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Effect of temperature on bean seed germination: vigor and isozyme expression

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ABSTRACT

This research aimed to evaluate the physiological quality of seeds and isozyme expression in seedlings of bean's genotypes under influence of germination temperatures. Seeds of Carioca, BRS Expedito and IPR Tuiuiú genotypes were subjected to five germination temperatures (15, 20, 25, 30 and 35 °C). Seeds were submitted to tests of germination, first count, germination speed index, cold test, isozyme expression and the total dry matter of seedlings. Germination was altered due to the different temperatures. As the temperature rose, seeds of IPR Tuiuiú and BRS Expedito had reduced germination. The increase on temperature significantly affected the germination speed index of the three genotypes, leading to a greater increase in the values of this attribute. Bands of esterase from the cold test showed less intensity compared to other tests. Due to the exposure to different temperatures, there are similarities between the number and intensity of bands on esterase, with lower enzyme expression at 30 and 35 °C, changes in the number and intensity of peroxidase expression at all temperatures, and practically none expression of the acid phosphatase enzyme in higher evaluated temperatures. Therefore, the seeds exhibit better performance when exposed to temperature of 30 °C compared to use of lower temperatures, while on temperatures above 30 °C isozymes expression is reduced for both genotypes.

Keywords:
Phaseolus vulgaris L., physiological quality, dry matter, isozymes

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Introduction

Beans (*Phaseolus vulgaris* L.) is a species of great importance in human food because its grains present high protein content and energy. It is a crop that has diversified farming systems, being produced in all Brazilian Regions. National production of beans in 2015/2016 growing season was 3.3281 million tons, an increase of 6.8% compared to the previous (CONAB, 2016). The low grain yield is related to the low rate of use of certified seed, corresponding to 19% in 2013/2014 growing season (ABRASEM, 2014). In order to obtain high quality seeds is necessary to have an appropriate and uniform stand of plants in the production field, as this can reflect on seed quality. The seeds have different quality attributes, among them stands out the physiological. Seed quality can be influenced by inherited genetic aspects of their parents, and environmental conditions of the growth site (ANDRADE et al., 2001).

Seeds physiological quality is a decisive point in seed development and can be reduced through the process of deterioration that occurs during germination (ABREU et al., 2014). Germination is the resumption of embryo growth in optimal conditions of temperature (PESKE et al., 2012), while the vigor is expressed in adverse conditions during seedling emergence (CARVALHO; NAKAGAWA, 2012), being influenced by conditions environment in which the seeds are produced.

Climatic variations affect plant development. The temperature is the climatic element that most influence the beans crop; it can delay / reduce seed germination and seedling emergence (TEIXEIRA, 2000). Temperatures below 15 °C negative influence on germination and hypocotyl elongation (ZABOT et al., 2008), while high temperatures can cause abscission of flowers and lower final retention of the pods (BARBOSA; GONZAGA, 2012).

Seed's exposure to high temperatures can reduce germination due to deteriorative processes (MARINI et al., 2013), protein denaturation and inactivation of enzymes (VIEIRA, et al., 2010). In lower temperatures, however, seeds' enzymatic activity is reduced, restricting the germination process (MATHEUS; LOPES, 2009). The stress caused in the seedlings by temperature can be associated with the hydrolysis of reservations (DEVI et al., 2007) and enzymatic expression. Enzymes act in the synthesis and degradation of molecules during seed germination and early seedling growth (MUNIZ et al., 2007; VEIGA et al., 2010; PIMENTEL, 2012). In recent years, unfavorable environmental conditions have been affecting the initial establishment and development of crops in the field, being essential to assess the effects caused by adverse temperatures on seed germination and isozyme profile of bean's seedlings.

In this context, the aim of this study was to evaluate the physiological quality of seeds and seedlings isozyme expression of different beans genotypes under germination temperature effect.

Material and methods

Seeds were produced in the city of Ametista do sul (Latitude 27 ° 20'20.98 "S and Longitude 53 ° 11'5.32" W), in the state of Rio Grande do Sul. Since the climate of the region characterized by being tempered with rains well distributed and hot summer, according to Köppen. After harvesting, quality analysis was performed in laboratory of the Graduate Program in Seed Science and Technology of Faculty of Agronomy "Eliseu Maciel", Federal University of Pelotas.

