American International Journal of Biology
December 2016, Vol. 4, No. 2, pp. 1-22
ISSN: 2334-2323 (Print), 2334-2331 (Online)

Published by American Research Institute for Policy Development
DOI: 10.15640/aijb.v4n2a1
URL: https://doi.org/10.15640/aijb.v4n2a1

Optimizing *in vitro* germination of *Capsicum baccatum* L. seeds through a multifactorial experimental design

Tiago Fidemann[1], Lia Bossard Nascimento[2], Milena Cristina Moraes[3], Mônica Rosa Bertão[4], Eutimio Gustavo Fernández-Núñez[5]

Abstract

The *Capsicum* genus comprises hundreds of varieties with fruits that differ widely in shape, size, color, and flavor. The Brazilian cuisine consumes *Capsicum baccatum* L. (pepper) extensively and few reports about chemical composition and biological properties about this pepper were published. The present study determined four factors numeric effects on germination *in vitro* of this specie, among them, the solution type for osmotic conditioning (water and 1% KNO_3 aqueous solution), germination medium (agar and agar + gibberellic acid at 1.88 mgL^{-1}), post-seeding time (15 and 30 days) and two genotypes (Pitanga and Cambuci) on two germination parameters (germination and cotyledon emerging rates), as first step for establishing a cell suspension culture to produce secondary metabolites. The methodology was a two-level full factorial experimental design (2^4). The results allowed polynomial equations definition which describes the germination phenomena as a function of the four factors under study. The genotype responses to osmotic conditioning and germination medium were different. The optimal combination of treatments for *in vitro* germination and development of seedlings for Pitanga and Cambuci was water + agar and water + agar-GA_3, respectively.

Keywords: Peppers, experimental design, gibberellic acid, potassium nitrate, plant cell culture.

[1] Laboratório de Biotecnologia Vegetal, Grupo de Engenharia de Bioprocessos, Departamento de Biotecnologia, Faculdade de Ciências e Letras, UNESP - Universidade Estadual Paulista, Assis, Brasil, Avenida Dom Antonio 2100, Bairro Parque Universitário, 19806-900, Assis, São Paulo, Brasil.
[2] Laboratório de Biotecnologia Vegetal, Grupo de Engenharia de Bioprocessos, Departamento de Biotecnologia, Faculdade de Ciências e Letras, UNESP - Universidade Estadual Paulista, Assis, Brasil, Avenida Dom Antonio 2100, Bairro Parque Universitário, 19806-900, Assis, São Paulo, Brasil.
[3] Laboratório de Biotecnologia Vegetal, Grupo de Engenharia de Bioprocessos, Departamento de Biotecnologia, Faculdade de Ciências e Letras, UNESP - Universidade Estadual Paulista, Assis, Brasil, Avenida Dom Antonio 2100, Bairro Parque Universitário, 19806-900, Assis, São Paulo, Brasil.
[4] Laboratório de Biotecnologia Vegetal, Grupo de Engenharia de Bioprocessos, Departamento de Biotecnologia, Faculdade de Ciências e Letras, UNESP - Universidade Estadual Paulista, Assis, Brasil, Avenida Dom Antonio 2100, Bairro Parque Universitário, 19806-900, Assis, São Paulo, Brasil. E-mail: mrbertao@assis.unesp.br, Telephone: +55-18-3302-5848
[5] Laboratório de Biotecnologia Vegetal, Grupo de Engenharia de Bioprocessos, Departamento de Biotecnologia, Faculdade de Ciências e Letras, UNESP - Universidade Estadual Paulista, Assis, Brasil, Avenida Dom Antonio 2100, Bairro Parque Universitário, 19806-900, Assis, São Paulo, Brasil.

1. Introduction

The *Capsicum* genus (Solanaceae) includes hundreds of varieties with fruits that differ widely in shape, size, color and flavor. Native from Central and South America, the *Capsicum* peppers have an important economic value (Manzur, Penella, & Rodríguez-Burruezo, 2013; Zimmer et al., 2012)being used as spice and condiment in cooking around the world. Have a high nutritive value besides being a rich source of vitamins C (ascorbic acid), A (β-carotene and provitamin A), B2 (riboflavin), B3 (niacin), E, K and B6, along with minerals like molybdenum, manganese and potassium (Kothari, Joshi, Kachhwaha, & Ochoa-Alejo, 2010).

