

# Imbibition and percentage of germination of cape gooseberry (*Physalis peruviana* L.) seeds under NaCl stress

Imbibición y porcentaje de germinación en semillas de uchuva (*Physalis peruviana* L.) bajo estrés por NaCl

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## ABSTRACT

In Colombia cape gooseberry is often grown on salt affected soils. The present study evaluated the effect of increasing NaCl concentrations on imbibition and percentage of germination of 'Colombia' ecotype cape gooseberry seeds. Under controlled laboratory conditions (25/20°C day/night temperature, 80% relative humidity, and a 12 hour photoperiod), the seeds were subjected to 0, 30, 60, 90 and 120 mM NaCl concentrations (corresponding to respective electrical conductivity levels of 0.8, 3.0, 6.0, 9.0, and 12.2 dS m<sup>-1</sup>), during an evaluation period of 299 hours. A significantly lower imbibition level, expressed as 35% of the fresh weight accumulated by the control seeds, was observed in the 120 mM NaCl treatment. At the end of the experiment, respective germination percentages of 97.6% and 96.4% were recorded in the salt-free seeds and in those exposed to 30 mM NaCl. In contrast, only 62.5% of those seeds treated with 120 mM NaCl germinated. Root malformations such as lack of elongation were observed in the highest NaCl concentration treatment. Regarding its germination process, cape gooseberry can be classified as moderately tolerant to sodium. In effect, after 299 h of treatment, there was no statistical difference in imbibition level or percentage of germination between the 0, 30 and 60 mM NaCl treatments.

**Key words:** electrical conductivity, salt tolerance, water uptake, root malformation.

## RESUMEN

En Colombia la uchuva está siendo cultivada, entre otros, sobre suelos afectados por sales. Con el fin de conocer el efecto del incremento de la concentración de NaCl sobre el grado de imbibición y el porcentaje de germinación de las semillas del ecotipo 'Colombia', estas se sometieron durante 299 horas a soluciones de NaCl 0, 30, 60, 90 y 120 mM (correspondientes a conductividades eléctricas de 0,8; 3,0; 6,0; 9,0 y 12,2 dS m<sup>-1</sup>, respectivamente), en condiciones controladas de laboratorio (25/20°C de temperatura diurna/nocturna, humedad relativa del 80% y un fotoperiodo de 12 h). Al tratar las semillas con una solución de NaCl 120 mM se observó una reducción significativa del grado de imbibición, evaluado a través del peso fresco acumulado de la semilla, que fue el 35% del acumulado por el control. Al terminar el experimento, mientras que las semillas no expuestas a la sal y las que fueron tratadas con NaCl 30 mM registraron porcentajes de germinación de 97,6 y 96,4%, respectivamente, aquellas tratadas con la solución 120 mM solamente germinaron en un 62,5%. A las concentraciones más altas de NaCl se observaron malformaciones de la raíz, tales como falta de elongación. Debido a que al final del experimento no se detectaron diferencias significativas en el grado de imbibición ni en el porcentaje de germinación a las concentraciones 0, 30 y 60 mM, la uchuva puede ser clasificada como una especie moderadamente tolerante al sodio con respecto al proceso de germinación.

**Palabras clave:** conductividad eléctrica, tolerancia a la sal, absorción de agua, malformación radical.

## Introduction

Abiotic stresses are major constraints on worldwide crop production, and salinity as one of the biggest problems affects about one-third of the irrigated land on earth (Mengel *et al.*, 2001). This has led to concentrate research efforts on salt tolerance of plants, in order to improve crop yield (Zhu, 2001).

For glycophytes, which include all the plant species cultivated for commercial purposes, salinity entails ionic stress (mainly

due to the effect of Na<sup>+</sup>, Cl<sup>-</sup>, and SO<sub>4</sub><sup>2-</sup>), as well as osmotic and secondary stresses such as nutritional and oxidative ones (Zhu, 2002). In cases of high substrate salinity, due to the many factors involved, it is not possible to assess the relative contribution of these mayor constraints to growth inhibition (Marschner, 2002). Said factors include ion concentration in the substrate, exposure time, plant species, cultivars and stock, phenological stage of organs and whole plant, and environmental conditions (Tobe *et al.*, 2002).