The experimental design was a randomized complete block in a factorial treatment design 3 x 5, where three genotypes (Carioca (Ca); BRS Expedito (Ex) and IPR Tuiuiú (Tu)) and five germination temperatures (15; 20; 25; 30 and 35 ºC) arranged in eight replications. To evaluate the physiological quality of seeds and isozyme expression of seedlings, they were subjected to the following tests:

Germination test (G): was conducted with eight replicates of 50 seeds, subjected to germination in rolls of germitest paper, moistened with distilled water 2.5 times the dry weight of the papers. After distributing the seeds, germitest paper was wound in order to form rolls, which were subsequently transferred to a growth chamber "B.O.D.". Using the temperatures described above, with light period of 12 hours. The evaluations were performed seven days after sowing and the results expressed as a percentage of normal seedlings, according to Rules for Seed Testing (BRASIL, 2009).

First germination count (FGC): conducted together with the germination test, where assessments were made four days after sowing, according to Rules for Seed Testing. The results were expressed as percentage of normal seedlings.

Germination speed index (GSI): was carried out together with the germination test, by counting the seedlings with minimal root protrusion of 3 to 4mm. Annotations were daily-obtained, at the same hour, and the assessment carried out up to stabilization of values. The GSI was calculated according to Vieira; Carvalho (1994).

Total dry mass (TDM): estimated by determining the mass of four replicates of 10 seedlings at the end of the germination test. Seedlings were placed in brown-paper envelopes and dried in forced-ventilation oven under temperature of 70 °C up to constant mass. Passed this time, dry mass of seedlings was assessed with precision scale, where results were expressed in milligrams per organ (organ mg^{-1}).

Cold test (CT): Carried out in four replications with four replicates of 50 seeds each, germinating in rolls formed by three sheets of germitest paper, wetted with distilled water 2.5 times the dry weight of the paper. After sowing, the rolls were kept in the type germination chamber B.O.D. under temperature of 10 °C for five days (KRZYŻANOWSKI et al., 1999). After this period, seeds were transferred to B.O.D. chamber, regulated at 25 °C, where assessments were carried out according to Rules for Seed Testing (BRASIL, 2009).

Isozyme expression: the expression of esterase, acid phosphatase and peroxidase isoenzymes was determined on 10 seedlings coming from germination and cold test and are expressed by vertical electrophoresis system on 7% polyacrylamide gels, applying 20µL of each sample. Coloring systems used were based on Scandalios (1969) and Alfenas (1998). The results were visually-evaluated in the gels by the presence or absence and intensity of bands' expression.

Data were subjected to two-way ANOVA by F test at 5% probability error aiming discovering interaction between genotypes x germination temperatures. When observed significant interaction, the traits were partitioned into simple effects. For quantitative factor, linear regression analysis was carried out considering the largest-polynomial degree significant.

Results and discussion

According to two-way ANOVA, it was observed significant interaction between genotype x germination temperature for germination (G), first count (PCG), germination speed index (GSI) and total dry matter (TDM).

Bean's seed germination under different temperatures showed quadratic curve trend being changed depending on germination temperature (Figure 1a). By analyzing IPR Tuiuiú genotype, germination increased with increasing temperature, whereas increase in temperature has reduced the germination of BRS Expedito. However, germination of Carioca genotype did not present significance.

Variations in the germination values for both genotypes can be related to the tolerance of different genotypes on wide temperature interval, due to the high temperatures cause denaturation of proteins and increase the fluidity of the lipid membrane (WAHID et al., 2007). Similar results were found by Machado Neto et al. (2006), studying the effect of temperature on germination of bean seeds, obtained a decrease of this attribute at a temperature of 35 °C, becoming null at the temperature of 39 °C.

First count values had fitted the quadratic curve for the three beans genotypes, with tendency to increase due to the increase in temperature (Figure 1b). The largest number of normal seedlings in the evaluation of the first count of germination occurred with the use of temperatures of 30.7; 31.2 and 35 °C, for the genotypes Carioca, BRS Expedito and IPR Tuiuiú respectively.

Sharp decrease in germination rate as the temperature gradually decreases, is explained by the low rate of water imbibition, affecting mobilization of reserves' seeds for embryo growth (MARCOS FILHO, 2015).

Germination speed index increased with rise in temperature (Figure 1c). The increase in temperature up to 35 °C had caused differences between GSI values of the three genotypes. The highest point was 35.0; 29.9 and 31.5 °C for IPR

Tuiuiú, Carioca and BRS Expedito genotypes, respectively. According to Bewley et al. (2014) GSI, is sensitive to temperature, generally increasing until it reaches the ideal temperature, and decreasing sharply above the optimum temperature.