Brazilian cuisine consumes different varieties of *Capsicum baccatum* L. extensively and few reports about chemical composition and biological properties on this pepper have been published. Recently, antioxidant and anti-inflammatory activities associated to its phenolic compounds and flavonoids have been demonstrated (Bertão, Moraes, Palmieri, & Pereira, n.d.; Zimmer et al., 2012). Nevertheless, in many cases, the natural availability of these compounds is low and the plant extraction methods require using some part or the entire plant. These practices are not sustainable, even more, when the plant is rare or endangered. The *in vitro* culture techniques can solve these drawbacks (Matkowski, 2008).

The plant cell and tissue culture is an essential tool for basic and applied academic studies as well as in industrial applications including several cultivation methodologies under sterile conditions such as seed germination, micro propagation, callus and cell suspension culture(Wilson & Roberts, 2012). *In vitro* propagation methods allow the selective, rapid and effective production of secondary metabolites avoiding seasonal, soil, geographical constrains as well as pests (Costa, Gonçalves, Valentão, Andrade, & Romano, 2013).

Micro propagation is one of the most important areas where *in vitro* culture has played a commercially remarkable role. *In vitro* propagation technologies when well-developed and/or adapted are critical for genetic resources and genetic improvement of the species conservation programs. A large number of important plants from horticultural, floricultural, and silvicultural have been successfully propagated. Among the most important *in vitro* technique applications, is the micro propagation of medicinal plants, species threatened and plants producing spices, flavoring and coloring substance (Matkowski, 2008; Purohit, 2013). Recent research on *Capsicum* cell culture also showed the great potential of *in vitro* cultivation techniques for understanding the cellular and molecular processes involved in the expression and production of metabolites of commercial interest (Kehie, Kumaria, Tandon, & Ramchiary, 2015).

In vitro seed germination is a decisive step for the success of numerous *in vitro* culture procedures and often allows a higher germ inability than in nurseries, greenhouses or field, probably because *in vitro* conditions are more suitable for germination processes and early seedling development. *In vitro* seed germination protocols are frequently used to produce sterile seedlings, which are source of shoot tips and stalk as explants for micro propagation (Kothari et al., 2010).

Some factors should be considered in order to optimize *in vitro* seed germination process and the subsequent seedling development, among these, plant growth regulator types and concentrations, culture medium nutrient composition, photoperiod, post-seeding time (Roy & Banerjee, 2001). Other important factors to take into account in the *in vitro* methodology are the pre-germination treatments with the major purpose of promoting seed priming and increase the physiological potential of seeds, uniformizingits vigor.

Several chemical strategies are available for *in vitro* seed germination in *Capsicum* and others different plant species (Bello-Bello, Canto-Flick, Balam-Uc, Gómez-Uc, & Robert, 2010; Bora, Gogoi, & Handique, 2014) and the influence of genotypes have been demonstrated (Shahzad & Sahai, 2014).

To determine the critical combination of factors that permit the improvement of *in vitro* germination in *Capsicum* seeds is an important strategy to develop multivariate protocols. Thus, this study aimed to evaluate the main effects of four factors potentially involved in the enhancement of *in vitro* seed germination in two genotypes of *C. baccatum*, the solution type for osmotic conditioning, germination medium, post-seeding time and genotypes, as well as the interactions between them on germination and cotyledon emerging rates, as a first step for establishing a cell suspension culture to produce secondary metabolites with commercial value in this species, besides demonstrate the potential of "two-level" full factorial experimental design for systemic establishing of the *in vitro* seed germination protocols.

2. Materials and Methods

2.1 Plant Material

The experiments were carried out in theLaboratório de Biotecnologia Vegetal, Departamento de Biotecnologia, UNESP – FCL, Assis-Brasil. Seeds of two *Capsicum baccatum* L. (Pepper) genotypes were used, Pitanga (GN1) and Cambuci (GN2), obtained in street markets from Assis, São Paulo State (-22º39'42''S, -50º24'44''W). Seeds were separated in two plots (200 seeds each) and used for assessing *in vitro* germination.

