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Yield and physiological quality of seeds of different bean genotypes produced in the off-season period in subtropical climate

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Abstract

The objective of this work was to evaluate the yield and physiological quality of seeds of different bean genotypes produced in the off-season period in the subtropical region of Rio Grande do Sul state. The work was divided in two experiments. The first one evaluated the number of pods per plant, number of seeds per plant, number of seeds per pod, thousand seed weight and seed yield per plant of five bean genotypes (IPR Tuiuiú, BRS Embaixador, Guabiju, Carioquinha and Mouro), during 2013 and 2014 off-season periods. In the second study, the physiological quality of the seeds such as germination, first germination count, shoot and root dry matter were measured under different osmotic potentials (0.0, -0.09, -0.18, and -0,27 MPa) at temperatures of 25 and 35°C. Moreover, the isoenzymatic expression of the seedlings at the temperatures 25 and 35°C, of the seeds from the 2013 crop was evaluated. The results showed that IPR Tuiuiú and Carioca genotypes have superiority in yield components, whereas the BRS Embaixador showed the highest Thousand seed weight. The physiological quality of the seeds was reduced in the lower osmotic potentials at both temperatures. The expression and intensity of isoenzyme peroxidase bands were higher in potencies of -0.09 and -0.18 MPa for all genotypes at both temperatures.

Keywords: *Phaseolus vulgaris*, agricultural crops, vigor, isoenzymes.

Abbreviations: PEG_ Poly Ethylene Glycol, G_ germination, PCG_first germination count, W_{PA}_ dry mass of aerial part, W_R_ root dry mass, Ve_ BRS Embaixador cultivar, Tu_ IPR Tuiuiú, Pe_Guabiju, Ca_Carioquinha, Mo_ Mouro, NPP_Means of number of pods per plant, NSP_number of seeds per plant, NS/P_number of seeds per pod, TGW_thousand grain weight, SY_seed yield per plant. of five common bean genotypes in the off-season period of the 2013 and 2014 agricultural years.

Introduction

The common bean (*Phaseolus vulgaris* L.) is a major source of protein for most of the human population, having economic and social importance. Common bean production in Brazil is approximately 3.3 million tonnes, reaching an average yield of approximately 1 t ha^{-1}. In the state of Rio Grande do Sul, the production is approximately 974,000 tons with an average yield of about 1.7 t ha^{-1} (Conab, 2017).

In Brazil, common bean is grown in three agricultural seasons and subjected to different environmental conditions (Melo et al., 2007). The yield of agricultural crops is likely to decrease because of adversities in temperature, luminosity, and excess or lack of rainfall, which are limiting factors for grain production (Fioreze et al., 2011). In Rio Grande do Sul, the occurrence of water deficit is common during the common bean growing period, in which the yield reductions for this crop are expected (Matzenauer et al., 2004).

The germination conditions of seeds in the soil such as water stress are sometimes adverse (Szareski et al., 2016). The reduction of seeds germination under water stress is attributed to the reduction of the enzymatic activities. On the other hand, the seeds (especially the driest ones) can absorb water quickly under full availability of water in the soil, causing ruptures in their tissues, with consequent damages to the germination. Beans are very sensitive to the lack of water after after sowing (Neto et al., 2006; Marrou et al., 2006). Values below (-0.35MPa) can dramatically reduce seed germination and elongation (Durval and Fancelli, 2000). However, in a laboratory experiment germination was occurred even after six days under water deficient condition at -1.5MPa in seeds of bean cultivars (Lemos and Machado Neto, 1999; Machado Neto et al., 2003).

Unfavorable environmental conditions, such as high temperatures and water stress cause effects on cultivated plants. Characterization of common bean genotypes subjected to these stressful environments can assist making decisions about the use of these genotypes in adverse conditions, maximizing yield and seed production with high quality.

The water absorption by seed is an essential physical-chemical process to increase the metabolic activity after physiological maturity. Water is capable of dissolving large amounts of compounds with high chemical potential and plays a key role in the germination process by allowing tissue rehydration, intensification of respiratory activity and other metabolic processes seeking the synthesis of new compounds, hydrolysis, and translocation of assimilates to the embryo (Peske et al., 2012).

The seed germination process can be compromised by negative water potential that influences water absorption by seeds (Lopes and Macedo, 2008; Peske et al., 2012) and in the expression of isoenzymes (Devi et al., 2007; Troyjack et al., 2017). The expression of seed vigor, initial plant growth, and isozymes expression may vary depending on the genotype and temperature (Facin et al., 2014; Dubal et al., 2016).

