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Effect of Osmotic Conditioning in Physiological Quality of *Cucumis sativus* L. and *Solanum lycopersicum* L. Seeds

P. V. Peña Alvarenga[1], Y. M. Lezcano Aquino[1], L. Ayala Aguilera[1], M. J. González Vera[1], W. N. Ortiz[1] & M. M. Romero Gavilán[1]

[1] Laboratorio de Análisis de Calidad de Semillas, Área de Producción Agrícola, Facultad de Ciencias Agrarias, Universidad Nacional de Asunción, San Lorenzo, Central, Paraguay

Correspondence: P. V. Peña Alvarenga, Laboratorio de Análisis de Calidad de Semillas, Área de Producción Agrícola, Km 11, Caja Postal 2160, Facultad de Ciencias Agrarias, Universidad Nacional de Asunción, San Lorenzo, Central, Paraguay. E-mail: pamepa89@hotmail.com

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Abstract

The human consumption of vegetables in different parts of the world is quite high, among them tomato stands out on a larger scale and not so far, also is cucumber, both with great economic importance. Due to the problems regarding the vigor of many seeds, techniques that seek to improve the establishment of the seedlings in a uniform way have been implemented. One technique is the osmotic conditioning to which the seeds are submitted, for which compound solutions such as polyethylene glycol or potassium nitrate can be used, with which very satisfactory results are obtained under laboratory and field conditions The objective of this investigation was to evaluate the effect of different doses and osmotic solutions on the physiological quality of seeds of tomato (*Solanum lycopersicum* L.) and cucumber (*Cucumis sativus* L.) in laboratory and field conditions. The evaluated variables were germination percentage, germination speed index, root length and emergence of seedlings. The tomato seeds were conditioned with PEG-6000 (-0.2, -0.4, -0.6 and -0.8 Mpa) and stored for a 30 days period, and as for the cucumber seeds with PEG-6000 (-0.2 and -0.1 Mpa) and with KNO^3 (100 mg and 300 mg) with a storage period of 45 days. It is concluded that, in tomato seeds, germination did not present statistical differences, however, the variables referring to vigor were favored with the conditioning in the presence of PEG-6000 with the (-0.2 Mpa) concentration, while in cucumber seeds the germination percentage was better using PEG 6000 compared to nitrate, while for vigor variables it behaved better in the presence of a concentration of (-0.2 Mpa) of PEG.

Keywords: osmotic conditioning, quality, seeds, tomato, cucumber

1. Introduction

Paraguayan peri-urban horticulture plays an essential role in the development of the local economy as it is a way of life, since it constitutes a market of proximity. In order to have competitiveness and access to the horticultural market system of the country, you must have specific objectives such as planning production based on the demand of the domestic market and the growing market demand of the region by improving the quality of horticultural seeds. For this matter we must guarantee the quality of the seeds used by the producers and in this way, guarantee the production and therefore the final retribution to the producer.

In what refers to the tomato crop, it stands out as one of the most consumed vegetables in the country and, therefore, one of the horticultural products of greater economic importance. Paraguay with more than 1.200 cultivated ha and a production that does not exceed from 40.000 tons, does not self-supply with this item, so it is required to import from countries such as Brazil and Argentina (Maldonado & Paredes, 2010). On the other hand, the cucumber is a vegetable of high economic potential because it is an export product that is cultivated and consumed all around the world.

According to Huerta (2015), the national yield is around 15 to 30 t/ha, which could be improved with the new technologies applications for greater yield and economic return.

Many of the vegetables seeds are unable of germinating under adverse conditions (Duran, 1989), so diverse techniques have been implemented, which allows to face the problems. A satisfactory treatment method that alleviates the dormancy of the seeds and increases the vigor by improving the synchronous seedling emergence and the yield of the crop is the osmotic conditioning or pre-germination with single or combined substances (Cortez-Baheza et al., 2011).

The osmotic treatment conditioning in seeds is reported as a technique to improve its physiological quality, through the uniformity in the germination percentage (Sánchez et al., 2007).

