

## CROSS TOLERANCE BY HEAT AND WATER STRESS AT GERMINATION OF COMMON BEANS

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**ABSTRACT** - Common beans are legumes of extreme importance for the food security of developing countries. Because it is widely cultivated in tropical and subtropical regions, it is subject to the stress effects caused by this type of climate, such as water stress and heat. Cross-tolerance allows plants to acclimate to a range of different stresses after exposure to a specific short-term stress. The present work had as objective to evaluate the induction of cross-tolerance by heat stress at 38°C and water stress simulated by polyethylene glycol (PEG) in 3 different osmotic potentials in the germination of the Brazilian cultivars IPR Uirapuru and IPR Campos Gerais in a randomized block design, setting up a 2 x 2 x 3 factorial scheme, with eight replicates of 25 seeds. The following variables were evaluated: percentage of germination, root and shoot length, and fresh root and shoot mass. The data were submitted to Tukey test with 5% significance. The results indicate a possible memory to the primary stress when the IPR UIRAPURU and IPR Campos Gerais varieties are subjected to an initial stress of 38°C, making them better adapt to an osmotic stress at the evaluated stages. The results indicated that there was a statistically significant difference of the treatments applied in relation to the germination of the cultivars where the cultivar IPR Uirapuru obtained higher germination indexes for all treatments evaluated when compared to the cultivar IPR Campos Gerais. The results indicate that in both cultivars there was induction of cross tolerance by heat followed by water stress.

**Keywords:** *Phaseolus vulgaris* L., Brazilian beans cultivars, food security, memory stress.

## TOLERÂNCIA CRUZADA POR CALOR E ESTRESSE HÍDRICO NA GERMINAÇÃO DE FEIJÃO COMUM

**RESUMO** - O feijão comum é uma leguminosa de extrema importância para a segurança alimentar dos países em desenvolvimento. Por ser amplamente cultivada em regiões tropicais e subtropicais, está sujeita aos efeitos de estresse causados por esse tipo de clima, como o estresse hídrico e o calor. A tolerância cruzada permite que as plantas se adaptem a uma série de diferentes estresses após a exposição a um estresse específico de curto prazo. O presente trabalho teve como objetivo avaliar a indução de tolerância cruzada por estresse térmico a 38°C e estresse hídrico simulado por polietilenoglicol (PEG) em 3 diferentes potenciais osmóticos na germinação das cultivares brasileiras IPR Uirapuru e IPR Campos Gerais, em delineamento em blocos casualizados, configurando esquema fatorial 2 x 2 x 3, com oito repetições de 25 sementes. Foram avaliadas as variáveis: porcentagem de germinação, comprimento de raiz e parte aérea e massa fresca de raiz e parte aérea. Os dados foram submetidos ao teste de Tukey, com significância de 5%. Os resultados indicam uma possível memória ao estresse primário quando as variedades IPR UIRAPURU e IPR Campos Gerais são submetidas a um estresse inicial de 38°C, adequando-as melhor a um estresse osmótico posterior. Os resultados indicaram que houve diferença estatística significativa dos tratamentos em relação à germinação das cultivares onde a cultivar IPR Uirapuru obteve maiores índices de germinação para todos os tratamentos avaliados quando comparados à cultivar IPR Campos Gerais. Os resultados indicam que nas duas cultivares houve indução de tolerância cruzada pelo calor seguida de estresse hídrico.

**Palavras-chave:** *Phaseolus vulgaris* L., cultivares brasileiros de feijão, estresse de memória, segurança alimentar.

### INTRODUCTION

Beans (*Phaseolus vulgaris* L.) are a legume with wide climatic adaptation and its grains are of great importance for the nutrition of the population (Silva; Wander 2013). Although between 2012 and 2014 world production was 23.8 million tons, Brazil contributes with

only 13% of this percentage (FAO, 2012), being cultivated by small, medium and large producers. Its planting occurs practically during all months of the year and its production comes from almost the entire national territory (JOHANN et al., 2010).

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Brazil is divided into five regions that have their own characteristics and different climatic conditions. It is not uncommon occurrence of frost and snow in some locations in the south of the country, as well as temperatures exceeding 35°C. Some regions, such as in northern Brazil, the climate is humid and semi-humid, and in regions such as the Midwest rainfall is poorly distributed, and this region has very dry winters (REBOITA et al., 2018).