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Wild plant germination and growth in saline soils takes place mainly during the rainy season, when the soil solution has been diluted and salts are washed out from under the root zone (Daubenmire, 2001). The first exposure of the crop to salinity conditions usually occurs at the germination stage, both in direct seeding and transplanting production (Passam and Kakouriotis, 1994). Deficient germination and decreased seedling growth result in poor establishment and eventual failure of the crop (Soltani *et al.*, 2006).

Seeds and seedlings are particularly vulnerable to increases in salinity because at that stage plants have not yet developed the physiological mechanisms to tolerate increasing salinity concentrations (Adam, 1990). In seeds, water is essential for enzyme activation, breakdown and translocation, as well as storage material utilization (Copeland and McDonald, 2001).

Authors have reported differences in the response to stress by seeds collected at different moments following anthesis. Thus, Demir and Mavi (2008) observed that pepper seeds harvested 70 d after anthesis had greater resistance to stress conditions than those harvested earlier.

In an attempt to understand plant response to saline stress, many studies have focused on the effect of NaCl on growth and ion absorption. Examples are the works of Costa-Franca *et al.* (2000) and Bayuelo-Jiménez *et al.* (2003) on *Phaseolus* species, Essa (2002) on *Glycine max*, El-Siddig *et al.* (2004) on *Tamarindus indica*, Alian *et al.* (2000) on *Solanum lycopersicum*, and Flórez *et al.* (2008) on *Solanum quitoense*. Regarding cape gooseberry, research on this topic is at the initial stage, although the production areas for this crop in Colombia are located in salt affected soils.

Maas and Grattan (1999) pointed out that reliable data on crop tolerance to salinity during emergence and seedling growth are extremely limited. Thus, as a first step, the present research on cape gooseberry response to salinity aimed to determine the effect of increasing NaCl concentrations on seed imbibition and germination percentage under laboratory conditions.

## Materials and Methods

### Seeds

*Physalis peruviana* (Colombia ecotype) seeds were obtained from the municipality of Granada, Cundinamarca (near Bogota) from plants selected for their sanitary

condition, vigour and productivity. Only fully mature fruits were used.

### Conditioning

The seeds were sterilized for 10 s with a mercury chloride solution, and then washed in distilled water several times. They were divided into five groups and placed in sterilized Petri dishes, each one having a paper filter on its base.

### Treatments

For a total period of 299 h, the seeds were dampened with 10 mL of distilled water in the control (treatment 1), and with four different NaCl concentration solutions (30, 60, 90 and 120 mM) (Tab. 1). Five replications of each treatment were placed in germination chambers at constant 25/20°C day/night temperature, 80% relative humidity and a daily 12 h light period (Sanyo Versatile Environmental test Chamber-Kasay, Etten Leur, The Netherlands). In order to keep the seeds adequately moistened, they were regularly dampened with distilled water or with the corresponding saline solution. They were considered germinated when the emerging radicle was approximately 1 mm long.

TABLE 1. Description of the NaCl solutions used in the study.

Treatment	NaCl (mM)	Electric conductivity (dS m <sup>-1</sup> )	Osmotic potential (MPa)
1	0	0.79	-0.02
2	30	3.00	-0.10
3	60	6.00	-0.21
4	90	9.00	-0.32
5	120	12.20	-0.43