2.2 Seeds Pretreatment and Disinfestation

Seeds of each *Capsicum baccatum* L. genotypes were submitted to pretreatment with 1%KNO_3 aqueous solution (w/v) for 10 minutes in order to evaluate its influence on osmotic conditioning. The remaining seeds were pretreated with distilled water (control group) for 10 minutes. Seeds groups were washed under running water and transferred to sterile environment. Pretreated seeds were surface sterilized in a laminar flow hood by soaking in 70% (v/v) ethanol for 2 minutes, rinsed with sterile distilled water, immersed in 1% (v/v) sodium hypochlorite for 20 minutes, and rinsed three times with sterile distilled water. After disinfestation, the seeds were kept submerged in sterilized distilled water until inoculation.

2.3 Seeding and Culture Medium

Sterilized seeds were aseptically inoculated in two different culture medium, M1, containing distilled water, 0.6% agar and 1.88 mg L^{-1}gibberellic acid (GA_3), and M2 (control), constituted of distilled water and 0.6% agar. For each culture medium were used 10 flasks of 500 mL containing 50 mL of medium (10 seeds /flasks), previously autoclaved at 121ºC, 1 atm for 15 minutes. Of the seeds pretreated with KNO_3 aqueous solution, 50 seeds were inoculated in M1 and 50 seeds in M2 and the same procedure was follow to the seeds pretreated with distilled water (control). All cultures were incubated at 25±2°C and 16 hours photoperiod.

2.4 Determination of Germination and Cotyledon Emerging Rates

Seeds were considered germinated when the radicle was 1 mm or greater. Germination rate was observed at 15 and 30 days and was calculated as the ratio between the number of seeds with radicle protrusion and the total number of seeds in each flask. The corresponding equation (1) used for germination rate was:

$$(1) \qquad Y_{g_i} = N_{gp_i}/n$$

Where:

Y_{g_i}: Germination rate in each flask (i=1, 2, 3, 4, 5); N_{gp_i}: number of seeds with radicle protrusion in each flask; n:total number of seeds in each flask (n=10).

Cotyledon emergingrate was also observed at 15 and 30 days, and it was determined as the ratio between the number of plants with cotyledon emerging and the total number of seeds in each flask. The mathematical expression (2) for calculate cotyledon emerging rate was:

$$(2) \qquad Y_{c_i} = N_{cp_i}/n$$

Where:

Y_{c_i}: Cotyledon emerging rate in each flask (i=1, 2, 3, 4, 5); N_{cp_i}: number of plants with cotyledon emerging in each flask; n: total number of seeds in each flask (n=10).

2.5 Experimental Design

In order to define the main effects of each factor, osmotic conditioning, gibberellic acid presence in germination culture medium, post-seeding time (15 and 30 days) and *Capsicum baccatum* L. genotypes (GN1 and GN2), as well as the interactions between factors on parameters germination and cotyledon emerging rates, a two-level full factorial design (2^4 experimental runs, five repetitions) was performed (Table 1). The level of significance (α) for statistical decisions was 0.05.

Experimental planning and data analysis were carried out in Design-Expert 6.0 Software (Stat-Ease, Inc.; Minneapolis, MN, USA).

Table 1: Factors as well as their classification and levels considered in full two-level factorial (2^4) design to describe germination and cotyledon emerging rates associated to pepper seeds considered in the present study.

Factor	Type	Range	
		Low	High
Genotype (A)	Categorical	GN1	GN2
Osmotic conditioning solution (B)	Categorical	Water	1% KNO_3 w/v
Germination culture medium (C)	Categorical	Agar	Agar + GA_3
Post-seeding time (days) (D)	Numerical	15	30

3. Results

Primary results related to a two-level full factorial design (2^4 = 16) and five repetitions of each experimental combination are presented in Table 2. The combination of distilled water (osmotic conditioning solution) +agar (germination culture medium) was the most efficient procedure for germination rate in genotype Pitanga (GN1) and this finding was independent of the total time of germination, reaching the maximum level of 74% efficiency. For genotype Cambuci (GN2), the best combination was distilled water + agar-GA_3 for initial germination (15 days) and after this period (30 days) irrelevant to the seedling development, with values ranging between 76 and 86% germination efficiency. For the seedlings development, the 1% KNO_3 (w/v) + agar-GA_3 combination showed greater efficiency in cotyledon emergence for GN1 to 30 days, reaching a maximum of 56% efficiency. For GN2 the highest cotyledon emergence efficiency was confirmed in GA_3 presence regardless the association with pretreatment.