This study aimed to evaluate the yield and seed physiological quality of different common bean genotypes grown in the off-season period in Rio Grande do Sul.

Results and Discussion

Yield of genotypes grown during the off-season period

The NPP was higher in Carioca genotype during the 2013 off-season period, while there were no differences between plants of the Carioca and IPR Tuiuiú genotypes during the 2014 off-season period (Table 2). In both off-season periods, Guabiju genotype had the lowest pod yield compared to the other genotypes. Pod production is attributed to the availability and incident of solar radiation on plants (Table 1), photoperiod and temperature, which influence the number and the development rate of the reproductive primordia (Jiang et al., 2011).

The traits NSP and NS/P showed different behavior among genotypes in both agricultural years for the off-season periods (Table 2). In the 2013 off-season period, Carioca genotype produced more number of seeds compared to the other genotypes. Meanwhile, in the 2014 off-season period, the greatest NSP and NS/P were obtained by IPR Tuiuiú genotype. Environmental conditions are important factors for the crop yield performance (Table 1), where different environmental conditions among years may interfere on the number of grains per area (Sousa and Lima, 2010).

The TGW was greater in the BRS Embaixador genotype in the off-season period in both agricultural years (Table 2). The lowest TGW among all genotypes was obtained in plants of the Carioca genotype grown in both off-season periods. These lower values are phenotypic characteristics and related to high seed production of this genotype, as seen by the largest NSP (Table 2).

The mass accumulation in seeds is related to the production of assimilates in the leaves and allocated in seeds (Lopes and Lima, 2015). Moreover, it is determined by genotype characteristics and environmental conditions of solar radiation and temperature (Mittler and Blumwald, 2010).

The seed yield per plant (SY) in 2013 off-season period was higher for the genotypes BRS Embaixador, IPR Tuiuiú, and Carioca (Table 2). By analyzing the 2014 off-season period, the SY per plant was greater for IPR Tuiuiú genotype. In the same agricultural year, the lower SY of BRS Embaixador genotype can be attributed to the decrease in the NPP and NSP combined with lower adaptation in the off-season cropping. Moreover, the decrease of temperature and solar radiation over time (Table 1) influenced the growth and seed filling (Cruz et al., 2010).

Common bean is sensitive to environmental changes during flowering (Didon and Silva, 2004) and may cause flower abortion and drop of pods (Silva et al., 2006). According to Stülp et al. (2009) and Alcantara et al. (2011), sowing outside the appropriate time can cause a reduction of 50% in SY of soybeans and change the chemical composition of the produced grains.

Quality of seeds under water restriction

From the analysis of the results, there was no significant interaction between water restriction and analyzed genotypes (Fig 1). The seed and seedling performance of the five common bean genotypes on the different water restrictions was simulated by polyethylene glycol (PEG).

Seed germination at 25°C was reduced in genotypes Guabiju, Mouro, and BRS Embaixador, respectively, by water restriction (Fig 1a). The genotypes IPR Tuiuiú and Carioquinha presented similar trend and the highest germination values observed under better water potential, when compared to other genotypes. Meanwhile, in temperature of 35°C (Fig 1b), the same results like those at 25°C were observed (Fig 1a). However, lower values were obtained by the Mouro genotype at 35°C.

Studying the seed germination and vigor of maize hybrids under water deficit, Kappes et al. (2010) found that the germination is reduced by lowering the osmotic potential. Similar results were found by Carvalho et al. (2013), where the seed performance of common bean evaluated under the action of different osmotic potentials simulated by polyethylene glycol.

The FGC of seeds of all genotypes showed a quadratic trend with high coefficients of determination (Fig 1c, 1d). There was a reduction in germination by decreasing the osmotic potential of the solution to all seeds of genotypes at 25ºC. More drastic results were observed in Guabiju, while the best results were obtained by the IPR Tuiuiú and Carioquinha (Fig 1c). Meanwhile, the highest values of FGC were found in IPR Tuiuiú and Carioquinha at 35°C (Fig 1d). However, the Mouro genotype presented the lowest values of FGC with the reduction of osmotic potential at this temperature (Fig 1d).

These results are probably due to the occurrence of low water availability for seed germination process, causing a negative influence on the process of respiration, mobilization of reserves, hydrolytic enzymatic activity and in the activation of different physiological processes related to germination and vigor (Peske et al ., 2012).

