Biotecnología Vegetal Vol. 21, No. 3: 140 - 148, julio - septiembre, 2021
Instituto de Biotecnología de las Plantas. UCLV. MES.
eISSN 2074-8647, RNPS: 2154

Characterization of the *in vitro* response of callus *of Phaseolus vulgaris* cultivar 'BAT-93' to PEG-6000-induced water stress

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ABSTRACT
Water stress is one of the major abiotic factors that limits the growth and productivity of many cultures including *Phaseolus*. In this research the *in vitro* callus response of cultivar 'BAT-93', in multiplication culture media with 6% of polyethylenglycol-6000 (PEG-6000) as water stress inductor was characterize. First, the callus response at 21 days of culture under water stress conditions was assessed. At the same time, the effect of subculture number on callus response with the use of two treatments was also determined. In the treatment I, the callus were subculture at 7 and 14 days of culture and then evaluated at 14 and 21 days respectively. In the treatment II, the callus were subcultivated at 14 days and subsequently assessed one week later. In each test, the degree of callus affectation as well as the frequency of its appearance was evaluated. The experimental design included 35 explants for both control as well as for every stress induction analysis. The callus growing in PEG-6000 free culture medium was considered as control. In this research, the water stress induction with 6% of PEG-6000 affected the *in vitro* response of 'BAT-93' callus. In the treatment II the use of only one *in vitro* callus subculture, reduced the affectation of them under stressing conditions respect to the use of two subcultures. The results constituted a step in advance in genetic focus on water stress tolerance in *Phaseolus*.

Keywords: abiotic stress, common bean, subculture number

Caracterización de la respuesta *in vitro* de callos de *Phaseolus vulgaris* cultivar 'BAT-93' a estrés hídrico inducido con PEG-6000

RESUMEN
El estrés hídrico es uno de los principales factores abióticos que limita el crecimiento y productividad de muchos cultivos incluyendo *Phaseolus*. En esta investigación se caracterizó la respuesta *in vitro* de callos del cultivar 'BAT-93', en medio de cultivo de multiplicación con 6% de polietilenglicol-6000 (PEG-6000) como inductor de estrés hídrico. Primero, se evaluó la respuesta de los callos a los 21 días de cultivo bajo condiciones de estrés hídrico. Al mismo tiempo, se determinó el efecto del número de subcultivos en la respuesta de los callos con el uso de dos tratamientos. En el tratamiento I, los callos fueron subcultivados a los 7 y 14 días de cultivo y posteriormente evaluados a los 14 y 21 días respectivamente. En el tratamiento II, los callos se subcultivaron a los 14 días y posteriormente evaluados una semana después. En cada ensayo se evaluó el grado de afectación de los callos así como la frecuencia de su aparición. El diseño experimental incluyó 35 explantes tanto para el control así como para cada

análisis de inducción de estrés. El crecimiento de los callos en medio de cultivo libre de PEG-6000 se consideró como control. En esta investigación la inducción de estrés hídrico con 6% de PEG-6000 afectó la respuesta *in vitro* de los callos de 'BAT-93'. En el tratamiento II la utilización de un solo subcultivo *in vitro* de los callos, redujo su afectación bajo condiciones estresantes respecto al uso de dos subcultivos. Los resultados constituyeron un paso de avance en los estudios de mejoramiento genético, enfocados hacia la tolerancia a estrés hídrico en *Phaseolus*.

Palabras clave: estrés abiótico, frijol común, número de subcultivos

INTRODUCTION

Water stress is one of the most important abiotic factor that affect crop productivity. Specifically, in *Phaseolus* genus this environmental factor cause yield losses of around 10–100% (Polania *et al.*, 2016). In Cuba, the cultivation of common beans is one of the most important grain legume crops for human consumption (Domínguez *et al.*, 2019). According to ONEI (2021) in 2020 in Cuba, about 73 thousand hectares of beans were harvested with a total production of 65 thousand tons and 0.89 t ha^{-1} average of agricultural yield.

Phaseolus vulgaris, because of its grown in very diverse habitats, is exposed to numerous biotic and abiotic factors that affect its yield. Among them, water stress is considered the greatest constraint to agricultural production worldwide (Kusvuran and Dasgan, 2017).