Data mentioned above report that the Brazilian climate presents conditions that can cause environmental stress for the bean production. These are limiting factors for productivity. Improving knowledge about how plants respond and can be affected by various environmental factors such as water and temperature deficits, as well as seeking new technologies for breeding is of paramount importance for the management of common bean production and can assist plant breeding programs (GHINI et al., 2011).

Water limitation and temperature rise affect crop productivity and limit the expansion of agriculture in many regions of the world (ZANDALINAS et al., 2017). Computational simulations predict an increase in temperature and decrease of available water in the coming years, which aggravates this scenario drastically (ZHAO; DAI, 2015).

Thus, as water limitation, stress caused by high temperatures causes numerous biochemical and metabolic changes in the cultivars, affecting growth and development by inactivating various metabolic pathways, as well as the reduction of their photosynthetic power (OSAKABE et al., 2014).

Water stress affects all aspects of shoot and root growth, usually causing reduction in cell expansion, stomatal conductance, photosynthesis and consequently dry matter accumulation (OSAKABE et al., 2014). In Latin America it is estimated that in 93% of the bean cultivation area, the water requirement of the plant during its entire development cycle is not satisfied (ROSALES-SERRA et al., 2004).

When plants are subjected to these conditions, several genes and proteins are activated, initiating signaling cascades that try to protect or defend the plant against these stresses. Studies suggest that many of these cascades are the same for both stresses, indicating the same type of perception of these events (PASTORY and FOYER, 2002; PEREZ and BROWN, 2014).

Studies with beans indicate that exposure to moderate stress followed by severe stress may prevent this plant from suffering further damage (HOSSAIN et al., 2018). This is due to the fact that both stresses share common signaling cascades, as previously reported. For this event, it is given the name of cross tolerance (RIZHSKY et al., 2002; PEREZ; BROWN, 2014). This may be an evidence of adaptive evolution forward the need for survival to various types of stress that plants are frequently submitted.

The literature shows several successful studies that demonstrate cross tolerance involving water deficit

and heat (RIZHSKY et al., 2002), heat and salt (SONG et al., 2005), heat and cold (TAKAHASHI et al., 1994), UV-B radiation and water deficiency (BORISOVA et al., 2001).

Despite numerous possibilities of studies for cross tolerance in Brazil for common bean, the combination of temperature and water stress is the most relevant for productivity. The high temperatures during the germinative process cause denaturation of proteins and alter the permeability of the membranes causing loss of cellular material. Therefore the importance of finding varieties with characteristics of resistance to different stresses. Thus, the objective of this work was to verify the existence of cross-tolerance in the Brazilian common bean cultivars IPR Uirapuru and IPR Campos Gerais against heat followed by water stress.

## MATERIAL AND METHODS

The work was conducted at the molecular biology laboratory of Paranaense University (UNIPAR), in the Umuarama city (Paraná), Brazil. The bean cultivars used were IPR Uirapuru and IPR Campos Gerais. Seeds of 2 lots, cultivars IPR Uirapuru and IPR Campos Gerais, of the company IAPAR (Instituto Agronômico do Paraná), harvest 2017/2018 were used.

For seeds surface disinfection, they were first immersed in distilled water. After washing in distilled water and 10% neutral detergent, followed by a 70% alcohol wash for 10 minutes, and a wash with 2.5% sodium hypochlorite for 15 minutes. After these procedures, seeds were washed 3 times consecutively in autoclaved distilled water.

The seeds were stored on 2 sheets of germitest paper, covered with one more sheet of the same paper. The paper was kept in roll form, and were moistened in the ratio of 2.5 times the dry mass (g) of the substrate (paper) (BRASIL, 2009).

A completely randomized design in a 2 x 2 x 3 factorial arrangement was used, two temperatures (20 and 38°C) and three osmotic potentials (0.0; -0.1 and -0.2 MPa) induced by PEG (polyethylene glycol) solution, with eight replications of 25 seeds each, with a total of 200 seeds per treatment. The seeds were submitted with a spacing of 5 cm in length and 4 cm in width, per block. The blocks were packed in a plastic bag. The seeds were all conditioned in greenhouse of biochemical oxygen demand (BOD), at a constant temperature of 20°C for 24 h. After that, half the treatments were subjected to thermal shock at 38°C for a further period of 24 h, while the other half remained at a temperature of 20°C.

