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EFFECT OF WATER STRESS SIMULATED WITH PEG 6000 ON TOMATO SEED GERMINATION (*Solanum* SECTION *Lycopersicon*)

Efecto del estrés hídrico simulado con PEG 6000 en la germinación en tomate (*Solanum* sección *Lycopersicon*)

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ABSTRACT. In the current investigation, the drought stress tolerance was evaluated on seed germination of 27 tomato accessions. This study was carry out first on two tomato accessions contrasting (Ciapan 31-5 and Campbell-28) subjected to different polyethylenglycol (PEG 6000) treatments (0,25; 0,5; 0,75 and 1 MPa), including a control treatment with distilled water. The results showed that the percentage of germination decreased with the increase of the osmotic potential from -0,5 MPa. This potential was used to evaluate the sample of germplasm composed of 27 accessions. A different response was obtained in the behavior of the accessions against simulated water stress with PEG-6000, being the accessions Ciapan-31-5, Rilia, Mex-121 A, Rojo Veracruz, LA-2128 and LA-1255 those that presented higher percentages of germination in these conditions. These accessions can be used as progenitors to increase tolerance to water stress in this phase of the crop.

Key words: abiotic stress, germination, drought, *Solanum lycopersicum* L.

RESUMEN. En el presente trabajo se evaluó la tolerancia al estrés hídrico en 27 accesiones de tomate durante la etapa de germinación. El estudio se efectuó primeramente en dos accesiones contrastantes (Ciapan 31-5 y Campbell-28) sometidas a diferentes tratamientos de polietilenglicol-6000 (PEG 6000) (0,25; 0,5; 0,75 y 1 MPa), incluyendo un tratamiento control con agua destilada. Los resultados mostraron que el porcentaje de germinación disminuyó con el incremento del potencial osmótico a partir de -0,5 MPa. Este potencial se utilizó para evaluar la muestra de germoplasma compuesta por 27 accesiones. Se obtuvo una respuesta diferenciada en el comportamiento de las accesiones frente al estrés hídrico simulado con PEG-6000, siendo las accesiones Ciapan-31-5, Rilia, Mex-121 A, Rojo Veracruz, LA-2128 y LA-1255 las que presentaron mayores porcentajes de germinación en estas condiciones. Estas accesiones pueden ser utilizadas como progenitores para incrementar la tolerancia al estrés hídrico en esta fase del cultivo.

Palabras clave: estrés abiótico, germinación, sequía, *Solanum lycopersicum* L.

INTRODUCTION

Climate change has a negative impact on agricultural production. In this sense, it is suggested that almost 90 % of the world's land surface is affected by abiotic stress factors at some point during the growth period (1), being the water stress critical for the development and productivity of the crops (2,3), hence, in the future, one of the alternatives will be to cultivate species that are tolerant of unfavorable conditions (1,4).

Most crops, including tomato (*Solanum lycopersium* L.) are sensitive to water stress at different stages of development, from germination to fruit set (5). The germination is the essential stage in the growth and development of the plants, because a good germination can guarantee the fast establishment and uniformity of the crops (6). That is why it is of great importance that the seeds are able to germinate quickly and uniformly in different environmental conditions, especially if the crop is established by planting seeds directly in the field instead of using transplants. Under optimal germination conditions, most tomato seeds germinate 2-5 days (7). However, under water stress

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conditions, germination is delayed or completely inhibited due to alterations during the imbibition of the seed and/or the activation of metabolic processes such as rehydration, repair mechanisms and the emission of the radicle. These alterations depend on the intensity and duration of stress, as well as the genetic background of the seed (8-10).

For this reason, several methods have been used to identify genotypes tolerant to water deficit during the germination stage (11). In this sense, the techniques of *in vitro* selection that involve the use of polyethylene glycol (PEG) to induce osmotic stress are the most used for screening genotypes and study water stress in various crops (11-16). Several reports have shown that these procedures are reliable for the selection of desirable genotypes, as well as to study in detail the water stress in plants (17,18).

The identification of tomato genotypes that can withstand drought conditions is vital to increase crop production and this can only be achieved by exploring tomato tolerant germplasm.

Taking into account the above, the present work was carried out with the objective of evaluating the effect of simulated hydric stress with polyethylene glycol-6000 (PEG 6000) on tomato germination to select drought-tolerant genotypes and their use in future breeding programs.