In Cuba, water stress has a direct impact on agriculture (Polón *et al.*, 2014). That is why the breeding and use of common bean cultivars with water tolerance, has become the greatest challenge to increase bean productivity. In this context, the application of plant tissue culture techniques offers many advantages respect to the problems associated with conventional breeding strategies (Pratap *et al.*, 2018). Thus, *in vitro* culture of plant cells and tissue has attracted considerable interest among scientist, as an important tool of improving crop tolerance (Al-Saedi and Abdulhalem, 2020).

Plant biotechnology in conjunction with *in vitro* breeding techniques, can facilitate the obtaining of water stress tolerant cultivars. In this sense, the development of research aimed at the induction of this type of stress at callus level, constitutes a feasible alternative to be considered. However, in *Phaseolus* the limited information about this topic is contrasting with the results obtained in other species. Authors like Mohamed and Tawfik (2006), reported the role of osmotic potential in stress resistance in *Phaseolus acutifolius* during a study on callus derived from six tepary bean lines with differential levels of water resistance in the field. Other authors have informed their successful results using the *in vitro* selection of callus to water stress and proposed the effectiveness of this strategy to speed up the improvement of water tolerance in *Oryza sativa* L. (Wani *et al.*, 2010), in *Triticum aestivum* L. (Mahmood *et al.*, 2012), in *Saccharum officinarum* L. (Rao and Jabeen, 2013) and in *Sorghum bicolor* L. (Tsago *et al.*, 2014).

The successful application of this strategy requires an efficient plant regeneration protocol. Thus, in the Instituto de Biotecnología de las Plantas, Collado *et al*. (2013) developed a regeneration protocol for *P. vulgaris* shoots via indirect organogenesis that included the cultivar 'BAT-93' (Sanchez-Valdez *et al.*, 2004). This cultivar has water susceptibility but it has high yield and disease resistance. For this reason, is part of the genetic improvement programs developed in Cuba.

In water stress selection, conventional methods of discrimination was rendered ineffective due to the simultaneous effect of other factors. Therefore, implementing tools that make it possible to accelerate and make improvement programs more efficient is a priority. Specifically, having *in vitro* techniques that allow the selection of promising individuals, from early stages would represent an initial step that would avoid the field evaluation of a large number of individuals and would improve the efficiency of selection (Tsago *et al.*, 2014). According to the above information, this research constitutes an approach for an early *in vitro* selection of individuals with better performance under adverse conditions such as water stress.

The aim of the present work was to characterize the callus response of cultivar 'BAT-93' to water stress induced with PEG-6000. This research constitutes a first step for the *in-vitro* screening of callus under these stressing conditions. It provides a more comprehensive understanding of the cultivar response to water stress, which constitutes an important tool in genetic improvement programs.

MATERIALS AND METHODS

The study was conducted in the plant tissue culture laboratory in the Instituto de Biotecnología de las Plantas (IBP).

Plant material and culture conditions

Mature seeds of the common bean cultivar 'BAT-93' with one month of having harvested at the greenhouse were used. They were obtained from the germplasm bank of CIAP (Centro de Investigaciones Agropecuarias) de la Universidad Central Marta Abreu de Las Villas. Disinfection of 250 seeds was follow according to the protocol described by Collado *et al*. (2017). The *in vitro* water stress induction with 6% PEG-6000 (SIGMA, Aldrich) as an osmotic agent was made. This concentration based on previous results obtained from seed germination studies in *Phaseolus* was chosen (García *et al.*, 2015).

Callus culture

The different culture medium used for *in vitro* culture of *Phaseolus vulgaris* were:

Germination medium: MS basal salts (Murashige and Skoog, 1962) 1.0 mg l^{-1} thiamine, 1.13 mg l^{-1} 6-bencylaminopurine (6-BAP), 3% sucrose (w/v) and 7.0 g l^{-1} agar (BIOCEN).

Callus induction medium: MS basal salts, B5 vitamins (Gamborg *et al.*, 1968) 0.2 mg l^{-1} thidiazuron (TDZ), 0.05 mg l^{-1} indole-3-acetic acid (AIA), 3% (w/v) sucrose and 6.0 g l^{-1} of agar (BIOCEN).

Callus multiplication medium: MS basal salts, B5 vitamins, 0.04 mg l^{-1} TDZ, 0.05 mg l^{-1} AIA, 2% (w/v) sucrose and 6.0 g l^{-1} agar (BIOCEN).

