* subspecies, which are normally sensitive to stress caused by low temperature (ADAMSKI et al. 2020).

Aerobic metabolism constantly generates reactive oxygen species (ROS). Under favorable conditions, ROS are produced at basal levels, which are eliminated by antioxidant machinery. However, under adverse conditions such as extreme temperatures, ROS generation is high, affecting the balance between generation and elimination of these molecules. ROS play an important role in signaling processes that control plant growth, development and response to biotic and abiotic stresses (DAS & ROYCHOUDHURY 2014). However, high levels of ROS cause damage to cellular constituents. GROHS et al. (2016) reported the ROS increase in rice seedlings under low temperature conditions, affecting the lipid peroxidation.

The main ROS are $^1O_2$, H_2O_2, $O_2^{\bullet-}$ and OH•, which are lethal and cause damage to proteins, DNA and lipids, affecting the normal functioning of cells. In plants, redox homeostasis is maintained by antioxidant machinery, which comprises enzymatic antioxidant components such as superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), glutathione-S-transferase (GST), catalase (CAT), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR), and low molecular weight non-enzymatic antioxidant compounds such as ascorbic acid (AA), reduced glutathione (GSH), α-tocopherol, carotenoids, phenolics, flavonoids, and proline (DAS & ROYCHOUDHURY 2014).

Phenolic compounds are secondary plant metabolites with antioxidant function. Phenolic compounds have more than 8,000 structural variants and denote many substances with aromatic rings containing one or more hydroxyl fractions. According to the structure, phenolic compounds can be grouped into phenolic acids, flavonoids, stilbenoids, lignans and polymeric lignins, tannins and diferuloylmethanes (HAN et al. 2007). Rice with pigmented seeds has a higher content of phenolic compounds and greater antioxidant capacity when compared to unpigmented rice. Black rice has an accumulation of anthocyanins, mainly cyanidin-3-glucoside and peonidin-3-glucoside (GUNARATNE et al. 2013). Anthocyanin is a flavonoid, being anthocyanin is in the form of glycoside while anthocyanidin is known as the aglycone (KHOO et al. 2017). Red rice has accumulation of proanthocyanidins and procyanidins (GUNARATNE et al. 2013). Procyanidins are derived from proanthocyanidins, also known as condensed tannins, are oligo or polymers of monomeric flavan-3-ols produced as an end-product of flavonoid biosynthesis (RUE et al. 2018, RAUF et al. 2019).

Anthocyanins are natural water-soluble pigments found in flowers, fruits, stems and leaves. These compounds have antioxidant capacity and in plants act to protect against various biotic and abiotic stresses (ZHANG et al. 2019). At low temperatures, increased expression of genes involved in the synthesis of anthocyanins, as well as an increase in the synthesis of this pigment was reported in plants and fruits at low temperatures, that is related to tolerance to this condition. The increase of anthocyanins was observed in *Mikania micranta* (ZHANG et al. 2019) and *Arabidopsis thaliana* (SCHULZ et al. 2015) under low temperature, which improved the tolerance of both species. In addition, seedlings of maize (*Zea mays* L.) (CHRISTIE et al. 1994) and purple head Chinese cabbage (*Brassica rapa* L.) (HE et al. 2020) also showed an increase in anthocyanin synthesis under low temperature. Similarly, at low temperatures during post-harvest, an increase in anthocyanins in mangoes (*Mangifera indica* L.) was observed, increasing tolerance to a chilling injury (SUDHEERAN et al. 2018). Likewise, anthocyanin content increase in blood oranges (*Citrus sinensis* L. Osbeck) under cold storage (LO PIERO et al. 2005, CRIFÒ et al. 2012, CARMONA et al. 2017). Also, low night temperatures increase anthocyanin accumulation in grapes (*Vitis vinifera* L.) (GAIOTTI et al.

2018).