Table 2: Primary data corresponding to a two-level full factorial design (24 = 16) performed with five repetitions of each experimental combination. Germination parameters are represented as mean ± standard deviation (n=5).

Run	Genotype (A)	Osmotic conditioning solution (B)	Germination culturemedium(C)	Time Days (D)	YG	YC
1	GN1	H2O	Agar	15	0.56±0.21	0.04±0.09
2				30	0.74±0.11	0.18±0.08
3			Agar-GA3	15	0.18±0.13	0.00±0.00
4				30	0.34±0.05	0.10±0.07
5		KNO3 1% (w/v)	Agar	15	0.45±0.35	0.05±0.06
6				30	0.53±0.38	0.20±0.18
7			Agar-GA3	15	0.48±0.22	0.16±0.11
8				30	0.72±0.24	0.56±0.17
9	GN2	H2O	Agar	15	0.66±0.11	0.30±0.28
10				30	0.76±0.05	0.32±0.31
11			Agar-GA3	15	0.75±0.10	0.45±0.13
12				30	0.83±0.05	0.58±0.13
13		KNO3 1% (w/v)	Agar	15	0.58±0.08	0.20±0.19
14				30	0.84±0.11	0.22±0.18
15			Agar-GA3	15	0.70±0.14	0.36±0.09
16				30	0.86±0.05	0.48±0.04

3.1 Germination Emerging

According to raw experimental data and confirmed by suitable statistical analysis, GN2 showed faster germination compared to GN1 (Table 3, Equation 3 and Figure1a). The significant and positive term A (genotype) validate this finding (Table 3, Equation 3).

Table 3: Analysis of variance for selected factorial models, which describe the cotyledon emerging rates and any detail related to in vitro germination emerging.

Source	In vitro germination parameter under consideration	
	Germination rate	Cotyledon rate
	Probability values	
Model	< 0.0001*	< 0.0001*
A	< 0.0001*	< 0.0001*
B	0.3039	0.4082
C	0.4422	0.0001*
D	< 0.0001*	0.0003*
AB	0.2606	0.0009*
AC	0.0086*	0.1236
AD	0.7806	0.0708
BC	0.0014*	0.0327*
BD	0.4231	0.2566
CD	0.9869	0.1809
ABC	0.0011*	0.0416*
ABD	0.4231	0.2426
ACD	0.4231	0.9449
BCD	0.7806	0.3705
ABCD	0.4422	0.3525
Lack of fit	0.3448	0.1762

*This symbol represents significant tests (model goodness of fit and lack of fit) or model terms. A, B, C and D represent variables: genotype, osmotic conditioning solution, germination culture medium and time, respectively.

However, the influence of germination culture medium was different for both genotypes under study. The supplementation of agar basal medium with gibberellic acid (GA_3) was only effective for GN2 and the corresponding seeds demonstrated superior germination rate (Figure 1a). A contrary effect for GN1 was observed. The post-seeding time (D) showed positive significant effects, for this reason, 30 days are necessary in order to increase germination rate *in vitro* for both genotypes (Table 3, Equation 3).

$$Y_G = 0.620 + 0.130 \cdot A + 0.082 \cdot D + 0.050 \cdot A \cdot C + 0.065 \cdot B \cdot C - 0.067 \cdot A \cdot B \cdot C \quad (3)$$

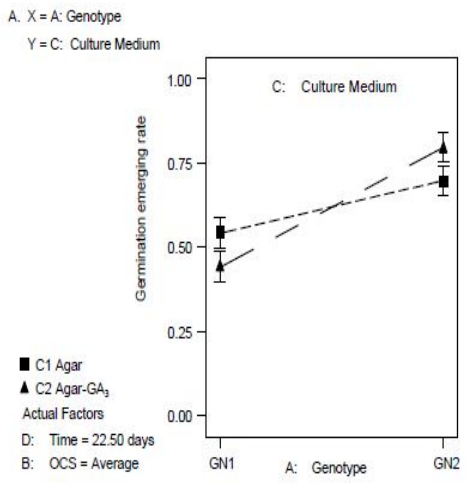
Where:

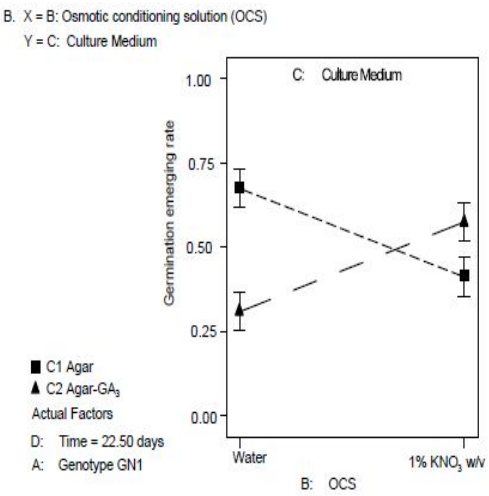
Y_G: Yield of any detail related to germination emerging; A: Genotype; B: Osmotic conditioning solution; C: Germination culture medium; D: post-seeding time (days).

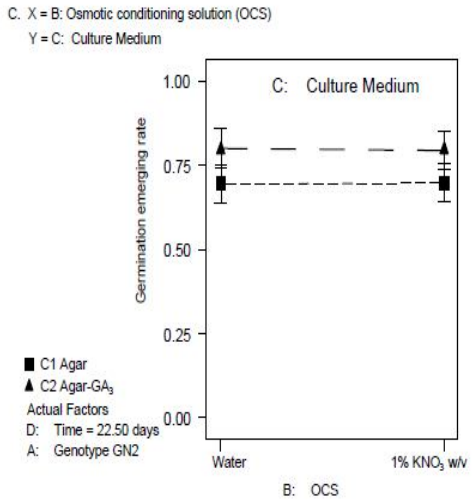
The water utilization as solution able to start and support seed germination (germination stimulating factor) demonstrated real positive effect only for GN1 (Figure 1b and 1c). For GN2, the impact of distilled water or 1% KNO_3 w/v, as solutions capable to start and support seed germination was the same on germination rate (Figure 1c).

For GN1, the positive influence of distilled water in the induction of *in vitro* germination was associated to the germination culture medium, when utilized the basal medium composed only of agar, the best germination rate was observed. Contrary effect was confirmed for 1% KNO_3 solution, its best performance was in association to agar supplemented with GA_3 (Figure 1b). This finding is also demonstrated for the significant interactions influence of $(B \cdot C)$ and $(A \cdot B \cdot C)$ in statistical model for germination rate (Table 3, Equation 3).

Figure 1: Interaction graphs for in vitro germination rate. A: genotype-culture medium associated to yield of any detail related to germination emerging. B: osmotic conditioning solution (OCS)-culture medium associated to yield of any detail related to germination emerging for GN1. C: osmotic conditioning solution (OCS)-culture medium associated to yield of any detail related to germination emerging for GN2.







3.2 Cotyledon Emerging

Statistical modeling was also performed to describe cotyledon emerging (Equation 4, Table 3). From equation 4, it's possible to conclude that genotype (A), germination culture medium (C) and post-seeding time (D) as well as binary interactions between $(A \cdot B)$ and $(B \cdot C)$ had significant influence on *in vitro* cotyledon emerging rate. As observed in germination, cotyledon emerging rate was higher at 30 days of the post-seeding time than to 15 days.

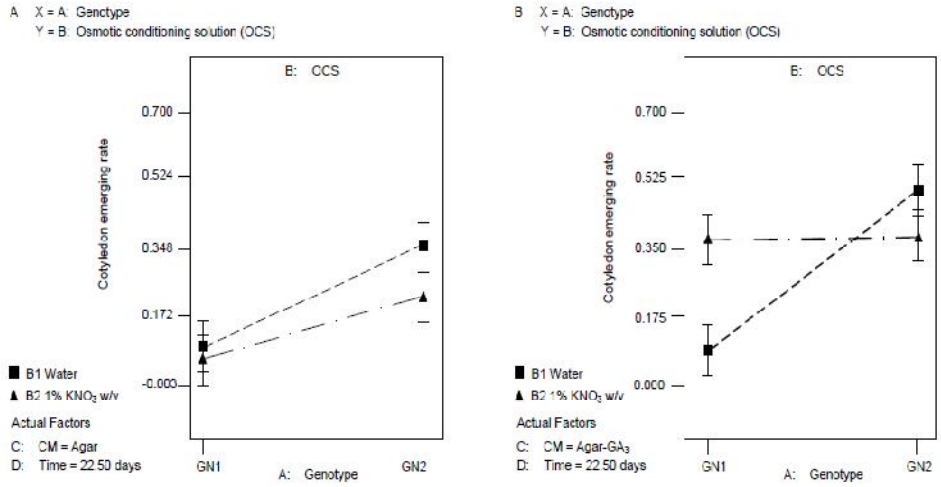
The *in vitro* germination conditions were not enough to reduce the time to cotyledon emerging. The influence of osmotic conditioning solution (B) was confirmed in combination of genotype (A) and germination culture medium (C).