After the period established for heat stress, the seeds that went through the heat stress, as well as those that did not pass, were transferred to 2 sheets of germitest paper, moistened with solutions of PEG for water stress simulation, covered with one more sheet of the same paper. The concentrations of PEG used were 0; 78.49 g L<sup>-1</sup> and 119.54 g L<sup>-1</sup>, simulating the osmotic potentials (OP) of 0; -0.1 and -0.2 MPa, calculated by the formula of Van't Hoff (BRAGA et al., 1999). The paper was kept in roll

form, and were moistened in the ratio of 2.5 times the dry mass (g) of the substrate (paper). After these procedures, all treatments were incubated in BOD at a temperature of 20°C, where they remained until the end of the experiment which lasted for another fourteen days.

Germination was evaluated considering asgerminated the seeds that developed primary root with a length equal to or greater than 2 mm. The number of germinated seeds was counted at the end of the experiment.

After fourteen days, the following evaluations were performed: weighing the seedlings with analytical balance to obtain their fresh weight and the length of the aerial part and the root, using a graduated ruler.

Data were subject to analysis of variance, using the F test, at 5% probability level. Means were compared by the Tukey's test at 5% probability level. Statistical analyses were performed using the software ASSISTAT

(version 7.7 beta) (SILVA; AZEVEDO, 2016) and Action Stat (version 3.5.152.34 build 4) (ESTATCAMP, 2014).

## RESULTS AND DISCUSSION

Seed germination capacity of cultivars IPR Campos Gerais and IPR Uirapuru were different ( $p < 0.05$ ). The effect of PEG and temperature on germination was not significantly.

The cultivars IPR Campos Gerais and IPR Uirapuru naturally presented different development parameters. When produced under control conditions (20°C and without PEG addition) the aerial part of the cultivar Uirapuru was 20% smaller than Campos Gerais (Table 1 and 2). Also, the cultivar Uirapuru had longer root length of 4.23 cm. Naturally the Campos Gerais cultivar presented highest aerial part and Uirapuru presented longer root. Thus the effect of temperature and osmotic potential on the plants development was analyzed separately for each cultivar.

**TABLE 1** - Seed germination of *Phaseolus vulgaris* IPR Uirapuru and IPR Campos Gerais variety submitted to different osmotic potentials (OP) and temperatures.

OP (Ψs)	Temperature (°C)	IPR Uirapuru variety	IPR Campos Gerais variety
0.0		22.50 a*	9.70 b
-0.1	20	21.00 a	11.60 b
-0.2		23.00 a	9.60 b
0.0		22.60 a	10.50 b
-0.1	38	21.50 a	10.50 b
-0.2		22.60 a	9.50 b
CV (%)		17.50	

\*Different letters in the same line differ statistically.

**TABLE 2** - Length and mass of shoot and root of *Phaseolus vulgaris* IPR UIRAPURU variety submitted to different osmotic potentials (OP) and temperatures.

OP (Ψs)	Temperature (°C)	Lenght (cm)		Mass (g)	
		Aerial part	Root	Aerial part	Root
0.0		12.33 a*	11.59 c	1.16 a	0.35 ab
-0.1	20	7.23 c	9.36 d	0.9 b	0.21 b
-0.2		12.15 a	14.07 ab	0.76 b	0.28 ab
0.0		9.52 bc	11.45 c	0.88 b	0.31 ab
-0.1	38	11.51 ab	14.88 a	0.93 b	0.39 a
-0.2		10.27 ab	12.78 bc	0.76 b	0.35 ab
CV (%)		16.31	10.52	15.39	29.20

\*Different letters in the same column differ statistically.

When we compared plants that endured osmotic stress (OP of - 0.1 Ψs), plants that did not undergo an initial temperature stress suffered a reduction of 59% in size. Zabot et al. (2008) reported in his study that the IPR UIRAPURU variety presented greater tolerance to stress at 38°C. This indicates that heat stress has helped the plant tolerate water stress. Plants submitted to osmotic stress (OP of - 0.2 Ψs) did not differ statistically in relation to the temperature difference.

When the parameter evaluated was root length, the temperature did not influence root growth without water stress (control at 20°C x 38°C). This result is

expected, since high temperatures first affect other parameters of development (JUMRANI; BHATIA, 2018).

For the plants submitted to osmotic stress (OP of - 0.1 Ψs), it was verified that those that had an initial high temperature stress at 38°C, had an increase of root growth in 58%, compared to those that did not suffer initial high temperature stress. This is an indication that plants undergoing initial stress better adapt to lack of water by elongating their root more efficiently than when the initial stress is not applied.

Some authors report that this better adaptation to a secondary stress is due to the fact that the plant acquires

a memory to the primary stress (ANTONIOU et al., 2016). One of the biochemical factors involved in high temperature cross-tolerance may be associated with Reactive Oxygen Species (ROS) accumulation, which play a role of cellular signaling along with MAPKS proteins, associated with cross-tolerance (GONG et al., 2001; TSUKAGOSHI, 2016).