Total dry mass was fitted by a quadratic curve, with coefficients of determination (R^2) above 0.70. The genotypes tended to show higher TDM with increase on temperatures tested (Figure 1d). Overall, for the three genotypes, TDM of seedlings showed an increase between 25 to 30 °C. The highest point was 28.8; 27.1 and 29.1 °C for Carioca BRS Expedito and IPR Tuiuiú genotypes, respectively.

Dry mass accumulation can be reduced by low temperatures (GUERRA et al., 2014). According to Marcos Filho (2015) at low temperatures, reduced seedling growth can occur, and this damage is usually proportional to the period of exposure at that temperature, and can also extend the problem during the rest of the crop development cycle.

Esterase isozyme showed similar number and intensity of bands for genotypes when subjected to different germination temperatures (Figure 2). In the cold test the intensity of the bands was lower than the other (Figure 2a) when can be observed low expression of esterase enzymes by allele view (EST1 and EST2). Already, at temperatures of 15, 20 and 25 °C bands expression was similar, with largest intensities (Figure 2b, 2c and 2d).

It was possible to identify the presence of three alleles at temperatures of 15 and 25 °C, and of two alleles using 20 ºC. However, when seedlings were exposed to high temperatures (30 to 35 ºC) there was a reduction in enzyme expression (EST11 up to EST15) (Figure 2e and 2f) observing reduction in expression of the *EST11* and *EST15* alleles in all genotypes. Given that, the esterase is an enzyme that acts on ester hydrolysis reactions, being directly related to isozymesthe metabolism of lipids (SANTOS et al., 2004), the reduction of the intensity of bands indicates a possible deterioration of seed, resulting from its exposure to high temperatures.

According to Basavarajappa et al. (1991), lipid peroxidation is a phenomenon related to damage membrane of the seeds; the changes may be expressing the occurrence of deteriorative events, which can result reduction on seed germination, to the extent that temperature and water content seed are increased.

Peroxidase isozyme had altered the number and expression of their bands when subjected to different temperatures of germination. There was no expression of peroxidase in seedlings coming from cold test and at temperatures of 15; 20 and 25 °C (Figure 3a, 3b, 3c and 3d). There was greater intensity of this bands in seedlings growing under higher temperatures for the three genotypes. However, changes in enzyme expression was markedly started at temperature of 30 °C, where it is observed the presence of four alleles (Figure 3e). At the temperature of 35 °C, however, there was decreasing in expression and under presence of only three alleles (Figure 3f).

The greater expression of peroxidase on temperature up to 30 °C may be related to lipid peroxidation and alterations in the permeability of cell membranes by temperature (ROSSI; LIMA, 2001).

Acid phosphatase isozyme showed a higher number and intensity of bands in seedlings at cold test and at temperatures of 15; 20 and 25 ºC, being possible to identify the presence of two alleles in each temperature (Figure 4a, 4b, 4c and 4d). When higher temperatures are used, there was reduction of acid phosphatase expression, and the presence of only one allele at 30 °C (Figure 4e) and two alleles at 35 °C (Figure 4f).

Acid phosphatase acts on ester hydrolysis and may act on phospholipid membranes, causing lipid peroxidation (SILVA et al., 2000). This enzyme is associated with the mobilization of storage proteins, especially during germination and seedling growth (GOMES et al., 2000). According to Spinola et al. (2000) the decrease in the number and intensity of bands of acid phosphatase enzyme, when corn seeds were subjected to accelerated aging is characterized by lipid peroxidation, fact that may have reduced the expression of this isoenzyme in bean seedlings in higher germination temperatures.

Generally, it appears that the use of temperatures up to 30 ° C favors the performance of seedlings,

thus causing increases in the first count of germination, germination speed index and total dry mass of seedlings. Thus, for conditions in which this research was performed, when higher it was the stress provided to seeds, smallest it was the expression of the isoenzymes.

Conclusion

Bean genotypes have different germination characteristics when subjected to elevated temperatures. Generally, the seeds exhibit better performance when exposed to higher temperatures, compared to use of lower temperatures, except for the BRS Expedito. The use of temperatures above 30 °C negatively affects the expression of esterase and acid phosphatase isozymes for all genotypes.