The APDM presented tendency with high coefficient of determination ($R^2 \geq 0.88$) to decrease with the osmotic potential at 25°C (Fig 1e). At this temperature, more severe results were imposed by the osmotic potential on growth attributes of BRS Embaixador, Mouro, Carioca, IPR Tuiuiú,

Table 1. Temperature (T), relative humidity (RH), and solar radiation (SR) incidents in Ametista do Sul - RS during the eoff-season periods of 2013 and 2014 agricultural years. Source: Federal University of Santa Maria (UFSM) - Campus of Frederico Westphalen - RS in 2014.

Months	T (ºC)		RH (%)		SR
	Maximum	Minimum	Maximum	Minimum	(kJ m⁻²)
	2013				
January	23.91	22.3	72.37	65.11	1,100.2
February	23.54	22.05	80.13	73.71	913.0
March	20.75	19.51	82.23	76.96	684.0
April	19.82	18.22	77.08	69.75	944.4
	2014				
January	25.01	23.51	80.52	73.42	1,111.8
February	25.46	23.82	73.20	65.33	1,038.75
March	22.02	20.64	80.54	74.22	806.24
April	20.14	18.97	81.99	76.89	752.78

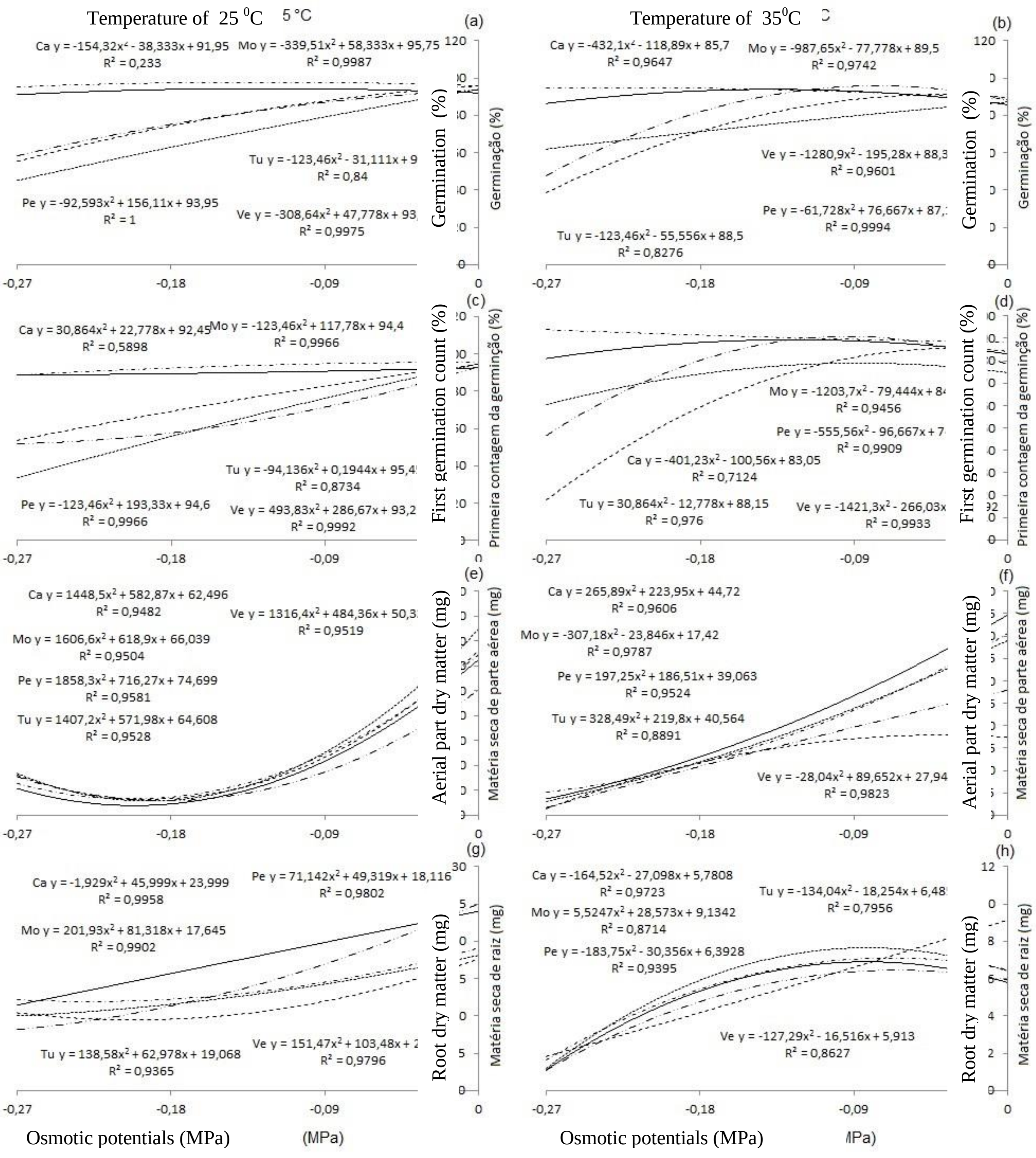

Fig 1. Germination (a, b), first germination count (c, d), aerial part dry matter (e, f); and root dry matter (g, h) of seeds and seedlings of common bean genotypes under different osmotic potentials in the temperatures of 25°C and 35°C.

Table 2. Means of number of pods per plant (NPP), number of seeds per plant (NSP), number of seeds per pod (NS/P), thousand grain weight (TGW), and seed yield per plant (SY) of five common bean genotypes in the off-season period of the 2013 and 2014 agricultural years.

Genotypes	NPP	NSP	NS/P	TGW(g)	SY(g)
2013					
Embaixador	10.6c[1]	38.2d	3.7c	450.9a	17.2a
Tuiuiú	12.8b	55.1b	4.8a	293.9c	17.5a
Guabiju	11.6bc	38.7d	3.3d	334.0b	12.9c
Mouro	11.2c	46.9c	4.2b	339.7b	14.3b
Carioquinha	16.9a	61.2a	3.9c	259.4d	18.2a
CV (%)	19.1	9.7	14.8	3.0	16.0
2014					
Embaixador	8.1d	25.9d	3.3c	391.3a	10.2d
Tuiuiú	19.4a	106.3a	5.4a	187.1e	19.6a
Guabiju	12.6c	41.5c	3.3c	297.5c	12.8c
Mouro	13.4c	44.8c	3.4c	374.6b	16.9b
Carioquinha	18.7a	79.9b	4.3b	208.9d	16.7b
CV (%)	9.2	13.1	9.7	2.9	13.9

[1]Means followed by the same letter do not differ among themselves by Tukey test ($p<0.05$).