The pH was adjusted to 5.7 with Sodium hydroxide (NaOH) or Hydrochloric acid (HCl) prior to adding the gelling agent (BIOCEN). The chemical esterilization of them with *Vitrofural* 0.114 g l^{-1} was done. Polypropylene flasks of 500 ml with 50 ml by flask were used.

Five seeds per vessel were put in germination culture media during three days in darkness. After that, the cotyledonary node with two cotyledons was taken (Collado *et al.*, 2013). Cotyledonary explants were grown on callus induction medium (CIM) during seven days in darkness and later on transferred to light conditions. Two subcultures in the same culture medium were made. Afterwards, the proliferated callus were subcultured onto a multiplication culture medium. The *in vitro* culture of explants was carried out in a growth chamber at 25 ± 2 °C under 16 h light/8 h dark photoperiod for 21 days. The light intensity was 45µmol m^{-2} s^{-1} from cold fluorescent lamps.

In vitro response of 'BAT-93' callus to water stress

Nodular callus (250 mg weight) after 21 days in multiplication culture media were selected and transferred on to the same culture media supplemented with 6% of PEG-6000. PEG-free medium was used as a control. On each assay, 35 explants (three callus by flask) were used and experiments were repeated twice.

In this research two test were considered. First, the *in vitro* response of 'BAT-93' callus, after 21 days of culture under stressing conditions induced with PEG-6000 was determined. For this purpose, the degree of callus affectation was evaluated according with the descriptive scale based on callus necrosis proposed by Bermúdez-Caraballoso *et al*. (2012). Degree scale: (1) no affectation, (2) 25% necrotic area, (3) 50% necrotic area, (4) 75% necrotic area and (5) total necrosis.

Effect of subculture number on callus selection

In a second test, the effect of subculture number in PEG treated callus of 'BAT-93' using two treatments was considered. In treatment I, callus were subcultured at seven and 14 days and evaluated at 14 and 21 days of culture respectively. However, in treatment II they were subcultured at 14 days and one

week later evaluated. Callus evaluation was done according with the previously described scale.

In both assay the callus color was determined with the hexadecimal code of color (http://www.cwp.linet.edu/cwis/cwp.html).

Statistical analysis

The experimental data from the descriptive analysis of callus affectation was processed with the Statistic Packaged for Social Science (SPSS) version 21.0 (SPSS Inc., Chicago, IL, USA) for Window, with previous confirmation of the suppositions of normality and variance homogeneity with a value $p \leq 0.05$. The non-parametric tests H of Kruskall-Wallis and U of Mann-Whitney was used, after having generated up to 10 000 samples with similar distribution to the real one by Monte Carlo technique, to estimate in this way the significance with 95% of trust.

RESULTS

In this study, the induction of water stress with 6% PEG-6000 affected the *in vitro* response of 'BAT-93' callus during multiplication phase. These stressful conditions produced changes in callus characteristics after 21 days of culture. In treated callus, different characteristics in terms of color and texture were observed respect to control (qualitative). Unstressed callus were nodular, compact with a green color mainly (hex code: #008000). However, most of treated callus lost their compactness and showed a mucilaginous texture and a sienna color (hex code: #A0522D) (Figure 1).

At 21 days of callus culture, differences on the degree of affectation in PEG treated callus respect to control were established. The stressed callus reached a degree between three and four, which corresponded with a necrotic area of 50% to 75%, according with the scale proposed by Bermúdez-Caraballoso *et al*. (2012). However, in control treatment the prevalence of degree two of the mentioned scale, which meant a lower callus damage, was observed.

The analysis of frequency data after 21 days of callus culture revealed that, the degree three of affectation with a 52% was the most frequent in this test. At this time, higher degrees with a lower frequency of appearance were in correspondence with a more severe callus damage. By other hand, in most of non-treated callus the degree two of the scale was present with the highest frequency (Figure 2).

The current study suggested the use of *in vitro* induction of water stress at callus level, as a useful tool in genetic improvement programs of stress tolerance. It will allow the early evaluation of different lines and/or cultivars obtained from the genetic improvement programs in *Phaseolus*.