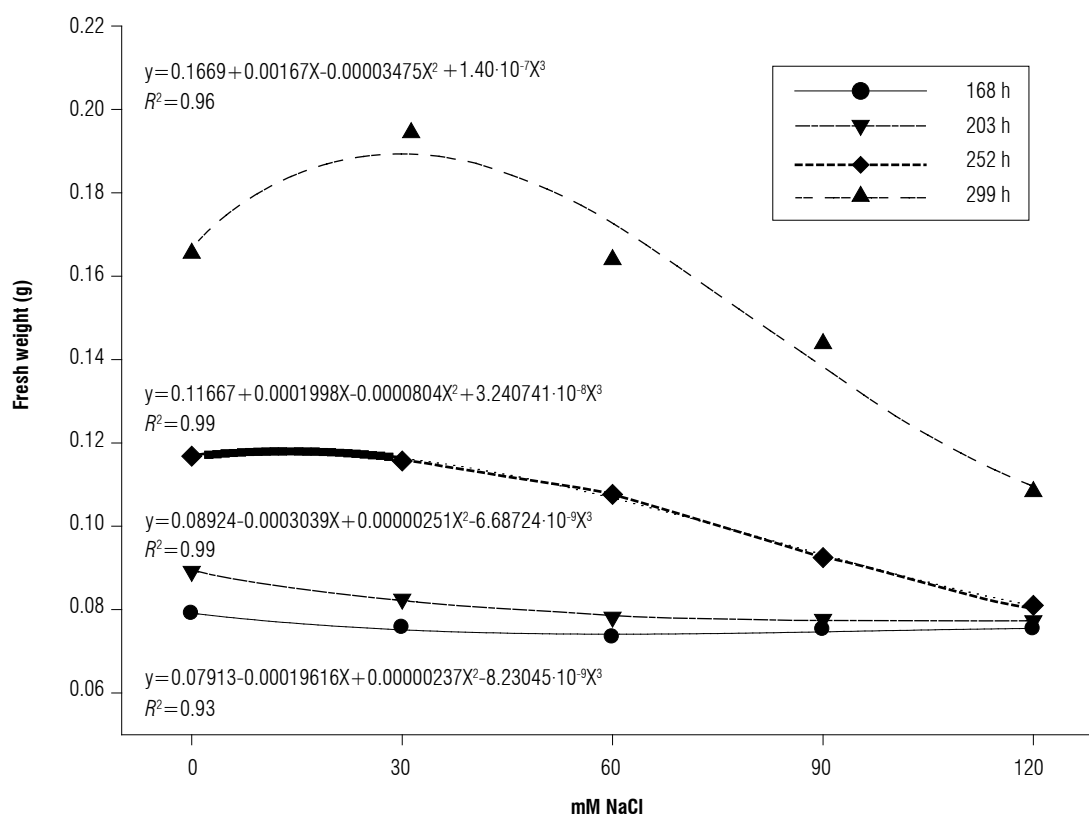
### Data analysis

The experiment was carried out using a completely randomized design with five replications and 50 seeds per treatment. Both imbibition (*i.e.*, water absorption represented as seed fresh weight increase) and percentage of germination were evaluated every 24 h until the end of the experiment. Means of five replications were subjected to variance analysis. Tukey's test was used for comparing averages ( $P \leq 0.05$ ).

## Results

### Imbibition

During the first 24 h, a high increase in average seed fresh weight, ranging from 0.025 to 0.075 g (for simplicity reasons, only results after 168, 203, 252 and 299 h are reported on tables), was generally observed due to



**FIGURE 1.** Accumulated fresh weight of cape gooseberry seeds after 168, 203, 252 and 299 h of imbibition, as a function of NaCl salinity levels.

imbibition. After 168 h of imbibition, average seed fresh weight oscillated from 0.073 g (60 mM NaCl) to 0.079 g (control). From then on, water salinity affected imbibition quadratically (Fig. 1).

The first significant differences ( $P \leq 0.05$ ) were observed after 203 h of treatment, when the unsalinized seeds had higher fresh weight than those treated with 60 to 120 mM NaCl solutions (Tab. 2). After exposing the seeds to NaCl for 252 and 299 h, those of the 0 and 30 mM NaCl treatments had taken up the most water. Thus, after 168 to 299 h of observation, the greatest weight increases were recorded in the control treatment (108.9%), followed by the 30 mM one (160.0%). In the meantime, the increase

was only 44.0% in the 120 mM treatment. At the end of the experiment, the seeds imbibited in the 30 mM solution had accumulated more water than the salt free ones (Fig. 1, Tab. 2); the 90 and 120 mM treatment seeds had respectively absorbed 26.7% and 44.6% less fresh weight than those in the 30 mM treatment, the differences being statistically significant ( $P \leq 0.05$ ); and the highest saline solution seeds (120 mM NaCl) had gained 34.5% less fresh weight when compared to the unsalinized seeds (Tab. 2, Fig. 1). In this way, the greatest seed weight was finally recorded in the 0 mM NaCl treatment, which allowed the seeds to gain 311% of their initial weight; and the lowest record corresponded to the maximum concentration treatment seeds, which increased their weight by 196% (Tab. 2).