MATERIALS AND METHODS

For the development of the present work seeds of two tomato accessions with different degree of tolerance to water stress were used (*Solanum lycopersicum* cv Campbell-28, commercial cultivar and Ciapan 31-5, tolerant accession, *S. pimpinellifolium* L. both accessions belong to the work collection of tomato germplasm conserved at the National Institute of Agricultural Sciences (INCA) .The seeds were disinfected for 5 minutes with sodium hypochlorite (3% v/v) and then washed three times with distilled water and were placed in petri dishes (10,5 x 0,8 cm) with 10 mL of PEG 6000 (PEG) solutions (*Sigma-Aldrich Chemie GmbH, Steinheim*, Germany) to simulate water stress, with filter paper (Whatman 42) as support.

The water deficit was caused by five levels of osmotic potential (control, -0,25, -0,5, -0,76 and -1,0 MPa), which were obtained from different concentrations of PEG. The control was with distilled water (without PEG) and for the rest of the osmotic potentials the concentrations of PEG were calculated according to the equation of Michel and Kaufman (19).

$$(\Psi s) = -(1{,}18 \times 10\text{-}2)\ C - (1{,}18 \times 10\text{-}4)\ C_2 + (2{,}67 \times 10\text{-}4)\ CT + (8{,}39 \times 10\text{-}7)\ C_2T$$

where:
Ψs osmotic potential (MPa)
C: Concentration of PEG-6000 (g kg^{-1} H_2O)
T: Temperature (°C).

Three petri dishes were used per treatment, and these were placed in a growth chamber at a temperature of 25 ± 2 °C, a photoperiod of 16/8 (light/dark) and a light intensity of 150 µmol m^{-2} s^{-1}. The experiment was repeated three times in time.

To evaluate the germination percentage of the seeds, daily counts of the number of germinated seeds were made during 15 days after their introduction in the growth chamber. The seeds were considered germinated when they presented an emission of 2 mm of radicle through the seminal cover (germination sensu stricto). A simple classification ANOVA, model fixed effects, was performed on the data obtained. The significant differences between the means of the different treatments were verified by the 95 % Tukey HSD test. The data processing was done in the SPSS software package version 21.0 on Windows. Once the analysis was carried out with the two contrasting accessions, germination was evaluated in 27 tomato accessions belonging to the INCA work collection (Table 1). For this, the percentage of germination and the average of days that these delayed in reaching 50 % of germination (G50) were evaluated. Only the control and osmotic potential treatments that showed the greatest divergence in the previous analysis were used.

RESULTS AND DISCUSSION

The effect of water stress induced by PEG on the germination percentage of the accessions Campbell-28 and Ciapán 31-5 is presented in Figures 1 and 2. The results show 100 % germination for the control treatment in both accessions, which shows uniformity and quality of the seeds used in the study. In this case, after three days approximately 71 % of the seeds of the tolerant accession germinated, while Campbell-28 only presented 49 % of germination, achieving 100 % of seeds germinated at six days in both accessions (Figure 2).

Similar behavior presented the seeds exposed to osmotic potential of -0,25 MPa. However, at higher osmotic potentials a significant decrease in germination percentage was observed with the increase in PEG concentrations used.

Table 1. Accessions used in the study and its origin

Accession	Specie	Origin
1. Amalia	*S. lycopersicum*	Cuba
2. AN-104-1	*S. lycopersicum*	España
3. Campbell-28	*S. lycopersicum*	EUA
4. Cl-1131-00-7-2-0-9	*S. lycopersicum*	Taiwan
5. Claudia	*S. lycopersicum*	Cuba
6. CO-7040	*S. lycopersicum*	Cuba
7. Lignon	*S. lycopersicum*	Cuba
8. Mara	*S. lycopersicum*	Cuba
9. Mariela	*S. lycopersicum*	Cuba
10. Mayle	*S. lycopersicum*	Cuba
11. Mercy	*S. lycopersicum*	Cuba
12. Rilia	*S. lycopersicum*	Cuba
13. Roma	*S. lycopersicum*	Italia
14. Santa Clara	*S. lycopersicum*	EUA
15. Tropic	*S. lycopersicum*	EUA
16 Vyta	*S. lycopersicum*	Cuba
17 Yaily	*S. lycopersicum*	Cuba
18. Nagcarlang	*S. lycopersicum* var. Cerasiforme	Filipinas
19. LA-2807	*S. lycopersicum* var. Cerasiforme	Bolivia
20. LA2871	*S. lycopersicum* var. Cerasiforme	Bolivia
21. P-531	*S. lycopersicum* var. Cerasiforme	Cuba
22. Ciapan 31-5	*S. pimpinellifolium*	México
23. Mex-121-A	*S. pimpinellifolium*	México
24. Rojo Veracruz	*S. pimpinellifolium*	México
25. LA-0094	*S. habrochaites*	Perú
26. LA-1255	*S. habrochaites*	Perú
27. LA-2128	*S. habrochaites*	Perú