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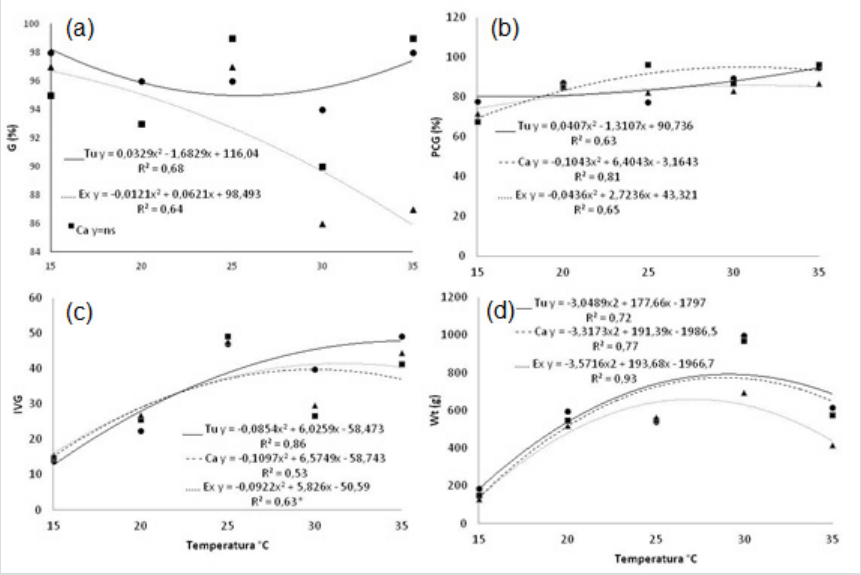


Figure 1 - Germination (a) first count (b) germination speed index (c) and total dry mass (d) of bean's genotypes subjected under different germination temperatures. Where: Carioca (Ca), BRS Expedito (Ex) and IPR Tuiuiú (Tu). ^{ns} = not significant.