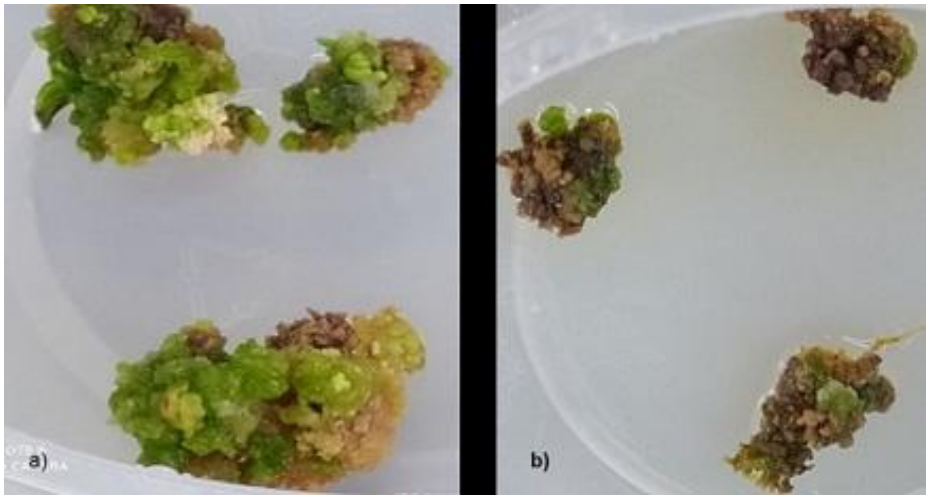


Figure 1. *In vitro* callus response in *Phaseolus vulgaris* L. cultivar 'BAT-93' under water stress conditions induced with PEG-6000 after 21 days of culture. a) Control and b) treated callus.

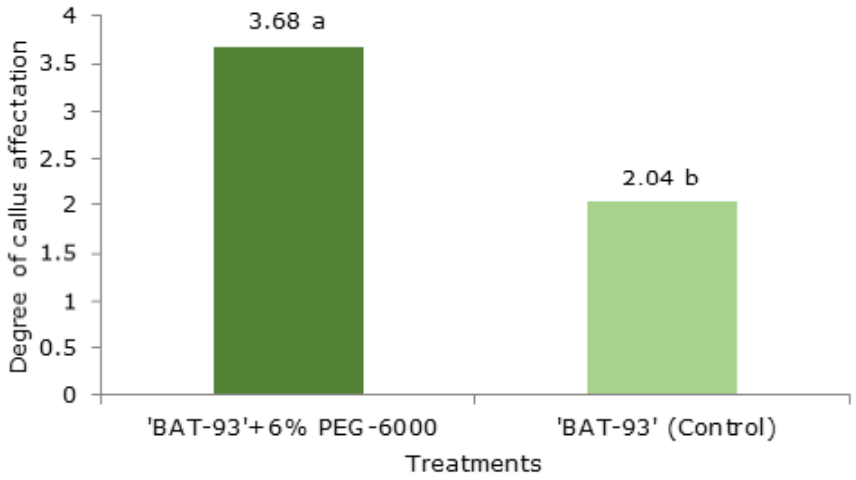
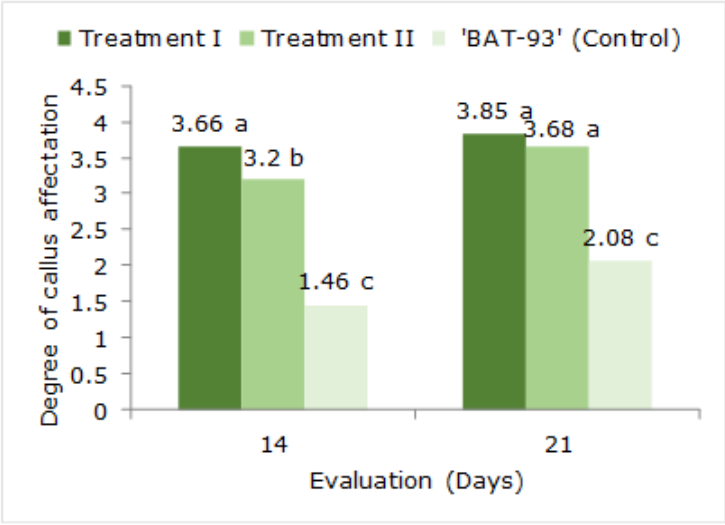


Figure 2. Frequency of appearance of the degree of callus affectation *in vitro*, in *Phaseolus vulgaris* L. cultivar 'BAT-93' callus under stress conditions after 21 days of culture with PEG-6000. Degree scale: (1) no affectation, (2) 25% necrotic area, (3) 50% necrotic area, (4) 75% necrotic area and (5) total necrosis.