Although the plants submitted to osmotic stress (OP of - 0.2  $\Psi$ s) did not differ statistically from each other, when the initial stress of 38°C was applied, the plants that underwent temperature stress had a root length decrease of 9% compared with the control. This result can be explained by the fact that severe osmotic stress greatly affects the bean crop, especially in moderately sensitive cultivars, and the plants present different responses according to the applied osmotic potential (COELHO et al., 2010).

**TABLE 3** - Length and mass of shoot and root of *Phaseolus vulgaris* IPR Campos Gerais variety submitted to different osmotic potentials and temperatures.

OP ( $\Psi$ s)	Temperature ( $^{\circ}$ C)	Lenght (cm)		Mass (g)	
		Aerial part	Root	Aerial part	Root
0.0	20	15.45 ab*	7.36 c	1.45 a	0.06 b
-0.1		15.29 abc	13.25 ab	1.15 b	0.10 a
-0.2		9.51 d	10.65 b	0.95 bc	0.04 b
0.0	38	16.60 a	12.24 ab	0.84 cd	0.07 b
-0.1		12.59 bcd	13.28 ab	1.16 b	0.10 a
-0.2		11.80 cd	15.05 a	0.67 d	0.04 b
CV (%)		17.51	17.49	15.05	29.53

\*Different letters in the same column differ statistically

The control plants, when grown at 20°C, did not differ statistically from the plants grown at 38°C in the shoot length parameter, however, they had a 66% increase in root length. This shows an immediate response of the plant when it is exposed to high temperatures, showing its potential for greater tolerance to this stress.

At 38°C, the O.P. of - 0.2  $\Psi$ s had ( $P \leq 0,05$ ) 24% higher shoot growth, and 41% longer root length, in relation to the plants submitted at 20°C (Table 2). This is an indication that plants undergoing initial stress better adapt to lack of water by elongating their root more efficiently than when the initial stress is not applied. Some authors (PIMENTEL and PEREZ, 2000; COLMAN et al., 2014) report in their studies on common bean that variation and morphology are affected.

Thus, the association between high temperature and osmotic stress promote greater adaptation of plants to stresses. This inference shows a cross-tolerance capability in germination of IPR Campos Gerais. This result is compatible with results found by Custódio et al. (2009), who also found evidence of cross tolerance in common bean.

The mass of the aerial part was also affected by the OP and the high temperature. Plants with OP of -0.2  $\Psi$ s had their size reduced by 29% ( $P < 0.05$ ) when compared to the same plants that were grown at 20°C. This result is expected, since the plants adapt their morphology

Bengough et al. (2011) reports that when there is an increase in severe osmotic potential, soybean root growth is adversely affected. Thus, it is possible that the cross tolerance can not meet the physiological needs of the IPR UIRAPURU variety in a more severe osmotic stress, as in the case of OP of - 0.2  $\Psi$ s. The results of the cultivar IPR Campos Gerais show a similar pattern to the pattern found for the IPR UIRAPURU variety.

The cultivar Campos Gerais presented differentiated growth when cultivated at different temperatures. Comparing the parameters at 20°C, the increase in temperature to 38°C increased the shoot length by 7.44% and the mass by 66.33%, respectively (Table 2). Thus, *P. vulgaris* Campos Gerais variety cultivated at 38°C presents aerial shoot elongation.

to root growth, however, the caliber of the root is impaired in this process. Water stress affects practically all aspects of shoot and root growth, generally causing reduction in cell expansion, stomatal conductance, photosynthesis and, consequently, dry matter accumulation (OSAKABE et al., 2014).

The results found in the literature on the behavior of the stature character of plants are divergent, and in some cases the genotypes presented reduction of plant height due to the fact that water stress reduces the turgor of the cells and, consequently, their growth (COSTA et al., 2008), but in others an increase occurs when submitted to water stress (AGUIAR et al., 2008).

## CONCLUSIONS

The results indicate that there was a significant statistical difference of the treatments with regard to the germination of the cultivars, and the cultivar IPR UIRAPURU obtained higher germination rates.

It is possible to infer a possible memory to the primary stress when the IPR UIRAPURU and IPR Campos Gerais varieties are subjected to an initial stress of 38°C, making them better adapt to an osmotic stress at the evaluated stages. However, more detailed studies are necessary to actually prove this event.

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