Proanthocyanidins are present in flowers, nuts, fruits, bark and seeds of various plants, and act in defense against biotic and abiotic stresses. Its astringency protects plants from pathogens and predators (RAUF et al. 2019). Furthermore, proanthocyanidins have an antioxidant capacity, which is significantly stronger than anthocyanins (GUNARATNE et al. 2013, GOURLAY & CONSTABEL 2019). JIA et al. (2012) demonstrated that proanthocyanidins act as antioxidants during germination of *Arabidopsis thaliana* seeds under oxidative stress conditions, and in addition, these compounds modulate the activity of enzymes that control ROS levels. The authors suggest that proanthocyanidins contribute as an adaptive mechanism that helps germination under environmental stress conditions. In plants of a hybrid poplar (*Populus tremula* × *P. tremuloides*) it was also verified that proanthocyanidins act in protection against oxidative stress (GOURLAY & CONSTABEL 2019).

Within this context, this study aimed to evaluate the effect of anthocyanins and proanthocyanidins, present in black and red rice seeds, respectively, on germination under low-temperature conditions.

MATERIAL AND METHODS

Rice genotypes

Rice seeds of the BRS Bojuru (*japonica* subspecies – tolerant to low temperatures during germination, with unpigmented seed) (VIGHI et al. 2016), BRS Pampeira (*indica* subspecies - sensitive to low temperatures during germination, with unpigmented seed) (STRECK et al. 2020), BRS 902 (red seed), SCS 119 Rubi (red seed) and SCS 120 Ônix (black seed) were used.

Total phenols quantification and antioxidant capacity in rice

Compound extraction

Initially, whole seed rice flour was made. In 50 mL Falcon tubes, 1.8 g of flour and 20 mL of methanol were added. The mixture was homogenized in a mechanical stirrer every 15 minutes until completing 1 hour. After extraction, the tubes were centrifuged at 5,000 rpm (Eppendorf - centrifuge 5810R) for 20 minutes. The supernatants were transferred to 15 mL Falcon tubes (wrapped with aluminum foil) and used as crude extracts to quantify total phenols and determine antioxidant capacity (SINGLETON & ROSSI 1965, BRAND-WILLIANS et al. 1995, RUFINO et al. 2007).

Quantification of total phenolic compounds

The quantification of total phenolic compounds was performed according to the methodology proposed by SINGLETON & ROSSI (1965) with modifications. Total phenols were determined on microplates in a spectrophotometer (SpectraMax 190 Microplate Reader). Each reaction consisted of 15 µL of the crude extract, added 240 µL of distilled water and 15 µL of 0.25 N Folin-Ciocalteu. The reaction was kept in the dark for 3 minutes and then 30 µL 1 N Na_2CO_3 was added, posteriorly the plate was shaken for 10 seconds and kept for 2 hours in the dark. The reading was performed in absorbance at 725 nm. To obtain the blank, the crude extract was replaced by 15 µL of methanol. The curve for the determination of phenolic compounds was prepared with a standard of gallic acid (GA) at concentrations in 25 and 500 µg mL^{-1} ($y = 0.0026x - 0.0064$). Results were expressed in milligrams of gallic acid equivalents per 100 grams of grain (mg GAE 100 g^{-1}).

Antioxidant capacity

The determination of the antioxidant capacity was performed by colorimetric reaction based on the scavenging capacity of the radicals DPPH – 2,2-diphenyl-1-picrylhydrazyl (BRAND-WILLIANS et al. 1995) and ABTS – 2,2′-azino-di-[3-ethylbenzthiazoline sulphonate (RUFINO et al. 2007), using the crude extract.

The reaction was carried out in microplates to determine the antioxidant capacity by the DPPH method. Each reaction consisted of 20 µL of crude extract added to 280 µL of solution containing DPPH (with absorbance adjusted to 1.1 nm ± 0.02) and the plate was shaken. The reaction remained in the dark for 24 hours and then read at 515 nm in a spectrophotometer (SpectraMax 190 Microplate Reader) (BRAND-WILLIANS et al. 1995). The crude extract was replaced by 20 µL of methanol to obtain the blank. Results were expressed as percent inhibition using the equation ((White Abs-Sample Abs)/White Abs)*100.