**TABLE 2.** Average fresh weight of cape gooseberry seeds imbibited in several saline solutions for 299 h.

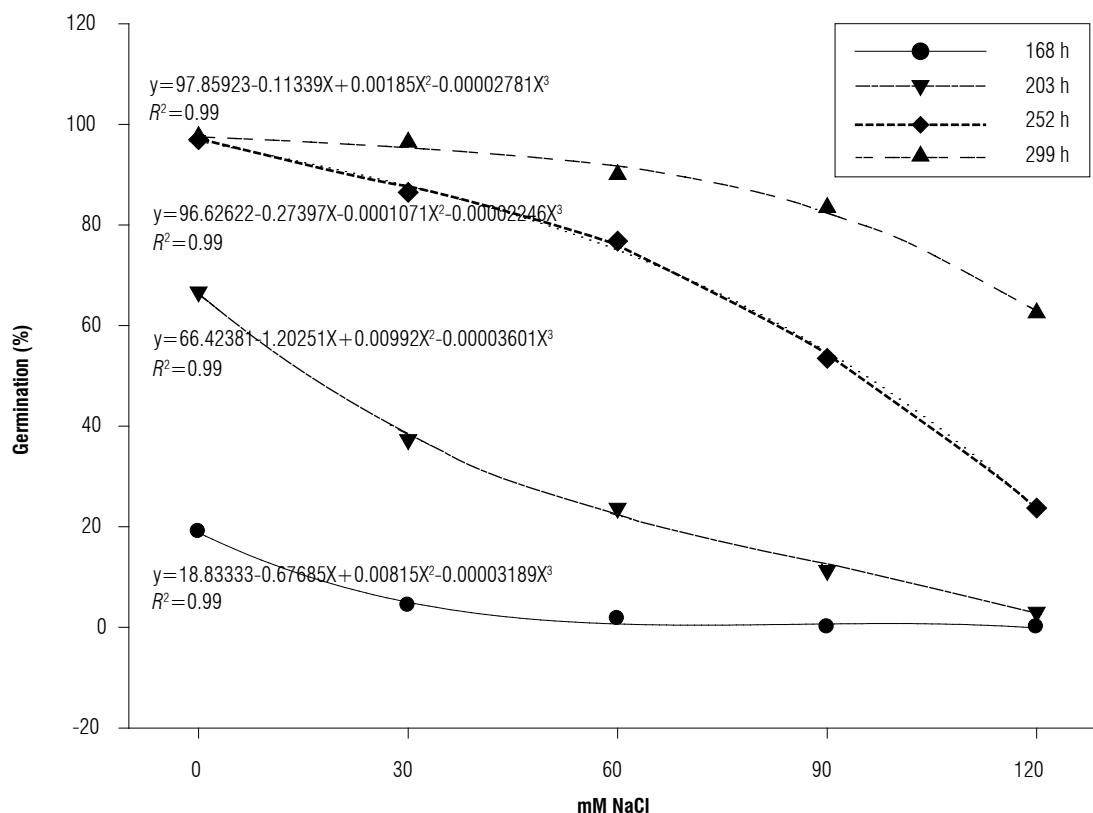
NaCl (mM)	Initial seed weight (g)	Fresh weight (g) progress in time after starting imbibition			
		168 h	203 h	252 h	299 h
0	0.054 a	0.079 a	0.089 a	0.116 a	0.165 ba
30	0.054 a	0.075 ab	0.082 ab	0.115 a	0.195 a
60	0.054 a	0.073 ab	0.078 b	0.107 b	0.164 ba
90	0.054 a	0.075 ab	0.077 b	0.092 bc	0.143 bc
120	0.054 a	0.075 ab	0.077 b	0.081 c	0.108 c

Means in the same column followed by the same letter are not significantly different according to Tukey's test ( $P \leq 0.05$ ).