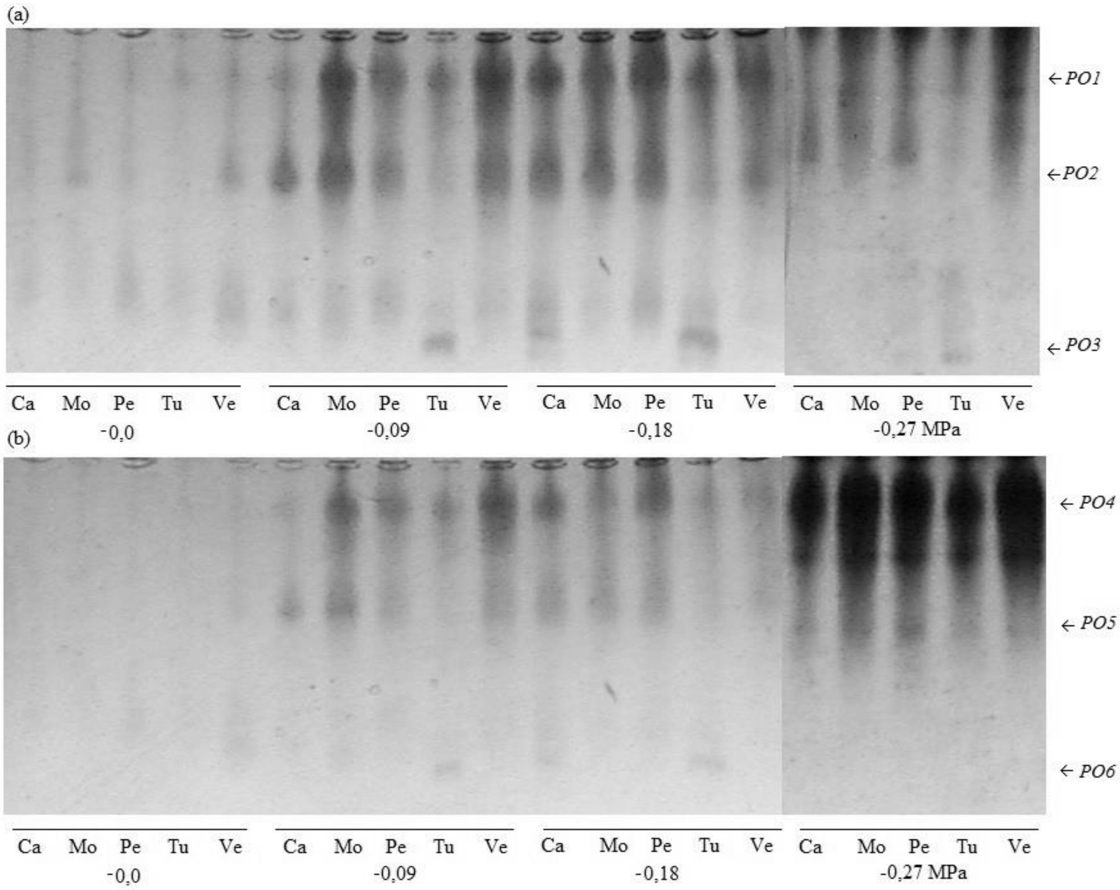

Fig 2. Expression of the peroxidase isoenzyme in seedlings of different common bean genotypes (BRS Embaixador (Ve), IPR Tuiuiú (Tu), Guabiju (Pe), Carioquinha (Ca), and Mouro (Mo) submitted to different water potentials with temperatures of 25°C (a) and 35°C (b), where PO1, PO2, PO3 indicate the band expression region of the peroxidase enzyme at temperature 25ºC. The PO4, PO5 and PO6 indicate band expression at temperature 35ºC.

and Guabiju, respectively (Fig1e). Meanwhile, the lowest growth was obtained by genotypes Mouro, BRS Embaixador, Guabiju, IPR Tuiuiú and Carioca at 35°C (Fig 1f). The RDM of seedlings of five genotypes subjected to water restriction and exposed to temperatures of 25ºC and 35ºC presented reduction in the osmotic potential of -0.27 MPa (Fig 1g and 1h). The lower dry matter values of seedlings in the lower osmotic potentials are explained by lower utilization of assimilates stored in the endosperm and by the lack of available water for growth (Peske et al., 2012).

Isoenzymatic expression

The peroxidase isoenzyme demonstrated higher expression in the number and intensity of bands, while the osmotic potential was reduced at temperatures of 25°C and 35°C (Fig 2a; 2b). There was the expression of three alleles for both seedlings produced in 25°C and 35°C, with greater intensity in the potentials of -0.09 and -0.18 MPa. Seedlings of IPR Tuiuiú genotype presented higher intensity of bands for both temperatures (25°C and 35°C). The increased expression such as number and intensity of peroxidase under more negative water potentials may somehow explain the reduction in germination of common bean seeds (Fig 1a, 1b). This indicates that these potentials cause a stress condition by exercising negative influence in the seeds during germination process. The peroxidase isoenzyme is related with defense mechanisms regarding several