Different letters on bars of the same cultivar indicate significant differences according to H of Kruskall Wallis and U of Mann-Whitney test for $p \leq 0.05$

Figure 3. Effect of subcultures number on the degree of callus affectation *in vitro*, in *Phaseolus vulgaris* L. cultivar 'BAT-93' under stress conditions at 14 and 21 days of culture with PEG-6000. Treatment I, callus subcultured at 7 and 14 days. Treatment II, callus subcultured at 14 days. Degree scale: (1) no affectation, (2) 25% necrotic area, (3) 50% necrotic area, (4) 75% necrotic area and (5) total necrosis.

In this research, the use of different subcultures number produced a differential *in vitro* response in PEG treated callus of 'BAT-93'. At 14 days of culture, significant differences on the degree of callus affectation among treatments were established. In treatment I, after two callus subcultures a degree of affectation between three and four according to the proposed scale was perceived. On the contrary, in treatment II the performance of only one subculture provided a better callus response, since the degree three of affectation was mainly observed. At the same time, in non-treated callus a lower level of callus damage was reached (Figure 3).

Later on, at 21 days of PEG-6000 treated callus culture, a similar response on callus affectation for both treatments was observed. At this time, most of them had a degree three or four of affectation in accordance with the proposed scale. This response was significant respect to control where the degree two of the scale was predominantly (Figure 3).

In treatment I, the analysis of frequency data at 14 days of PEG treated callus culture, revealed that after two subcultures the degree four of the scale was the most representative with a 50% of appearance. This value was in correspondence with a 75% of damage in the functional area of the callus. Simultaneously, it was observe the presence of degree three of affectation in a high frequency as well. Besides, the degree five in a lower frequency produced the total necrosis of a certain number of callus. In an opposite way, the performance of a single subculture, allowed a better response of PEG treated callus to the induced stress. This was highlighted by the higher frequency of appearance of degree three with an 80% over degree four. Most of non-treated callus had no affectation while the rest of them showed the degree two in a lower frequency (Figure 4).

The data analysis after 21 days of PEG treated callus culture, showed a differential response in the frequency of appearance of degree three to five between treatments (Figure 5). In treatment I, a huge affectation in callus proliferation because of the prevalence of degree four with a 53.9% of appearance, in conjunction with degree five was observed. In a different way, in treatment II, the higher frequency of appearance for degree three, evidenced a better callus response to stress after one subculture. The degrees four and five were also present but in a lower frequency which caused a serious damage in the functional area of the callus around 75% or more. In control, the degree two of the scale was highlighted in accordance with a lower callus affectation. These results suggested the possible use of one callus subculture at 14 days of culture, for the *in vitro* selection of water stress.

DISCUSSION

The research showed that 'BAT-93' callus proliferation *in vitro* was affect under water stress conditions induced with 6% PEG-6000. The callus damage observed after 21 days of culture was in accordance with previous studies, where callus growing was also compromise under stressful conditions. In this