Actually, the water stress simulated with PEG only exceeded the G50 with water potentials of up to -0,5 MPa, from this potential none of the two accessions achieved 50 % germination (Figure 1). It is noteworthy that at this osmotic potential (-0,5 MPa) there were significant differences with respect to the control in Campbell-28, not in the Ciapán-31-5 accession, which indicates that it has a greater degree of tolerance to water stress. In both accessions the germination time was prolonged, being shorter in Ciapán 31-5 (Figure 2).

The decrease in the percentage of germination with increases in water potential may be due to changes in the metabolic and enzymatic processes present in the seeds, such as the development of metabolites induced by stress in the generation of reactive oxygen species, to a reduction in the diffusion of the water through the tegument and the absorption of water by the seeds that causes a hydration deficit (8,20,21).

Different authors point out that water stress greatly affects the germinative process and indicate that germination is the critical phase for the establishment and development of plants, as it guarantees their survival (20,22,23). It is also reported that polyethylene glycol is an efficient osmolyte to simulate water stress in germination studies, as it is an inert and non-toxic compound (12,13).

Once known the differential response to water stress of the accessions Campbell-28 and Ciapán 31-5 the percentage of germination in the work collection composed of 27 tomato accessions was evaluated. For this, the osmotic potential of -0,5 MPa was used, since higher potentials inhibited this process.

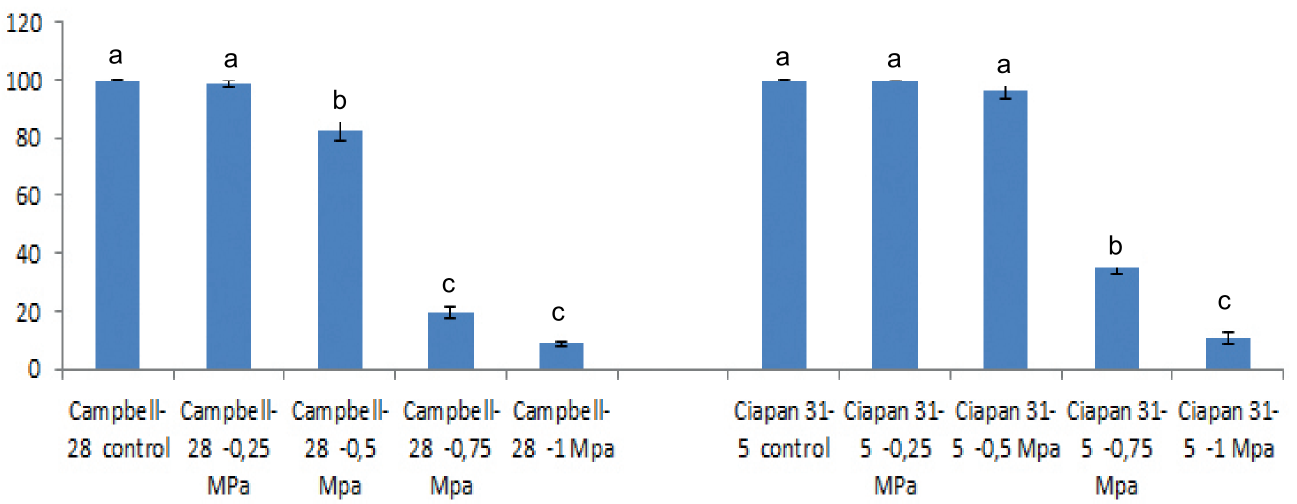

Figure 1. Effect of water stress induced by different concentrations of PEG 6000 in the germination percentage of the seeds of the Campbell 28 and Ciapan 31-5 accessions