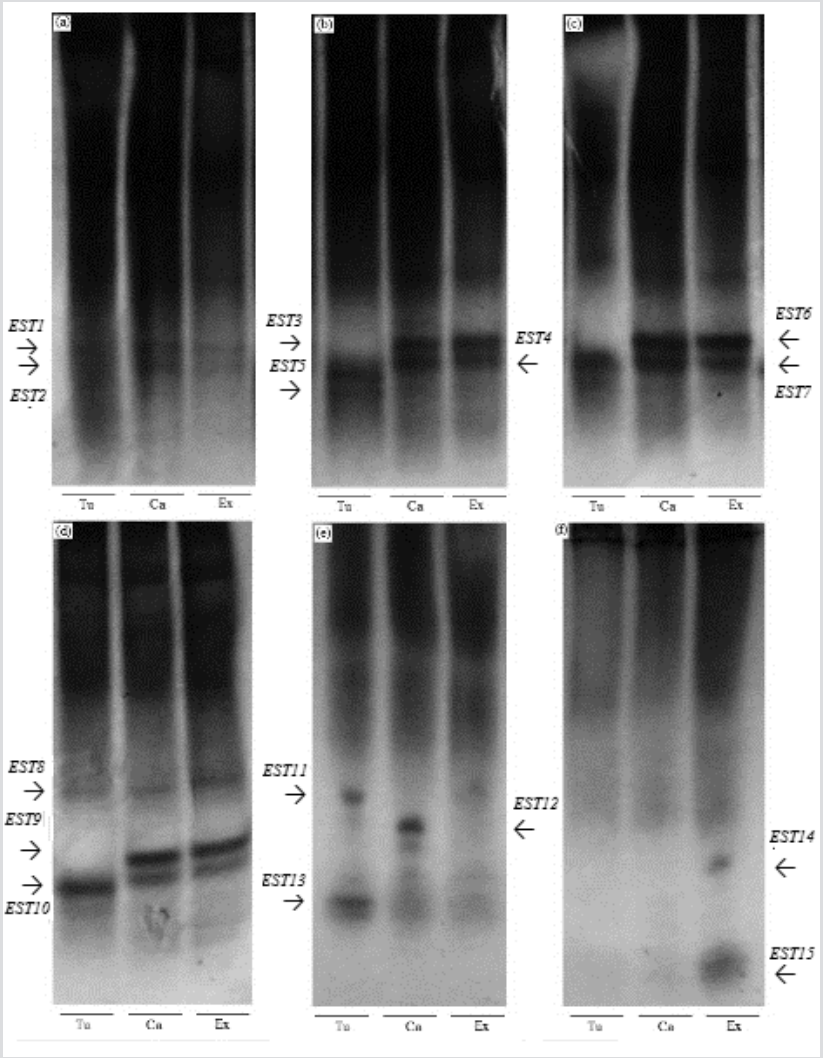


Figure 2 - Isozyme expression of esterase activity in bean seedlings growing under different temperatures. Where: Cold test (a), 15 (b), 20 (c), 25 (d), 30 (e) and 35 °C (f). Where: Carioca (Ca), BRS Expedito (Ex) and IPR Tuiuiú (Tu).