The reaction was carried out in microplates to determine the antioxidant capacity by the ABTS method. Each reaction consisted of 20 µL of crude extract added to 280 µL of solution containing ABTS (adjusted to 0.7 nm ± 0.05) and the plate was shaken. The reaction was kept in the dark for 6 minutes and then reading at 734 nm in a spectrophotometer (SpectraMax 190 Microplate Reader) (RUFINO et al. 2007). Then, the crude extract was replaced by 20 µL of methanol to obtain the blank. Results were expressed as percent inhibition using the equation ((White Abs-Sample Abs)/White Abs)*100.

Rice germination under low-temperature conditions

The disinfestation process was carried out by placing the seeds for 1 minute in 70% alcohol, and after

washing them in distilled water. After, the seeds were washed in a 2% sodium hypochlorite solution for 1 minute, then the seeds were washed in distilled water.

The seeds of each genotype were uniformly distributed in a gerbox-type box containing a sheet of Germitest® paper moistened 2.5 times its weight with distilled water. The boxes were kept in growth chambers at 25 ºC (control) and 13 ºC (low temperature) and a photoperiod of 16/8 hours light/dark. The experiment was carried out until the seedlings reached the S3 stage. At the S3 stage, the emergence of coleoptile prophyll occurs (COUNCE et al. 2000), and coleoptile growth is the most used method for identifying genotypes tolerant to low temperature (CRUZ & MILACH 2004, VIANA et al. 2021).

The experiment was completely randomized, using five genotypes (BRS Bojuru, BRS Pampeira, BRS 902, SCS 119 Rubi and SCS 120 Ônix), two treatments (control and low temperature) and four replications of each treatment for each genotype. For each repetition, 50 seeds were used. The parameters analyzed were: Days to germination (DG), were considered germinated seeds those at S1 stage; Days from sowing to S3 stage (DS3); and Days from germination to S3 stage (DGS3).

Seedlings in stage S3 were characterized for shoot length (SL) and root length (RL), using a caliper (Starrett, ± 0.05 mm precision), as described by BRESOLIN et al. (2019) and results expressed in millimeters. After, shoots and roots were separated and placed in a forced air oven for 96 hours at 80 ºC. Subsequently, shoot dry weight (SDW) and root dry weight (RDW) were determined using an analytical balance (Shimadzu auw220d, precision: 0.00001 g), and the results were expressed in milligrams. Afterwards, considering that the studied genotypes present intrinsic developmental characteristics, the data were presented analyzing the relative performance (RP = (measurement of the variable in the low temperature condition/measurement of the variable in the control condition) *100).

In the control condition, the first count was performed at 3 days, when the germination of the first genotype occurred, and the final count was carried out at 14 days when all genotypes germinated. In the low temperature condition, the first count was performed at 16 days, on germination of first genotype, and the final count was performed at 27 days, when all cultivars germinated.

Statistical analysis

The data were analyzed using R software. Days to germination and days until reaching the S3 stage were presented in bar graph. For variables with significant differences in ANOVA (data not show), the comparison of means test was performed (Tukey ($p \leq 0.05$).

RESULTS AND DISCUSSION

It is already known that rice seeds with black and red pigments have anthocyanins and proanthocyanidins, respectively, which are phenolic compounds with antioxidant capacity (GUNARATNE et al. 2013). In this study, using seeds under non-stressful conditions, SCS 120 Ônix (black seed) had the highest phenolic compounds content, followed by BRS 902 and SCS 119 Rubi (red seed), but SCS 119 Rubi did not differ significantly of BRS Bojuru (not pigmented and cold tolerant). The lowest total phenolic compound content was found in BRS Pampeira (not pigmented and cold sensitive) seeds, but did not differ from BRS Bojuru (Table 1). For both detection methods, the highest antioxidant capacity was detected in BRS 902 and SCS 120 Ônix seeds, followed by SCS 119 Rubi and BRS Bojuru, which showed similar profile. BRS Pampeira was the one with the lowest antioxidant capacity in seeds (Table 1). It can be clearly observed that the content of total phenolic compounds is directly correlated with the antioxidant capacity. Similar results were reported by LOURENÇO et al. (2015), that also quantified total phenols in these genotypes.