**TABLE 3.** Germination percentages of cape gooseberry seeds subjected to several saline concentrations for 299 h.

mM NaCl	Germination % progress in time after starting imbibition			
	168 h	203 h	252 h	299 h
0	19.00 a	66.66 a	96.93 a	97.58 a
30	4.33 b	37.33 b	86.46 a	96.47 ab
60	1.66 b	23.66 bc	76.87 ab	90.03 ab
90	0.00 b	11.33 cd	53.49 b	83.45 b
120	0.00 b	3.00 d	23.71 c	62.53 c

Means in the same column followed by the same letter are not significantly different according to Tukey's test ( $P \leq 0.05$ ).



**FIGURE 2.** Germination percentages of cape gooseberry after 168, 203, 252 and 299 h of imbibition in different concentration NaCl solutions.

### Percentage of germination

During the first 156 h, seed germination percentages were very low and did not differ among treatments. Then, they started to increase with time.

After 168 h of treatment, NaCl concentrations affected ( $P \leq 0.01$ ) the percentages of germination quadratically (Fig. 2). At that moment, the latter were significantly higher for the control seeds (19%), followed by those of the 30 mM treatment, whilst the greater salt concentration ones had not germinated yet (Tab. 3). After 203 h, a significantly

higher percentage of germination was observed in the distilled water treated seeds, whereas increased NaCl concentrations had reduced it (Tab. 3).

After 252 to 299 h, the percentages of germination remained nearly constant in the control seeds (Tab. 3). During this evaluation period, no statistical differences were observed among unsalinized and 30 to 60 mM treatments seeds. Those exposed to the 90 and 120 mM solutions germinated in significantly lower percentages ( $P \leq 0.05$ ) than those observed at lower salt concentrations. In the two last

evaluations, the 120 mM NaCl exposed seeds reached the lowest percentages of germination, which were significantly different from those observed in the 90 mM treatment (Tab. 3, Fig. 2).

## Discussion

### Water uptake by the seeds

The results reveal that the saline solutions had significant effects on imbibition. Accumulated fresh weight of seeds was highly reduced when they were subjected to the 120 mM NaCl solution. This result is contrary to observations by Gill *et al.* (2003) on sorghum seeds, which were found to gain fresh weight at increased salinity. The tendency (although not statistically significant) to take up more water than the control seeds, observed in the 30 mM treatment after 299 h of experiment, could be related to the moderate tolerance to sodium exhibited by this species, as reported by Ulloa *et al.* (2006) regarding growth stimulation of cape gooseberry plants exposed to 30 mM NaCl.

Similar results were obtained by Murillo-Amador *et al.* (2002), who demonstrated that water uptake in cowpea (*Vigna unguiculata*) seeds was inhibited after the first 24 h of exposure to imbibition, whereas in our study it was hampered after 168 h. Mehra *et al.* (2003) and Murillo-Amador *et al.* (2002) found out that either dryness or salinity in growth medium affected seed germination by reducing water absorption. Under these stresses there is a decrease in water uptake during imbibition. Furthermore, salt stress may cause excessive uptake of ions (Murillo-Amador *et al.*, 2002).

### Percentage of germination

Germination is more successful in salt-free settings, or in those having extremely low saline conditions (Larcher, 2003), but in the present experiment cape gooseberry seeds germinated well at 30 and 60 mM NaCl concentrations, especially after 252 to 299 h, confirming the tolerance of this species to low and moderate salt concentrations (Ulloa *et al.*, 2005, Miranda, 2010). Larcher (2003) defined the upper limit for germination and subsequent growth at an EC of 15–20 dS m<sup>-1</sup> or less, which is only somewhat higher than that of our 120 mM NaCl (12.2 dS m<sup>-1</sup>) treatment, the one that allowed 62.53% of the seeds to germinate. According to Prado *et al.* (2000) and Patanè *et al.* (2009) the decrease in germination may be attributed to an apparent osmotic dormancy developed under saline stress conditions, which may constitute an adaptive strategy aimed at preventing germination in stressful environments.