The method consists in the seeds hydration under controlled conditions (Sampaio, 1992), in a solution of determined concentration for an established period of time, in which is sought to condition the seeds by retarding their physiological deterioration. The main substances applied in pre-germination treatments are compound solutions such as polyethylene glycol and saline solutions, such as potassium nitrate. Likewise, different authors have developed different methods or models to condition seeds, with which very satisfactory results are obtained to increase the germination and emergence of crops under laboratory and field conditions (Nascimiento, 2009).

Considering that horticultural crops in the country are mostly cultivated by small producers and knowing that they do not have advanced technology to facilitate the production, It is very important to have easy-to-apply production alternatives, making able to help the producer to advance the transplant, being able to shorten the days that are generally necessary and achieve a better establishment of seedlings.

The objective of this investigation was to evaluate the effect of different doses and osmotic solutions on the physiological quality of seeds of tomato (*Solanum lycopersicum* L.) and cucumber (*Cucumis sativus* L.) in laboratory and field conditions.

2. Materials and Methods

2.1 Date and Place of Work

The study was carried out in the Seed Analysis and Quality Laboratory of the Faculty of Agricultural Sciences, National University of Asunción (FCA-UNA). It was executed in the period between the months of May to July 2018.

2.2 Vegetal Material

Seeds of the species *Cucumis sativus* L. (cucumber) and *Solanum lycopersicum* L. (tomato) cv. Pyta'i, obtained from local commerce. Before the selection of the seed batch to be used, a germination pretest was performed on the species in study, which threw yield results of 75% for tomatoes and 70% for cucumbers.

2.3 Osmotic Conditioning

The conditioning treatment was performed using the traditional method proposed by Heydecker, Higgins, and Gulliver (1973). The osmotic solution used to condition the tomato and cucumber seeds was Polyethylene Glycol-PEG-6000, initially the relationship between the amount of Polyethylene Glycol and the water potential was determined following the proposal of Michel and Kaufmann (1973), modified by Hardegree and Emmerich (1990); where finally the equation to be used was:

$$\Psi = 0.130[PEG]2T - 13.7[PEG]2 \quad (1)$$

The cucumber seeds were also conditioned in potassium nitrate (KNO3), where the osmotic potential was calculated using the equation proposed by Wiggans and Gardner (1959):

$$G = (PVm)/(RT) \quad (2)$$

Where, G = grams of solute to use, P= desired osmotic pressure, V = volume in liters, m= molecular weight of the chemical used, R = 0.0825 atm and T = K.

Different concentrations of PEG-6000 (-0.20, -0.40, -0.60 and -0.80 Mpa) were used for tomato and (-0.20 and -0.10 Mpa) for cucumber, in addition for cucumber seeds two doses of potassium nitrate were used (100 and 300 mg KNO_3).

The seeds were previously immersed in the solutions, in the case of cucumber for a period of 20 hours, and in the case of tomato for 48 hours, during this period they were kept at 25 °C. Once the time elapsed, the osmotic material was discarded and the seeds were washed with distilled water to remove the residues of the product, subsequently were dried with absorbent paper, and finally placed in a stove at 35 °C for 24 hours (time required to reach approximately the initial fresh weight of the seeds). Once the drying process was finished, the seeds were placed in ziploc-type hermetic bags and stored at a 10 °C temperature for 45 days (cucumber) and 30 days (tomato).

2.4 Germination Percentage

100 seeds were sown per repetition in gerbox type plates, previously disinfected with 70% alcohol, as a substrate, 2 sheets of absorbent paper previously sterilized in a stove at 105 °C were used, which were moistened with distilled water (2 times the weight of the dry paper). Later they were placed in a germination chamber with alternate temperatures of 20 °C for 8 hours and 30 °C for 16 hours. The final reading was made after 8 days, counting the normal seedlings containing all their essential structures.

2.5 Germination Rate Index

To measure the GRI, the procedure used was the one proposed by Krzyzanowski (1999), for the data obtention the samples corresponding to the germination test were used, the final calculation was made using the formula described by Maguire (1962), which states that the higher the value obtained, the higher germination speed rate is assumed, consequently greater vigor, since the calculated index estimates the average of normal plants germinated per day.