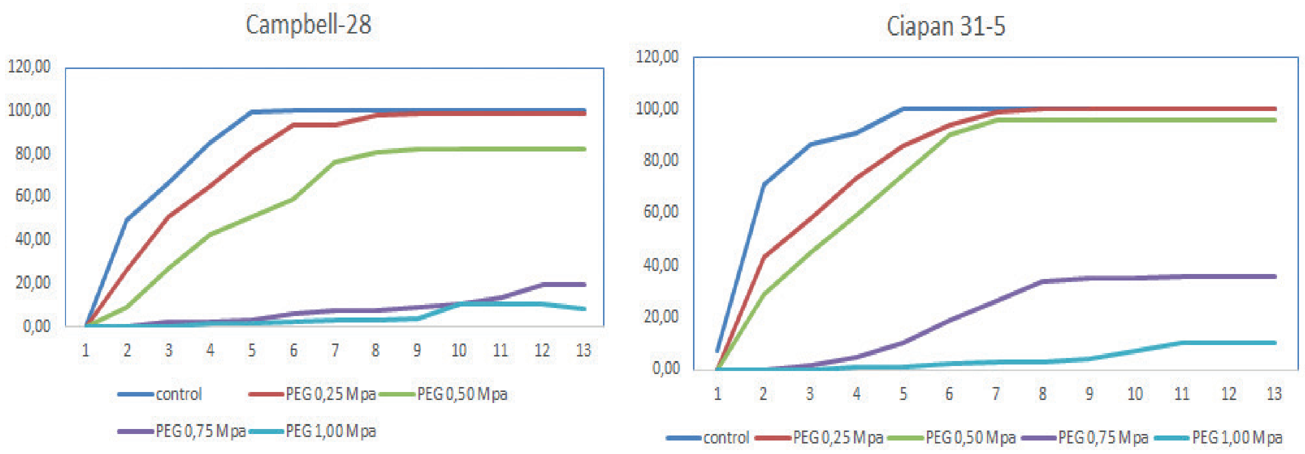

Figure 2. Effect of water stress induced by different concentrations of PEG 6000 in the prolongation of seed germination of the Campbell 28 and Ciapan 31-5 accessions

As shown in Table 2, no significant differences were found for the germination percentages and days to reach 50% germination in the control treatment, which indicates that the seeds used in the study had good physiological quality. All the accessions showed germination percentages higher than 98 % and achieved 50 % germination before four days. However, when the seeds were treated with PEG there were significant differences for these indicators.

Under conditions of simulated stress with PEG 6000, the germination time of all the accessions was increased and a decrease in germination percentage was observed in most of them; only two accessions Ciapán-31-5 and LA-1255 of *S. pimpinellifolium* and *S. peruvianum*, respectively, showed no affectations for this character. With respect to the days to germination, it can be observed that 'Ciapán-31-5', 'Mex-121 A', 'LA-2871', 'LA-1255' and 'LA-2128' were the ones that took less days in achieving 50 % germination, while 'Santa Clara' was the worst underperforming, followed by 'CO-7040' and 'Mercy', which took approximately 10 days to achieve 50 % germination. Also, the most tolerant accessions were 'Rilia', 'Ciapán-31-5', 'Mex-121 A', 'Rojo Veracruz', 'LA-2871', 'LA-1255' and 'LA-2128', the which had the highest percentages of germination, while 'Santa Clara' and 'CO-7040', only achieved 58,67 and 60 % germination, respectively. The genotypes 'Tropic', 'Mercy', 'Mayle' and 'AN-104-1', showed affectations of approximately 30 % of the germination, with respect to the control. It is worth noting the high germination percentages found in the accessions of *S. pimpinellifolium*, a species that has tolerance to water deficit (7).