abiotic stresses (Lee et al., 2001; Hsu and Kao, 2003; Aisenberg et al., 2016) and constitute antioxidative protection (Rossi et al., 1997) on the exposed seedlings against adverse environmental conditions. The reduction in the activity of this isoenzyme may affect the seed performance (Henning et al., 2010; Pedó et al., 2016), as observed with reduced seed quality (Fig 1).

Materials and Methods

The experiment was carried out in the city of Ametista do Sul, RS. Southern Brazil located at latitude of 27°20'20.98"S and longitude of53°11'5.32"W, and altitude of 322 m. According to Köppen climate classification, the climate of region is Cfa, ie, humid subtropical with an average annual temperature of 19.1°C, varying with maximum of 38ºC and minimum of 0°C (Alvares et al., 2013). The soil of the experimental area is classified as Oxisol distrofic typical, clayey, deep and well drained. For sowing the adjustment of the fertilization was carried out according to the fertilization and liming manual (Embrapa, 2006; CQFS RS/SC, 2004). Soil chemical analysis showed pH of 6.3, CEC at pH7 of 21.1cmol$_c$ dm^{-3}, base saturation of 83%, organic material of 2.4%, texture at 3, clay at 31 %, phosphorus of 50.3 mg dm^{-3}, potassium of 190 mg dm^{-3}, calcium of 13.6 cmol$_c$ dm^{-3}, magnesium of 3.5cmol$_c$ dm^{-3}, and sodium of 13 mg dm^{-3}. Data of temperature, relative humidity, and solar radiation were obtained from the bulletin of the Agrometeorological Station of the Federal University of Santa Maria, campus of Frederico Westphalen – RS (Table 1). The research was divided into two phases, as follows:

Yield assessment of genotypes grown during the off-season period

The experimental design was randomized blocks with five replications per treatment of 25 plants. The treatments consisted of five genotypes assessed for yield performance during two agricultural off-season periods (2013 and 2014).