2.6 Root Length

20 seeds were sown in 4 repetitions per treatment, distributed in 3 sheets of paper per repetition (2 bases and one lid), the papers were moistened with distilled water, 2 times the weight of the dry paper, the seeds were arranged in the upper part of the paper in a longitudinal way taking care that the micropyle was directed towards the lower part of the paper in order to guide the root growth as rectilinear as possible, in order to facilitate its measurement. After sowing, the samples were kept in a germinator chamber with light and constant temperature of 25 °C. After 4 days later of sowing, the roots of the normal plants were measured with the help of a millimeter ruler, expressing the results in cm.

2.7 Seedling Emergency

The previously conditioned seeds were sown in plastic trays (100 seeds/tray), using as a substrate sterilized sand in stove at 200 ºC/2 hs, after being cooled, each experimental unit was moistened (10% of distilled water regarding to the dry weight of the substrate), they were maintained under uncontrolled environmental conditions, irrigated according to need in a uniform way.

Statistical analysis: The data collected from each variable were subjected to ANOVA (analysis of variance) and in the cases where statistical differences were detected, the Tukey test was applied at a 5% error probability.

3. Results and Discussion

The results obtained in this research are exposed in tables where the variables studied on cucumber seeds are shown in Table 1 for the different concentrations effects of PEG 6000 and KNO_3 used as osmotic solutions in the seeds treatment after 45 days storage. And Table 2 shows the variables for tomato seeds where the effect of different concentrations of PEG-6000 used as osmotic solutions in the seeds treatment after 30 days storage is observed.

Table 1. Effect of osmotic conditioning of cucumber seeds on germination percentage (G), germination rate index (GRI), root length (RL) and seedlings emergence (SE). San Lorenzo, Paraguay, 2018

Treatment	G (%)	GRI (pl/day)	RL (cm)	SE (%)
T1 PEG (-0.2 Mpa)	96 a	20 a	7.29 a	90 a
T2 PEG (-0.1 Mpa)	96 a	16 b	6.23 b	78 b
T3 KNO_3 (100 mg)	68 b	10 c	4.39 c	44 c
T4 KNO_3 (300 mg)	58 c	8 d	3.47 d	44 c
T5 Control	38 d	4 e	2.50 e	18 d
Mean	**71.2**	**11.6**	**4.77**	**55**
CV (%)	**6.96**	**10.22**		**14.52**

Note. G: germination; GRI: rate index; RL: root length and SE: seedlings emergence. Averages followed by the same letter in the column do not differ statistically by Tukey test at 5% probability. CV: coefficient of variation.

3.1 Germination Percentage

It can be observed that the germination for the treatments with PEG-6000 was the ones with higher percentages of normal seedlings obtained, being higher in 28% to 38% with respect to the treatments with KNO_3. Although, if we compare the KNO_3 conditioned seeds with the control without osmotic conditioning and also stored for 45 days, the KNO_3 presented a superiority of 30% to 20% more germinated seeds, which shows that the germination percentage of the seeds without previous conditioning is reduced during storage.

According to Sánchez et al. (2007), in a study carried out with *Allium cepa* seeds, osmotically conditioned with PEG, KNO_3 and KCl, significant statistically differences were found, with the highest percentage in germination being PEG with 81%, surpassing the nitrate and potassium chloride. However, statistically the control (untreated) was superior to the osmotic agents used in this test.

Sanchez et al. (2001), investigated about the seeds conditioning treatments with water, at different times of imbibition on the germination and the establishment of two varieties of seeds of *Cucumis sativus*, the tested treatments accelerated and significantly increased the seeds germination. In the present investigation osmotic conditioners were used (PEG and KNO_3), which are characterized by a water absorption pattern that stabilizes the germination period of the seeds in phase 2 of imbibition just before issuing the radicle, this allows better control of the phases of water absorption, thus also presenting significant increases in the percentage of germination.