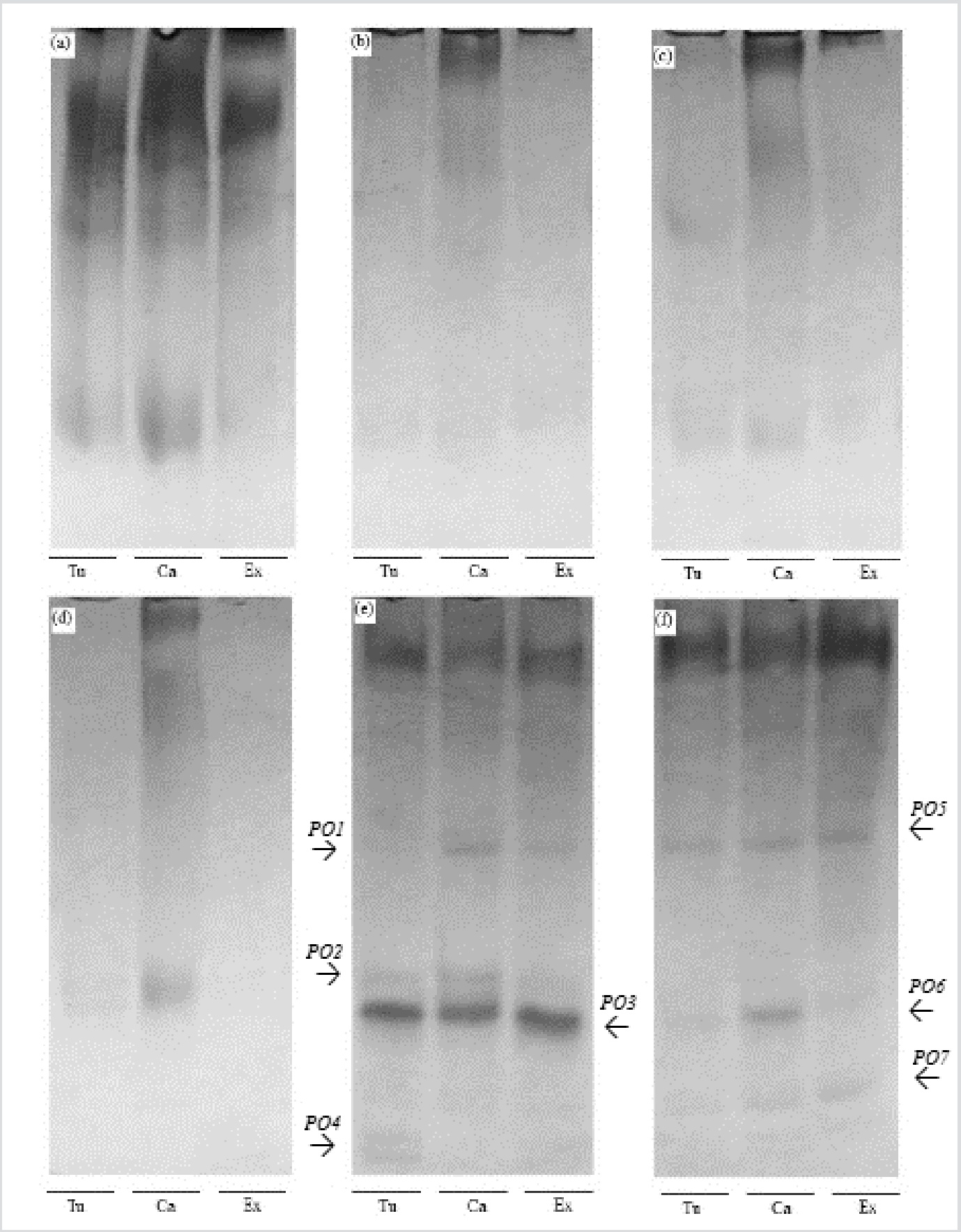
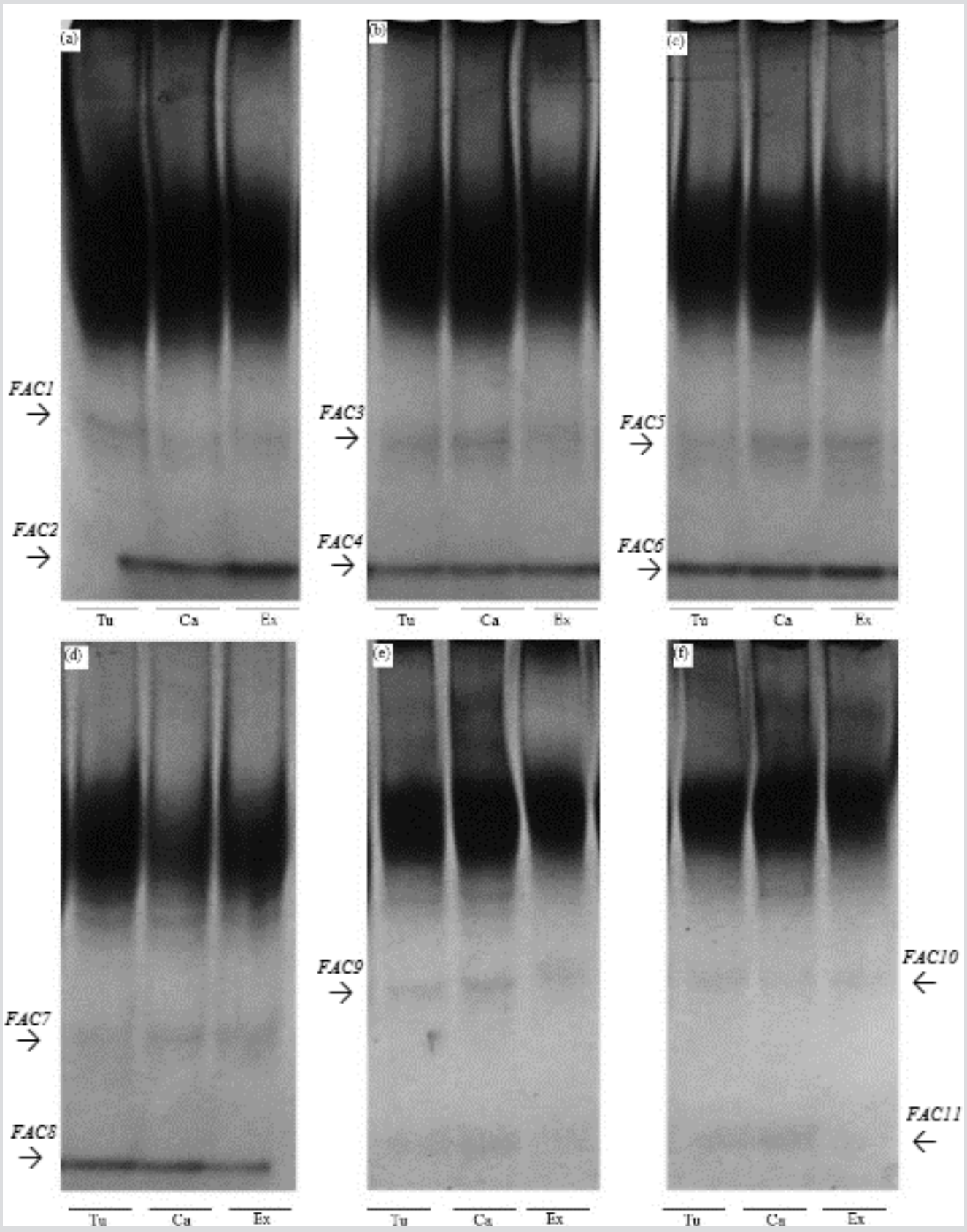


Figure 3 - Isozyme expression of peroxidase activity in bean seedlings growing under different temperatures. Where: Cold test (a), 15 (b), 20 (c), 25 (d), 30 (e) and 35 °C (f). Where: Carioca (Ca), BRS Expedito (Ex) and IPR Tuiuiú (Tu).



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