Table 1. Mean total phenol content and scavenging capacity of ABTS and DPPH in pigmented rice seeds.

Genotype	Total phenolic content (mg GAE 100 g^{-1})	ABTS radical scavenging activity (%)	DPPH radical scavenging activity (%)
BRS Bojuru	245.4 cd*	55.0 b	21.7 c
BRS Pampeira	177.3 d	34.0 c	8.4 d
BRS 902	499.9 b	92.6 a	79.3 a
SCS 119 Rubi	272.8 c	59.0 b	27.2 b
SCS 120 Ônix	582.5 a	92.5 a	78.3 a
CV (%)	4.8	2.6	2.9
General mean	355.6	66.6	43.0

*Means followed by the same letter do not differ by Tukey's test ($p \leq 0.05$).

BRS Bojuru and BRS Pampeira are contrasting for low temperature tolerance during germination (VIGHI et al. 2016, STRECK et al. 2020) and therefore were used as control in this study. In the control condition, all genotypes initiated germination between two and three days. This result is interesting, because in *Arabidopsis thaliana* was shown that in the absence of oxidative stress, the presence of proanthocyanidins delays germination (GOURLAY & CONSTABEL 2019), which was not observed in this study, including the cultivar SCS 119 Rubi, with accumulation of proanthocyanidins, was the one with the fastest germination. Under low temperature conditions, germination of BRS Bojuru occurred 16 days after sowing, and for SCS 119 Rubi, germination occurred 17 days after sowing. The germination of BRS Pampeira, BRS 902 and SCS 120 Ônix, occurred 20 days after sowing (Figure 1). The faster germination in SCS 119 Rubi under stress can be explained by the presence of proanthocyanidins, as observed in *Arabidopsis thaliana*, which, under oxidative stress, seeds with proanthocyanidins germinated faster than seeds without this pigment (GOURLAY & CONSTABEL 2019).

In the control condition, all cultivars reached the S3 stage in four or five days after sowing and two days after germination (Figure 1). Under low-temperature conditions, the tolerant cultivar (BRS Bojuru) reached S3 stage 23 days after sowing; the sensitive cultivar (BRS Pampeira), together with the SCS 120 Ônix cultivar, needed 31 days after sowing to reach S3, and the other cultivars reached 30 days after sowing (Figure 1). The period between germination and S3 stage was of seven days for cultivar BRS Bojuru and 10 days or more for all other cultivars (Figure 1).