3.2 Germination Rate Index

A better stand of plants per day was found for the treatment with low concentrations of PEG-6000 having a difference of up to 12 plants less per day in the treatment that was conditioned with KNO_3. In addition, it was observed that the control (untreated) of the cucumber seeds had a low GRI compared to the other conditioned treatments, which suggests that the use of osmotic conditioning is opportune when the cucumber seeds are stored for a long time and it is desired to standardize and accelerate the germination of them.

In the results presented by Nascimento (2005), who worked on vigor tests to evaluate cucumber seeds, shows that for the GRI, positive responses were obtained with the conditioning in water of the seeds without a subsequent drying in comparison to the conditioning plus the drying, however, this did not differ statistically from the control

3.3 Radicular Length

The data regarding root length for the cucumber seeds observed in Table 1 reveal a significant statistically difference of up to 3.82 cm between treatments, were PEG-6000 has the lowest concentration with -0.2 Mpa which reacted best to the treatment with a 7.29 cm length. On the other hand, the treatments with KNO_3 that obtained low root lengths and the control (untreated) being the one that presented the lowest root length with only 2.50 cm.

Kilian (2005), observed negative effects on root length in tomato seeds that were osmotically conditioned with solutions of NaCl (11.98 g/100 ml) and KCl (4.02 g/100 ml), which could be also observed in cucumber seeds that were conditioned with KNO_3 at a 300 mg concentration, presenting the lowest root length with only 3.47 cm.

These results give us an important tool such as the seeds osmotic conditioning, for when adverse situations are presented as in the case of water stress since longer and more vigorous roots will be able to resist better these conditions.

3.4 Seedlings Emergence

In the last variable studied for cucumber seeds, it was shown that there are significant statistical differences, where the T1 with a -0.2 Mpa concentration of PEG-6000 was the one that presented the highest result, with an average of 90% of emergency of seedlings compared to the KNO_3 and the control being those the ones that presented the lowest emergency values.

The results obtained from the control seeds (untreated) and stored for a 45 days period, presented decreasing seedlings, where only 18% of emerged seedlings were obtained, which suggests that prolonged storage of cucumber seeds is not recommended.

Table 2. Effect of osmotic conditioning of tomato seeds on germination percentage (G), germination rate index (GRI), root length (RL) and seedlings emergence (SE). San Lorenzo, Paraguay, 2018

Treatment	G (%)	GRI (pl/day)	RL (cm)	SE (%)
1 PEG (-0.2 Mpa)	90 a	17 a	4.90 a	87 a
2 PEG (-0.4 Mpa)	90 a	15 ab	4.54 a	78 ab
3 PEG (-0.6 Mpa)	91 a	17 a	4.42 ab	75 ab
4 PEG (-0.8 Mpa)	87 a	14 b	2.43 c	61 b
5 Control	90 a	14 b	3.82 b	77 ab
Mean	**87**	**11.6**	**4.02**	**76**
CV (%)	**4.66**	**10.22**		**20.87**

Note. G: germination; GRI: rate index; RL: root length and SE: seedlings emergence. Averages followed by the same letter in the column do not differ statistically by Tukey test at 5% probability. CV: coefficient of variation.

3.5 Germination Percentage

In this variable none significant statistical differences were detected in any of the treatments conditioned with different concentrations of PEG-6000.

Liptay and Tan (1985), found that in seeds of tomato with a osmotic conditioning treatment with PEG and in conditions of severe water stress, the percentage of germination was markedly superior in regard to the untreated seeds and under conditions of not too severe stress, the germination was faster and more uniform, although the final germination percentage was not superior in comparison to the control.

In an investigation carried out in seeds of husk tomato, with treatment periods of 48, 72 and 96 h, significant differences were observed in the control compared with all the evaluated osmotic substances such as; potassium nitrate, potassium chloride and polyethylene glycol, being the osmotic conditioning with potassium nitrate for 48 hours the one that favored the physiological quality of the husk tomato seed cv. Rendidora, effect that persisted up to 180 days after treatment (Marin et al., 2007).