The reduction of germination percentage in the presence of simulated water stress with PEG-600 has also been reported in the crop by several authors (11,13,24). A similar response has been found in wheat (3,14,25,26), barley (27), cowpea (15), corn (28), canola (29) and others. In this regard, it is reported that the lower affectation in the germination of the seeds of one accession with respect to the other, offers advantages for an establishment, growth and effective development of their seedlings in conditions of water deficit (3,21). These authors indicate the potential usefulness of these indicators in the selection of drought tolerant materials, an aspect of great importance, since the successes of the evaluation of tolerance to water stress in the field are low and highly variable, due to the high environmental influence (7); therefore, laboratory methods are a valid option to discriminate genotypes in early stages of improvement programs. In this sense, it has been indicated that one of the most widespread methods to determine the tolerance of plants to water stress is the observation of the germinative capacity of the seeds under these conditions (14,21,26).

The results obtained correspond to those found by various authors in the crop (5,9) who point out that the majority of commercial tomato cultivars are sensitive to this stress during this stage. However, the sources of tolerance have been identified in wild species, including *S. pennellii* and *S. pimpinellifolium*, mainly (7), can be of great help in future breeding programs. For this reason, good performance during germination is important to guarantee the establishment of fast and uniform crop plants (8,30).

Table 2. Percentage of final germination and time to achieve 50 % germination (G50) of tomato accessions under stress conditions

	PEG Concentration			
	0 MPa		0,5 MPa	
	G50	Germination (%)	G50	Germination (%)
1. Amalia	2,00	100,00	6,82 defg	89,33 bcde
2. AN-104-1	3,11	99,33	7,59 gh	70,00 j
3. Campbell-28	2,99	100,00	5,17 bc	82,12 efgh
4. Cl-1131-00-7-2-0-9	3,29	98,67	8,96 hij	74,00 hij
5. Claudia	2,56	100,00	6,08 cd	78,67 ghij
6. CO-7040	2,47	100,00	10,36 jk	60,00 k
7. Lignon	3,28	99,33	6,04 cd	84,00 cdef
8. Mara	2,09	100,00	6,16 cdef	90,67 bcde
9. Mariela	2,32	100,00	5,95 cd	90,00 bcde
10. Mayle	2,41	100,00	7,92 ghi	72,67 ij
11. Mercy	2,65	100,00	9,53 jk	70,67 j
12. Rilia	2,67	99,33	7,51 efg	92,00 abc
13. Roma	2,25	100,00	6,09 cde	88,00 bcdef
14. Santa Clara	3,73	100,00	10,65 k	58,67 k
15. Tropic	3,68	98,67	9,03 ij	70,00 j
16 Vyta	2,43	100,00	8,05 ghi	82,67 defg
17 Yaily	2,31	100,00	5,72 cd	91,33 bcd
18. Nagcarlang	2,20	100,00	7,08 defg	89,33 bcde
19. LA-2807	2,83	100,00	9,88 jk	80,00 fghi
20. LA2871	2,91	100,00	4,92 abc	90,00 bcde
21. P-531	3,20	100,00	7,57 fgh	89,33 bcde
22. Ciapan 31-5	1,81	100,00	3,77 ab	100,00 a
23. Mex-121-A	2,00	100,00	4,19 ab	96,00 ab
24. Rojo Veracruz	3,20	100,00	5,16 bc	94,67 ab
25. LA-0094	3,18	100,00	6,77 defg	87,33 bcdef
26. LA-1255	2,06	100,00	3,63 a	100,00 a
27. LA-2128	2,19	98,67	4,93 abc	95,33 ab
Media	2,66 ns	99,78 ns	6,88	83,88 ***

Equal letters do not differ statistically according to the Tukey test (α <0.05)

In this study it was shown that there are potential sources of tolerance to water deficit during germination in the accessions studied, which can be used as progenitors in future improvement programs for this purpose, so that the monitoring of tolerance to water stress in this phase can be useful to avoid the losses that occur under water deficit conditions (31).

CONCLUSIONS

- Simulated water stress with PEG-6000 affects the germination of tomato seeds from osmotic potentials of -0,5 MPa. While higher potentials inhibited germination of the seeds.
- A differentiated response of the different accessions to water stress was observed. The accessions 'Rilia', 'Ciapán-31-5', 'Mex-121 A', 'Rojo Veracruz', 'LA-2871', 'LA-1255' and 'LA-2128' presented the highest percentages of germination in these conditions, so they can be used as parents in future improvement programs.
- The utility of PEG-6000 for the selection of materials tolerant to water deficit in the early stages of improvement programs is evident.

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