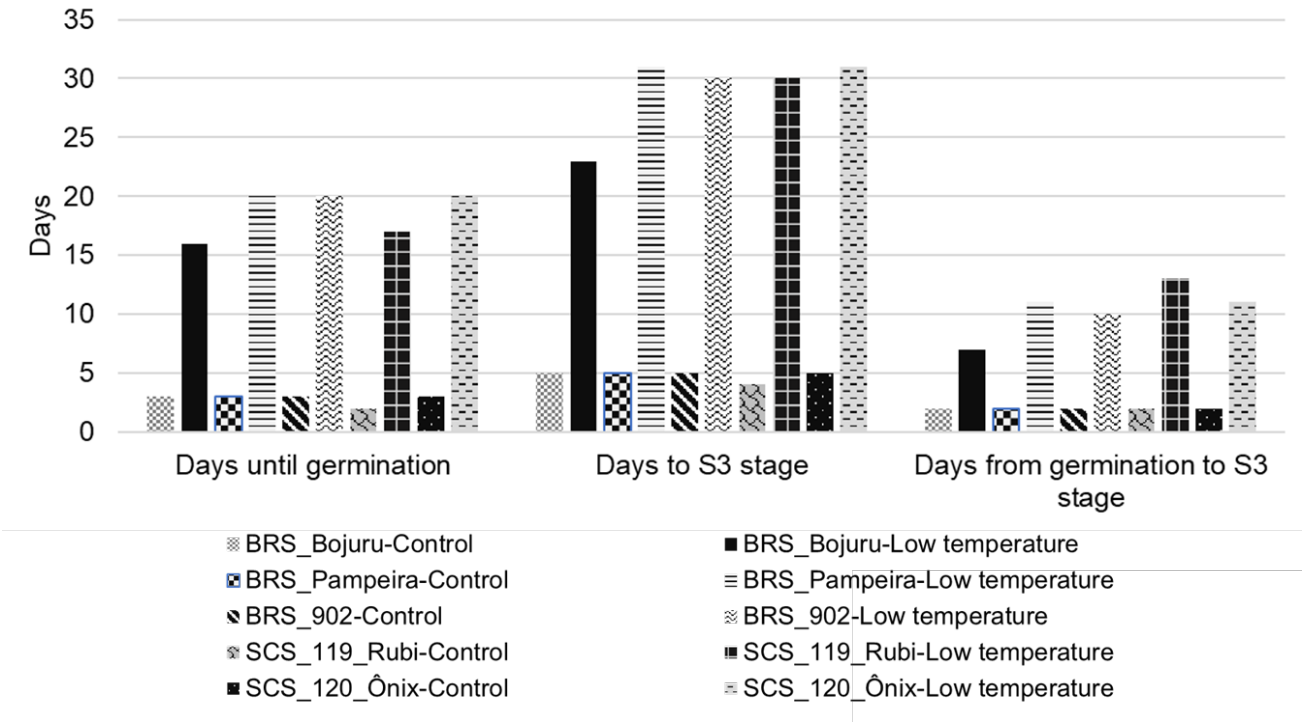

Figure 1. Days for germination and days until reaching the S3 stage of rice cultivars in the control (25 ºC) and low temperature (13 ºC) conditions.

In a study with other contrasting rice cultivars for low temperature tolerance, SCSBRS 113 Tio Taka (sensitive) and Oro (tolerant), DA MAIA et al. (2017) and VIANA et al. (2021) found results similar to those obtained for the cultivars BRS Bojuru (tolerant) and BRS Pampeira (sensitive). As the cultivars with black or red seeds presented a profile similar to the cultivars BRS Pampeira and SCSBRS 113 Tio Taka, sensitive to low temperatures, these are the first indications that the cultivars SCS 119 Rubi, BRS 902 and SCS 120 Ônix are sensitive to this condition.

Evaluating the germination percentage three days after sowing (first count) in the control condition, it is verified that the studied cultivars present different germination speed. At 14 days after sowing (second count), most cultivars had a germination percentage equal to or greater than 90%, except for the cultivar SCS 120 Ônix, with 78% (Table 2). Red pigmentation, that is related to proanthocyanidins, has been reported to be linked to seed dormancy, as the transcription factor bHLH that controls the accumulation of flavonoids in the pericarp also controls abscisic acid levels. In *Arabidopsis thaliana*, the flavonoids present in seed testa are the main determinants of dormancy (GALLAND et al. 2014).

In the low temperature condition, the first count to determine the germination percentage was performed 20 days after sowing, when 95% of germination was observed in the BRS Bojuru cultivar and below 65% in the other cultivars. At 27 days after sowing (second count), 96% germination was observed in the cultivar Bojuru and below 75% in the other cultivars. Low temperatures significantly reduce the germination ability of rice, and this result was also verified by PEYMAN & HASHEM (2010), SHARIFI (2010), DA MAIA et al. (2017), DIEN & YAMAKAWA (2019), TEIXEIRA et al. (2021), VIANA et al. (2021). The percentage and germination speed can be influenced by several factors such as water, light and temperature. Temperature acts directly on seed imbibition and on biochemical reactions that regulate the metabolism involved in the germination process (TEIXEIRA et al. 2021).

Table 2. Germination percentage (%) of rice cultivars in the control (25 ºC) and low temperatures (13 ºC) conditions.