The effect of the highest saline concentrations of our study (90 and 120 mM NaCl) was to delay germination. Such effect was observed mainly after 168 and 203 h of treatment, coinciding with observations by Sonaike and Okusanya (1987), who demonstrated that increased salinity generally resulted in reduced germinability and delayed germination rate in seeds of *Lufa aegyptica*. Similarly, Murillo-Amador *et al.* (2002) registered delayed germination rates in black-eyed pea seeds treated with saline solutions, although at the end of the evaluation period, 100% germination was reached at all osmotic potentials. Chartzoulakis and Klapaki (2000) demonstrated that 50 mM salinity in the external medium delayed germination in two pepper hybrids, but did not reduce the accumulated percentage of germination observed at the end of the experiment; whilst the ability to germinate became significantly reduced at 100 and 150 mM NaCl concentrations. Seeds of tomato (another Solanaceae species) have been found to need 50% more time to germinate in an 80 mM NaCl solution than in a salt-free-medium; and almost 100% more time when the concentration is raised to 190 mM (Cuartero and Fernández-Muñoz, 1999).

Reductions in both germination rates and plantlet emergence under salinity conditions have been demonstrated by Villagra (1997), Cony and Trione (1998), Murillo-Amador and Troyo-Diéguez (2000), Murillo-Amador *et al.* (2002) and Soltani *et al.* (2006). Both osmotic and salt toxic effects, have been implicated in germination inhibition (Machado *et al.*, 2004). Soares *et al.* (2002) found that salinity delayed the germination process in yellow passion fruit, but a relative reduction was only observed above an EC of 4.43 dS m<sup>-1</sup>. Foolad and Lin (1997) examined the germination of tomato seeds in different ionic and non-ionic germination mediums with identical osmotic potentials. They concluded that tomato germination rate was mainly affected by the osmotic effect of the medium, and just secondarily by its ionic effect.

Our results reveal important reductions in germination rates in those seeds subjected to the highest NaCl concentrations after 252 and 299 h of imbibition. This may indicate that seed osmotic adjustment was affected and that stress had possibly favoured the entering of other ions into the seeds. Smith and Comb (1991) attributed this to low humidity content, which may have increased saline stress, caused cessation of metabolism or inhibited certain stages in the germination metabolic sequence. Shokohifard *et al.* (1989) found two ways in which saline stress negatively affected radish seed germination: i) osmotically, by reducing water absorption, and ii) ionically, by accumulating Na<sup>+</sup> and Cl<sup>-</sup>,

thereby altering nutrient uptake balance and causing a toxic effect.

Besides their reduced germination, the cape gooseberry seeds treated in the present work were seen to produce deformed and shortened roots. Similar results were obtained by Chartzoulakis and Klapaki (2000) in several pepper cultivars, and by Ungar (1996) in *Atriplex patula* (spear saltbush). Salt damage is thought to affect seed germination through several factors such as reduced water availability, changes in storage material mobilization and protein structure disturbance (Foolad and Lin 1997; Almansouri *et al.*, 2001; Machado *et al.*, 2004). Stored carbohydrate mobilisation occurs during early germination, especially after radicle emergence. However, some mobilisation may occur between growth regions (*e.g.* embryo axis) before germination is completed (Bewley and Black 1994). Once high molecular weight carbohydrates are mobilised, they acquire soluble forms (*e.g.*, sucrose, glucose or fructose) which are immediately transported to sites where they are required for growth (Mayer and Poljakoff-Mayber, 1989).

Ungar (1996) presented the hypothesis that the tolerance of seeds to salinity works in two levels, given by the seed's ability to germinate under high salinity conditions; and to recover and germinate once such conditions have been released. Our results are in agreement with the first point of this hypothesis, since in the highest NaCl concentration treatments the first radicular protrusions were delayed in more than 36 h. This restricted germination pattern persisted until the end of the experiment.

According to our findings, the ability of *P. peruviana* to germinate and emerge under low saline stress conditions could indicate that the species possesses certain salt tolerance genetic potential, at least during its early development stage. This does not necessarily indicate that plantlets initiated in saline stress conditions can continue to grow and complete their adult plant life cycle in these circumstances. Said contrast has already been observed in other species (Norlyn and Epstein, 1984). However, studies carried out by Ulloa *et al.* (2006) on adult cape gooseberry plants exposed to increasing NaCl concentrations have demonstrated that this species is moderately tolerant to salinity as it manages to perform well even at conductivity values of up to 6 dS m<sup>-1</sup>.

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