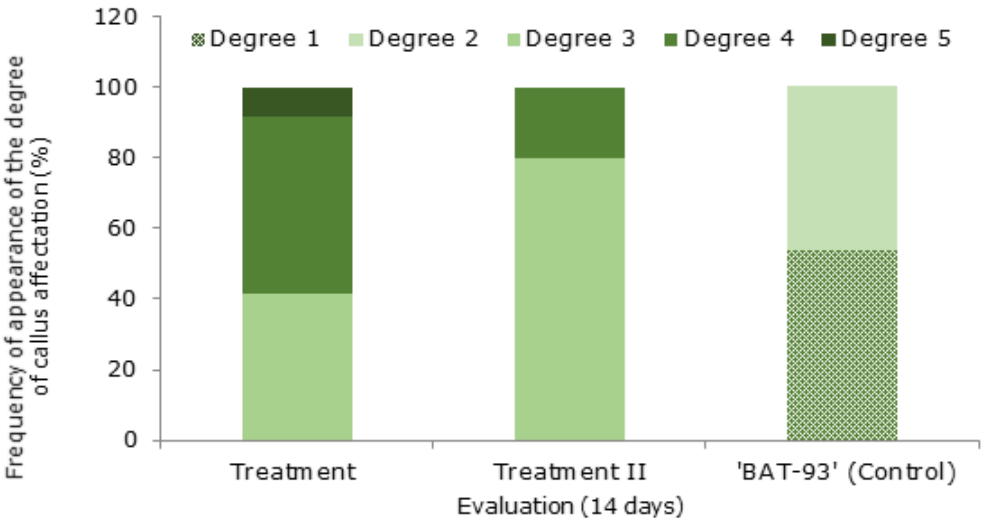


Figure 4. Frequency of appearance of the degree of affectation *in vitro*, in *Phaseolus vulgaris* L. cultivar 'BAT-93' callus under stress conditions after 14 days of culture with PEG-6000. Treatment I, callus subcultured at seven and 14 days. Treatment II, callus subcultured at 14 days. Degree scale: (1) no affectation, (2) 25% necrotic area, (3) 50% necrotic area, (4) 75% necrotic area and (5) total necrosis.

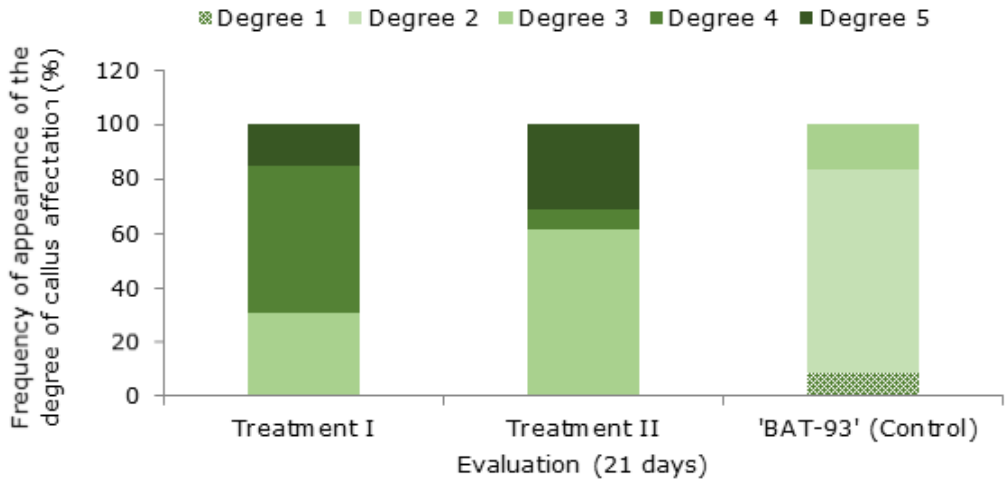


Figure 5. Frequency of appearance of the degree of affectation *in vitro*, in *Phaseolus vulgaris* L. cultivar 'BAT-93' callus after 21 days of culture with PEG-6000. Treatment I, callus subcultured at seven and 14 days. Treatment II, callus subcultured at 14 days. Degree scale: (1) no affectation, (2) 25% necrotic area, (3) 50% necrotic area, (4) 75% necrotic area and (5) total necrosis.

sense, some authors during the *in vitro* selection of tolerant plants to water, informed a significant declined in callus growing when increasing concentrations of PEG-6000 were used (Wani *et al.*, 2010; Mahmood *et al.*, 2012; Rao and Jabeen, 2013). In the same way, Tavangar *et al*. (2021) while studying the effect of water stress in *Trigonella foenum-graecum* L. founded identical results.

The addition of PEG-6000 to the culture medium produces an osmotic stress affecting cell division, with a subsequently reduction of callus growth through a drop in water availability to cells. This response could be related with the increase of reactive oxygen species (ROS) because of oxidative stress activation (Guo *et al.*, 2018; Sarmadi *et al.*, 2019). The 'BAT-93' callus response, after 21 days of culture on the stressful medium confirmed the role of the osmotic agent, since a decreased in growth under this conditions was observed.

In the course of this research, in PEG-6000 treated callus of 'BAT-93' necrosis associated with tissue browning was observed. This phenomenon is related with the phenolic compounds oxidation via PPO (Polyphenol oxidase) enzyme activity (Król *et al.*, 2014). Authors like Hosseini *et al*. (2020) in *Salvia leriifolia* Benth and Yunita *et al*. (2020) in *Oryza sativa* L., informed the negative effect of PEG in the morphological characteristics of callus. They founded that browning intensity increased with increasing concentration of PEG.