Genotype	PCG_C[1]	Ger_C	PCG_ LT	Ger_ LT
BRS Bojuru	57.5 bc*	96.5 ab	95.0 a	96.5 a
BRS Pampeira	78.0 ab	90.5 c	47.5 bc	65.5 b
BRS 902	72.0 abc	95.0 bc	33.5 cd	56.5 b
SCS 119 Rubi	99.5 a	99.5 a	63.5 b	72.0 b
SCS 120 Ônix	43.0 c	78.0 d	28.0 d	62.5 b
CV (%)	19.5	3.94	15.3	15.6
General mean	70	91.9	53.5	70.6

[1]PCG_C: first germination count, at 3 days, in the control (%); Ger_C: final germination count at 14 days, in the control (%); PCG_ LT: first germination count, at 20 days, in cold condition (%); Ger_ LT: final germination count, at 27 days, in cold condition (%). *Means followed by the same letter do not differ statistically according to Tukey's test (p≤0.05).

The effect of low temperatures on the S3 stage seedlings phenotype was clearly visible. BRS Bojuru seedlings had the small phenotype changes when germinated under low temperature conditions (Figure 2). Likewise, considering shoot growth, BRS Bojuru showed the best germination relative performance under low temperature, but did not differ significantly from SCS 120 Ônix (Table 3).

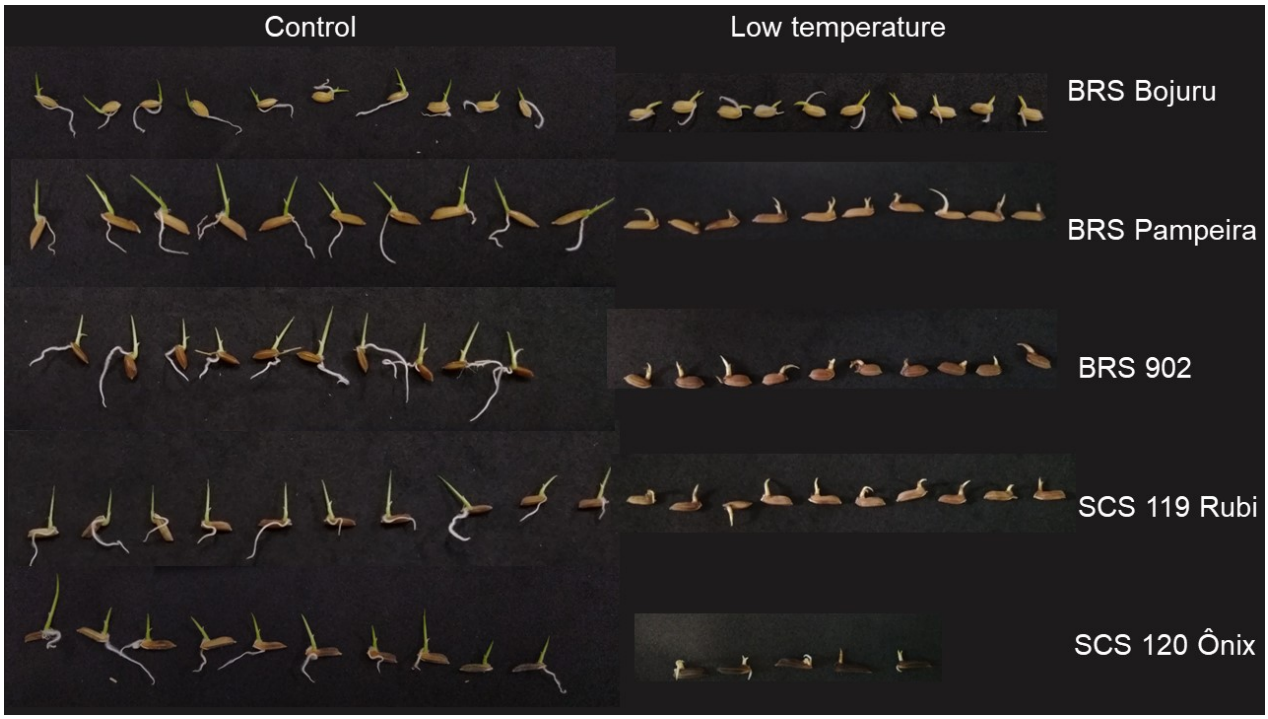

Figure 2. Rice seedlings at S3 stage under germination in the control condition (25 ºC) and in the low temperature condition (13 ºC).