On the other hand, Oluoch and Welbaum (1996) stated that the effectiveness of these treatments depends on the degree of maturation that the seeds have at the moment of its obtaining, which agrees with Bray (1995), and Chojnowski, Corbineau, and Côme (1997) where concluding that the germination increase by the osmotic conditioning of the seeds is not only due to the activation of the germinative and membrane repair mechanisms presents in the crops, but apparently other processes related to the physiological maturation of the seeds are also involved.

3.6 Germination Rate Index

the highest concentrations of PEG-6000 used did not favor the uniformization of the plants stand, not like the osmotic potentials with -2 Mpa of PEG-6000 which throwed the highest values with an average of 17 plants per day.

The GRI of tomato seeds followed a similar behavior to the germination percentage, where we can say that the most effective treatments to accelerate and standardize the germination were the osmotic conditioning with the lowest concentration of PEG-6000, which obtained better results.

Sánchez et al. (1999) mention that such effect could be due to the water absorption barrier imposed by the osmotic solutions, thus balancing the adsorption of water by the seeds to the point of allowing them to quickly reach a suitable moisture level in a large proportion of seeds and, with that, , standardize and accelerate the germination start time.

These results show that polyethylene glycol as an osmotic seed conditioner does not have a negative effect on tomato seeds and that with small concentrations, favorable results can be obtained, such as the increase of seedlings per day.

3.7 Radicular Length

When comparing different concentrations of PEG-6000, significant statistical differences were obtained between treatments, being higher the T1 with an average of 4.90 cm of root length, in addition it was found that higher concentrations such as -0.8 Mpa can be harmful for tomato seeds, with a difference of up to 2.47 cm less.

McDonald (2000), reports that one of the benefits of osmotic seed conditioning is the stimulation of root growth, which coincides with the results obtained in this investigation.

In these results is shown that the osmotic conditioning of seeds gives positive results with PEG minimum concentrations and in high doses there can be a decrease in root length compared to the control. This could be due to the fact that during phase 2 of imbibition of the germination before emitting the radicle in the process of essential organs repair, part of the cotyledons reserve material is gradually becoming available for the growth of the embryonic axis and this may determine the growth or appearance of roots when the doses of osmotic solutions are high.

3.8 Seedlings Emergence

There were significant statistical differences in PEG-6000 concentrations between T1 (lowest concentration: -0.2 Mpa) that obtained high percentages of emergence of seedlings and T4 (highest concentration: -0.8 MPa) that presented a decrease in the emergency. On the other hand, the control did not present significant statistical differences in relation to the four treatments.

Orta et al. (1998), performed an experiment with different osmotic conditioning treatments, the seeds conditioned with PEG and H_2O at 25 ºC for 3 days increased the emergence and the vigor of seedlings.

In an investigation carried out by Marin (2007), he emphasizes that the period of osmotic conditioning must be short; with prolonged periods there is a risk that the ions of the osmotic agents penetrate the seed and damage the embryo and although we know that for short periods reduces the storage time, which does not agree with the data obtained in this investigation, where the physiological quality of the tomato peel seed was maintained as the storage period was prolonged, in addition higher physiological quality was observed in conditioned seeds than in the control (untreated), this affirms that the osmotic conditioning facilitates the repair of damage associated with disturbances in the membranes, oxidation, mitochondrial dysfunction and enzymatic inactivation; fact that could be evidenced in the present investigation, since a smaller number of abnormal seedlings was obtained

4. Conclusion

With the results obtained and under the conditions which the experiment was performed it is concluded that:

Tomato seeds conditioned osmotically with different concentrations of PEG-6000 and stored for 30 days, was favored with the lowest concentration (-0.2 Mpa) of PEG-6000 as it uniformed and accelerated germination, increased the length of the roots and the percentage of emergence of seedlings.

The cucumber seeds osmotically conditioned with different concentrations of PEG-6000 and KNO_3 and stored for 45 days, obtained positive results with the osmotic solution of PEG-6000 at -0.2 Mpa in the germination variables and in all the vigor variables.

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