$$(4)\quad Y_C = 0.260 + 0.100 \cdot A + 0.072 \cdot C + 0.068 \cdot D - 0.061 \cdot A \cdot B + 0.041 \cdot B \cdot C - 0.038 \cdot A \cdot B \cdot C$$

The choice of osmotic conditioning solution (distilled water or 1% KNO_3 w/v) was indifferent for cotyledon emerging rate in GN1 when water + agar was utilized as germination culture medium. On the other hand, this *in vitro* germination parameter was improved by distilled water compared to 1% KNO_3 w/v (higher than two-fold) for seeds corresponding to GN2 in the same culture medium (Figure 2a). Contrary findings were confirmed in culture medium supplemented with GA_3 (Figure 2b). In this case, the GN2 cotyledon emerging rate was not influenced by osmotic conditioning solution and GN1 showed higher cotyledon emerging rate when 1% KNO_3 w/v was used as osmotic conditioning solution (higher than two-fold, using distilled water as a reference).

Figure 2: Interaction graphs for *in vitro* cotyledon emerging rate. A: genotype-osmotic conditioning solution (OCS) associated to yield of cotyledon emerging for agar as germination culture medium.

B: genotype-osmotic conditioning solution (OCS) associated to yield of cotyledon emerging for agar-GA_3 as germination culture medium.



4. Discussion

The natural process of seed germination reflects a physiological sequence of events that is strongly influenced by external and internal factors, such as environmental conditions, dormancy, germination inhibitors, or inducers. Such factors may act alone or integrated in order to transform the germination process in a biological event that determines the retake of metabolic activity and embryo plant growth with consequent emergence and development, radicle, hypocotyl and cotyledons (Bewley, Bradford, Hilhorst, & Nonogaki, 2013).

The parameters that determine the germination process and seedling development to establish the *in vitro* culture conditions should follow the natural process sequence. Therefore, it is necessary to seek a balance between physiological seed quality, utilized genotype, the culture medium composition, and the *in vitro* development itself. This study aimed to evaluate in an integrative approach the germinability of two *Capsicum baccatum* genotypes under variations in seed priming and composition of the culture medium.

Our results show that genotypes Pitanga (GN1) and Cambuci (GN2) from *Capsicum baccatum* had quiescent seeds in suitable stage of physiological maturation and vigor and that germinated in a relative short period (averaging 22 days, data not shown), producing healthy seedlings able to be use for explants extraction necessary for initiation of the *in vitro* callus culture. Such inference is based on Baskin and Baskin (2004)who stated a quiescent seed is capable of germinating in its fullest practicable extent for the physical environment factors, considering the imposed limits by their genotype.

In general, the germination period can be long and variable between different pepper species and the process could be uneven. The optimum germination temperature is about 30ºC and no specific light condition seems necessary for germination. However, several pretreatments can be used to enhance seed germination and seedlings emergence as e.g. potassium nitrate (KNO_3), gibberellic acid (GA_3) and sodium hypochlorite(Paul W. Bosland & Votava, 2012; Cano-Vázquez et al., 2015; Cortez-Baheza et al., 2011). *Ex vitro* pre-germination treatments has been used on different plant seeds species in order to increase the seeds germination, reducing the time between sowing and seedling emergence and enhance the seed tolerance to adverse environmental conditions. In this context, the priming is a promising treatment and it involves soaking seeds in natural or synthetic solutions under controlled conditions (temperature, light and time) followed or not by drying the seeds to the initial conditions of moisture content.

The seed hydration to a certain level, allows the initial germination stages of the process to happen, however, without radicle protrusion. This process tends to result in even and quicker germination (Ibrahim, 2016; Manonmani, Junaithal, & Jayanthi, 2014; Paparella et al., 2015; Singh et al., 2015).