For shoot dry weight, no significant differences between the relative performance of cultivars was detected (data not show). The seedlings of BRS Pampeira, BRS 902, SCS 119 Rubi and SCS 120 Ônix showed a highly affected phenotype when germinated at low temperature, with whitish color, reduced shoot growth and absence of roots (Figure 2, Table 3).

Table 3. Relative performance of shoot length, root length and shoot and root dry weight of rice seedlings subjected to germination at low temperature.

Genotype	RP_SL[1]	RP_RL	RP_RDW
BRS Bojuru	62.9* a	47.5 a	44.5 a
BRS Pampeira	42.9 b	0.0 c	0.0 b
BRS 902	37.3 b	0.0 c	0.0 b
SCS 119 Rubi	41.5 b	0.0 c	0.0 b
SCS 120 Ônix	52.1 ab	29.3 b	44.2 a
CV (%)	17.1	15.0	52.1
General mean	47.3	38.4	44.4

[1]RP_SL: relative performance of shoot length; RP_RL: relative performance of root length; RP_RDW: relative performance of root dry weight. *Means followed by the same letter do not differ statistically according to Tukey's test ($p \leq 0.05$).

GOTHANDAM (2012) also reported that seedlings subjected to low temperature present several physiological disorders, which include reduced leaf expansion, wilting and chlorosis (yellowing of the leaves), which can lead to necrosis (death of tissue). Similarly, SHARMA et al. (2021) found that seedlings of some rice cultivars have few or no roots when germinated at low temperatures. Additionally, in a study developed by HSU & HSU (2019), with a low temperature period of four days during rice germination, the authors observed a reduction in shoot and root length. The presence of roots, even with reduced growth, found by HSU & HSU (2019), can be explained by the fact that only the initial period of sowing was subjected to a low temperature (four days), and later germination occurred under optimal conditions. This study demonstrates that even short periods of low temperature after sowing negatively affects seedling growth.

The results obtained in this study suggest that the presence of anthocyanins and proanthocyanidins in seeds does not provide tolerance to low temperature during germination of cultivars SCS 119 Rubi, BRS 902 and SCS 120 Ônix, which present a very similar profile to the sensitive cultivar, BRS Pampeira.

During germination, the embryo's scutellum synthesizes and secretes gibberellin into the aleurone layer, which induces the synthesis and secretion of hydrolytic enzymes into the endosperm. The embryo uses the sugars released by the degradation of starch and proteins as a source of energy, providing carbon and nitrogen for germination and establishment of the seedlings. In addition to serving as a source of nutrients and mechanical protection for the embryo, the endosperm is able to detect environmental signals and produce and secrete signals to regulate embryo growth (YAN et al. 2014). However, black rice anthocyanins and red rice proanthocyanidins mostly accumulate in the bran layer (GOUFO & TRINDADE 2014, SHAO et al. 2014) and apparently are not remobilized to the embryo or seedling during germination. SHAO et al. (2014) showed in black rice that 97% of anthocyanins accumulate in the bran and only 3% in the embryo. In the case of red rice, proanthocyanidins were also detected in the embryo and bran (UPANAN et al. 2019). The amount of anthocyanins and proanthocyanidins present in the embryo is not enough to prevent damage caused by low temperature.

In a study developed with rice cv. Nipponbare, without the anthocyanins and proanthocyanidins pigments, the dynamics of flavones (another type of flavonoid) during germination was characterized (GALLAND et al. 2014). It was observed that germination does not change the flavonoid profile (stability of flavonoids in different seed compartments), but there is the induction of many genes involved in the biosynthesis of these metabolites in this period. The authors suggest that the flavones present in the embryo confer a protective effect. However, here it was verified that anthocyanins and proanthocyanidins present in the embryo are not sufficient for a protective effect under low temperature conditions.

CONCLUSION

The presence of pigments (anthocyanins or proanthocyanidins) in rice cultivars BRS 902 (red), SCS 119 Rubi (red) and SCS 120 Ônix (black) does not confer tolerance to low temperatures during germination.

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