In this study, a better callus response to water stress using only one *in vitro* subculture at 14 days, constitutes a step forward to continue searching water tolerant plants. Selection and development of stress tolerant cultivars by using *in vitro* culture techniques is essential for *Phaseolus* improvement. However, in spite of the relevance that it may have at callus level, knowledge is still limiting. The collected data from this research constitutes a first approach towards a better understanding of common bean response under stressing conditions of culture.

CONCLUSIONS

From the present investigation, it can be concluded that water stress induction with PEG-6000 produces some morphological changes at callus level in *Phaseolus vulgaris* L. cultivar 'BAT-93'. These changes could be use as selection criteria in genetic improvement programs. The use of only one *in vitro* callus subculture at 14 days is propose for early stress selection.

ACKNOWLEDGEMENTS

This work was funded through the project: Genetic improvement of *Phaseolus vulgaris* L. for the search for resistance to biotic and

abiotic stresses (code P131LH001.30), financed by the Producción de Alimentos y su Agroindustria. The funders were not involved in the design of the study, the collection and analysis of the data, the decision to publish or the preparation of the manuscript is that of the institution and the collective of authors of the project.

Conflict of Interest

The authors declare that they are not interest conflict.

Author contributions

Conceptualization NV, Formal analysis NV, MFMR and AMR, Investigation MFMR, NV, DT, LR and SH. Methodology NV and MFMR, Writing-Original MFMR and NV, Writing-Review and Edition MFMR and NV

REFERENCES

Al-Saedi RKM, Abdulhalem AG (2020) Callus induction and shoot formation for mexican red bean (*Phaseolus vulgaris* L.) Pinto cultivar *in vitro*. Iraqi Journal of Science 61(8): 1887-1893; doi: 10.24996/ijs.2020.61.8.5

Bermúdez-Caraballoso I, Collado R, García LR, Veitía N, Martirena A, Torres D, Romero C, Angenon G (2012) Determinación de la concentración mínima inhibitoria de Glufosinato de amonio en callos organogénicos de *Phaseolus vulgaris* L. cv. CIAP7247F'. Biotecnología Vegetal 12(4): 203-210

Collado R, Veitía N, Bermúdez-Caraballoso I, García LR, Torres D, Romero C, Rodríguez Lorenzo JL, Angenon G (2013) Efficient *in vitro* plant regeneration via indirect organogenesis for different common bean cultivars. Scientia Horticulturae 153: 109-116; doi: 10.1016/j.scienta.2013.02.007

Collado R, Carabeo Pérez A, Poveda Martínez I, Rojas LE, Leiva-Mora M, García LR, Veitía N, Martirena A, Torres D, Rivero L (2017) Diferenciación de cultivares de *Phaseolus vulgaris* L. mediante respuesta del tejido foliar expuesto a estrés hídrico y salino. Biotecnología Vegetal 17(1): 25-32

Domínguez A, Darias Rodríguez R, Martínez Dávalos Y, Alfonso Negrín E (2019) Tolerancia de variedades de frijol común (*Phaseolus vulgaris*) a condiciones de sequía en campo. Centro Agrícola 46(3): 22-29

Gamborg OL, Miller RA, Ojima K (1968) Nutrient requirements of suspension cultures of soybean root cells. Experimental Cell Research 50(1): 151-158; doi: 10.1016/0014-4827(68)90403-5

García LR, Leiva-Mora M, Carabeo Pérez A, Collado R, Poveda Martínez I, Veitía N, Martirena A, Torres D, Rivero L (2015) Efecto del estrés hídrico inducido con PEG 6000 sobre la germinación *in vitro* de semillas de *Phaseolus vulgaris* L. cv. 'ICA Pijao'. Biotecnología Vegetal 15(4): 243-249

Guo YY, Yu HY, Yang MM, Kong DS, Zhang YJ (2018) Effect of drought stress on lipid peroxidation, osmotic adjustment and antioxidant enzyme activity of leaves and roots of *Lycium ruthenicum* Murr. seedling. Russian Journal of Plant Physiology 65(2): 244-250; doi: 10.1134/S1021443718020127

Hosseini NS, Hagh ZG, Khoshghalb H (2020) Morphological, antioxidant enzyme activity and secondary metabolites accumulation in response of polyethylene glycol-induced osmotic stress in embryo-derived plantlets and callus cultures of *Salvia leriifolia*. Plant Cell, Tissue and Organ Culture (PCTOC) 140(1): 143-155; doi: 10.1007/s11240-019-01718-z