In *Capsicum annuum* Kikuti et al. (2005) showed that conditioning with KNO_3 is effective to improve seed performance and the increase of exposure time to the priming solutions favors the process of *ex vitro* germination of sweet pepper. Batista et al. (2015)demonstrated that priming for the *ex vitro* germination of *Capsicumfrutescens* is active in the emergence speed and the KNO_3 usage increases the seedlings dry weight, however the seeds quality is related to the germination treatments results.

Under the *in vitro* evaluated conditions, the genotype Pitanga (GN1) germinated better once priming with distilled water while genotype Cambuci (GN2) showed no difference in response to the pre-treatment with distilled water and KNO_3. For GN1 the KNO_3 did not influence the germination, water was enough to start the seeds germination. In contrast, GN2 showed indifferent behavior to priming treatments. Such behavior reflects the fact that each genotype has its own needs to start the germination process, determined by the conditions of seed formation and genetic composition, reflecting therefore divergence in the germination response(Popinigis, 1985).

As to cotyledons emergence, the genotype, the germination culture medium and post-seeding time as well as their interactions were more effective at 30 days post-seeding time, though such conditions were not sufficient to reduce this time. For GN1 the use of distilled water or 1% KNO_3 w/v was indifferent for cotyledon emerging rate when agar was utilized as the germination culture medium, indicating that this genotype is more vigorous and their genetic composition allows its development without the direct action of inductors or development stimulators.

For the germination behavior in the culture medium, was observed that the gibberellic acid (GA_3) presence influenced differentially seed germination and seedling growth.

For GN2 (Pitanga) the culture medium containing gibberellic acid was effective in seed germination, but such treatment had no influence for GN1 (Cambuci). According to Vipranarayana et al. (2012), GA_3 also works effectively on *in vitro* seed germination of *Pterocarpussantalinus (Fabaceae* - medicinal plant), probably breaks the dormancy and stimulates seed germination via synthesis of α-amylase and other hydrolyses.

According to Kucera et al. (2005) the dormancy process and germination are complex and controlled by various plants hormones encoded by a large number of genes affected by both developmental and environmental factors. The hormonal interactions determine the trigger mechanisms or control these events. In this context, gibberellic acid (GA_3) plays a fundamental role in the release of dormancy, increasing the embryo growth potential and overcoming the mechanical restraint conferred by the seed-covering layers, by weakening of the tissues surrounding the radicle. Gibberellic acid promotes germination being required for embryo cell elongation, for overcoming coat restrictions to germination of non-dormant and dormant seeds, and for inducing endosperm weakening (Gupta & Chakrabarty, 2013).

In the present study we choose the combination of hydropriming (in water) and halopriming (in solution of inorganic salts, i.e. KNO_3) as indicated in studies on the *ex vitro* seed germination of several species including *Capsicum* and that have shown variable germination patterns and seedling emergence (Aloui, Souguir, & Hannachi, 2014; Batista et al., 2015; Cortez-Baheza et al., 2011; Maiti, Rajkumar, Jagan, Pramanik, & Vidyasagar, 2013; Smith & Cobb, 1991). Among the most diverse treatments suggested in the literature (water, NaCl, $CaCl_2$, KCl, PEG, KNO_3, GA_3, e.g.) those involving potassium nitrate (KNO_3) and gibberellic acid (GA_3) have shown promising results (Paul W. Bosland & Votava, 2012), because such compounds assist in expression of the physiological seeds potential (Nascimento, 2005).

Regeneration *in vitro*studies in *Capsicum* has mostly evaluated the behavior in commercial genotypes of *Capsicumannuum*, *Capsicumchinense* and *Capsicumfrutescens*. These studies have resulted in different protocols and observed a significant diversity of morphogenetic responses dependent on genotype, the explant type and the culture medium conditions(Akram, Gaafar, Safwat, & Diab, 2011; Bora et al., 2014; Gogoi, Acharjee, & Devi, 2014; Grozeva, Rodeva, & Kintzios, 2009; Manzur et al., 2013; Orlinska & Nowaczyk, 2015; Raj, Glint, & Babu, 2015; Valadez-Bustos et al., 2009; Verma, Dhiman, & Srivastava, 2013). Among the various types of explants for the initial establishment of *in vitro* cultures, the seeds have advantages such as rapid growth, rapid availability of material for subsequent cultivation stages. However, regard the *in vitro* germination involving *Capsicum* species, different protocols available have directed attention to the seeds surface sterilizing procedures and the composition of the culture medium for germination. Most common sterilizing processes are the application of 0.1% HgCl and 70% ethanol, followed by inoculation in Murashige and Skoog basal culture medium. Hormones are mostly used after seedling is developed also in MS basal culture medium, not intending to reduce growth time, but to induce multiple shoot bud, callus formation, plant regeneration, flower induction, fruit development and rooting. Considerations for seed viability in germination rate or even seeds pretreatment, which can trigger or enhance germination, were not still reported(Bora et al., 2014; Manzur et al., 2013; Orlinska & Nowaczyk, 2015; Verma et al., 2013).