The seeds were sown in early January during the off-season period during the 2013 and 2014 agricultural years. Seeds of five common bean genotypes were used: BRS Embaixador (large dark red commercial group), IPR Tuiuiú and Guabiju (black commercial group), Carioquinha (carioca commercial group), and Mouro (brindle trade group). The seed harvest was carried out when they reached humidity of 16%. Plants were harvested and processed manually with removal of inert material and visually damaged seeds. The post-harvest storage of seeds of genotypes occurred in a cold and dry chamber (10°C/45%) during the period of performing experiments.

The following variables were used to assess the yield attributes:

Number of pods (NPP) per plant and number of seeds per plant (NSP) were obtained by direct counting of the number of pods and seeds per plant.

Number of seeds per pod (NS/P) was determined by dividing the total number of seeds per plant by the total number of pods per plant;

Thousand grain weight (TGW) was obtained by counting eight replications of 1000 seeds and expressed in grams (Brazil, 2009).

Seed yield per plant (SY) was determined from the seed weight, expressed in grams per plant.

Evaluation of the physiological quality of seeds

For evaluation of the physiological and isoenzymatic quality, only the seeds produced in the 2013 crop were used. The seeds were subjected to the same post-harvest procedure used in "phase a" of this research. In order to assess water stress tolerance, five genotypes were used (IPR Tuiuiú, BRS Embaixador, Guabiju, Carioquinha, and Mouro) and four osmotic potentials (0.0, -0.09, -0.18, and -0.27 MPa) at two temperatures (25°C and 35°C) were applied during seed germination.

Water restriction was established from preliminary trials through solutions composed by distilled water and different concentrations of polyethylene glycol (PEG-6000), using the ratio between mass and volume (m/v). The following tests were performed in order to evaluate the influence of water deficit on the seed physiological performance and isozyme expression in common bean seedlings:

Germination test (G) was performed with eight replications of 50 seeds disposed to germinate in rolls formed by three *germitest* paper sheets, moisturized with distilled water in the amount of 2.5 times the dry weight of the dry paper. The rolls were transferred to the B.O.D. germination chamber at 25°C and 35°C and photoperiod of 12 hours. The assessments were performed at nine days after sowing (DAS) and the results expressed as a percentage of normal seedlings, according to the Rules of Seed Analysis (Brazil, 2009).

First germination count (FGC) was performed together with the germination test at five DAS, according to the Rules of Seed Analysis (Brazil, 2009). The results were expressed as a percentage of normal seedlings.

Aerial part dry matter (APDM) and root dry matter of seedlings (RDM) were obtained by the measurement of four samples of 10 seedlings at the end of the germination test. The seedlings were placed in brown paper envelopes and dried in forced ventilation oven at a temperature of 70°C for 72 hours. The results were expressed in milligrams per organ (mg organ^{-1}).

Isoenzymatic expression

Isoenzyme expression: the plant material used for the determination of isoenzyme expression was obtained by collecting 10 seedling of the germination test at nine DAS. The expression of peroxidase isoenzyme was determined by vertical electrophoresis system in polyacrylamide gel. The electrophoresis was performed in 7% polyacrylamide gels, applying 20 µL of each sample. In this study, the coloring systems described by Scandálios (1969) and Alfenas (1998) were used. Furthermore, the interpretation of the results was through visual inspection of the gels, with the presence or absence of expression and intensity of band expression.

Statistical procedures

The data were subjected to the Shapiro-Wilk test for normality (1965) and variation of variances homogeneity by Hartley (Ramalho et al., 2012). Subsequently, they were subjected to analysis of variance. If the data was significant by F test, the means were compared by Tukey test at 5% probability ($p<0.05$). While the data related to the germination and initial growth were subjected to analysis of variance and if significant at 5%, they were adjusted by orthogonal polynomials.

Conclusion

The IPR Tuiuiú and Carioca genotypes showed superiority in yield components, whereas the BRS Embaixador showed the highest thousand seed weight. The physiological quality of the seeds was reduced in the lower osmotic potentials at both temperatures. The expression and intensity of isoenzyme peroxidase bands were higher in potencies of -0.09 and -0.18 MPa for all genotypes at both temperatures.

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