Król A, Amarowicz R, Weidner S (2014) Changes in the composition of phenolic compounds and antioxidant properties of grapevine roots and leaves (*Vitis vinifera* L.) under continuous of long-term drought stress. Acta Physiologiae Plantarum 36(6): 1491-1499; doi: 10.1007/s11738-014-1526-8

Kusvuran S, Dasgan HY (2017) Effects of drought stress on physiological and biochemical changes in *Phaseolus vulgaris* L. Legume Research 40(1): 55-62; doi: 10.18805/lr.v0i0.7025

Mahmood I, Razzaq A, Hafiz IA, Kaleem S, Khan AA, Qayyum A, Ahmad M (2012) Interaction of callus selection media and stress duration for *in vitro* selection of drought tolerant callus of wheat. African Journal of Biotechnology 11(17): 4000-4006; doi: 10.5897/AJB11.2610

Mohamed MF, Tawfik AA (2006) Dehydration-induced alterations in growth and osmotic potential of callus from six tepary bean lines varying in drought resistance. Plant Cell Tissue and Organ Culture 87(3): 255-262; doi: 10.1007/s11240-006-9159-2

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum 15(3): 473-497

ONEI (2021) Oficinal Nacional de Estadística e Información. Anuario estadístico de Cuba. Available in: http://www.one.cu/aec2015.htm. Accessed 4/1/2021

Polania J, Poschenrieder C, Rao I, Beebe S (2016) Estimation of phenotypic variability in symbiotic nitrogen fixation ability of common bean under drought stress using ^{15}N natural abundance in grain. European Journal of Agronomy 79: 66-73; doi: 10.1016/j.eja.2016.05.014

Polón R, Miranda A, Ramírez MA, Maqueira LA (2014) Efectos del estrés de agua sobre el rendimiento de granos en la fase vegetativa en el cultivo del frijol (*Phaseolus vulgaris* L.). Revista Ciencias Técnicas Agropecuarias 23(4): 33-36

Pratap A, Prajapati U, Singh CM, Gupta S, Rathore M, Malviya N, Tomar R, Gupta AK, Tripathi S, Singh NP (2018) Potential, constraints and applications of *in vitro* methods in improving grain legumes. Plant Breeding 137(3): 235-249; doi: 10.1111/pbr.12590

Rao S, Jabeen F (2013) *In vitro* selection and characterization of polyethylene glycol (PEG) tolerant callus lines and regeneration of plantlets from the selected callus lines in sugarcane (*Saccharum officinarum* L.). Physiology and Molecular Biology of Plants 19(2): 261-268; doi: 10.1007/s12298-013-0162-x

Sanchez-Valdez I, Acosta-Gallegos JA, Ibarra-Perez FJ, Rosales-Serna R, Singh SP (2004) Registration of 'Pinto Saltillo' common bean. Crop Science 44(5): 1865-1866; doi: 10.2135/cropsci2004.1865a

Sarmadi M, Karimi N, Palazón J, Ghassempour A, Mirjalili MH (2019) Improved effects of polyethylene glycol on the growth, antioxidative enzymes activity and taxanes production in a *Taxus baccata* L. callus culture. Plant Cell, Tissue and Organ Culture (PCTOC) 137(2): 319-328; doi: 10.1007/s11240-019-01573-y

Tavangar A, Karami L, Hedayat M, Gholamreza A (2021) Effect of salinity and drought stress on morphological and biochemical properties of two Iranian fenugreek (*Trigonella foenum-graecum*) populations. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 49(2): 12038-12038; doi: 10.15835/nbha49212038

Tsago Y, Andargie M, Takele A (2014) *In vitro* selection of sorghum (*Sorghum bicolor* (L.) Moench) for polyethylene glycol (PEG) induced drought stress. Plant Science Today 1(2): 62-68; doi: 10.14719/pst.2014.1.2.14

Wani SH, Sofi PA, Gosal SS, Singh NB (2010) *In vitro* screening of rice (*Oryza sativa* L.) callus for drought tolerance. Communications in Biometry and Crop Science 5(2): 108-115

Yunita R, Dewi IS, Lestari EG, Purnamanengsih R, Rahayu S (2020) Formation of upland rice drought-tolerant mutants by mutation induction and *in vitro* selection. Biodiversitas Journal of Biological Diversity 21(4): 1476-1482; doi: 10.13057/biodiv/d210427

Recibido: 14-01-2021
Aceptado: 06-05-2021