In this study we investigated the germination phenomena using a system's approach, as a function of the four essential parameters on*in vitro* cultivation (genotype, seed pretreatment, germination medium and time) and was confirmed that the best responses for the two *Capsicum baccatum* L. genotypes under consideration were different in germination rate terms, water + agar for Pitanga (GN1), and water + agar-GA_3 for Cambuci (GN2).

On the other hand, the seedling development was stimulated by the combination of 1% KNO_3 + agar-GA_3 for genotype Pitanga (GN1), whereas the same optimal combination for rate of germination events was observed for Cambuci (GN2). In general, for both genotypes, the post-seeding time was not reduced using the osmotic conditioning solutions and germination culture media assessed.

The use of inducing agents or enhancers such as water, KNO_3 and/or GA_3, can be very effective to raise the germination process and enhance embryo development in different *Capsicum baccatum*genotypes. However, these parameters should be considered in an integrated manner so that it is possible to enhance or maximize the germination rate and lead to obtaining aseptic healthy seedling that can be employed as the explant source for *in vitro* culture protocols.

We concluded that for genotypes Pitanga(GN1) and Cambuci (GN2) both *Capsicum baccatum* L. the optimal treatments combinations were water (osmotic conditioning solution) + agar (germination culture medium) and water + agar-GA_3, respectively; and that the two-level full factorial experimental design (2^4) was effective to show differential responses for genotypes front of four main factors involved at *in vitro* germination phenomenon. It was established the most suitable conditions for the early bioprocess stage for producing secondary metabolites from *in vitro* culture of this specie, using a system's approach.

Acknowledgments

The firstauthorwouldliketothank Fundação de Amparo à Pesquisa do Estado de São Paulo/Brazil (FAPESP) for Master'sfellowship (2014/26997-4).

5. References

Akram, O., Gaafar, M., Safwat, G., & Diab, A. (2011). Comparative Study among the Germination and Propagation of Different Capsicum Annuum Cultivars using Tissue Culture Techniques. Nature & Science, 9(7), 183–190. Retrieved from http://www.sciencepub.net/nature/ns0907/22_6213ns0907_183_189.pdf\n http://search.ebscohost.com/login.aspx?direct=true&profile=ehost&scope=site&authtype=crawler&jrnl=15450740&AN=65959168&h=rW/ur6odmg/vtB9oQNHv5ZApboi56BEaAY+xQLCb/Gm7TaYpciFJm087pDC

Aloui, H., Souguir, M., & Hannachi, C. (2014). Determination of an optimal priming duration and concentration protocol for pepper seeds (Capsicum annuum L.). Acta Agriculturae Slovenica, 103(2), 213–221. http://doi.org/10.14720/aas.2014.103.2.6

Baskin, J. M., & Baskin, C. C. (2004). A classification system for seed dormancy. Seed Science Research, 14(1), 1–16.

Batista, T. B., Da Binotti, F. F. S., Cardoso, E. D., Bardiviesso, E. M., & Costa, E. (2015). Aspectos fisiológicos e qualidade de mudas da pimenteira em resposta ao vigor e condicionamento das sementes. Bragantia, 74(4), 367–373. http://doi.org/10.1590/1678-4499.0133

Bello-Bello, J. J., Canto-Flick, A., Balam-Uc, E., Gómez-Uc, E., & Robert, M. L. (2010). Improvement of In Vitro Proliferation and Elongation of Habanero Pepper Shoots (Capsicum chinense Jacq .) by Temporary Immersion, 45(C), 1093–1098.

Bertão, M. R., Moraes, M. C., Palmieri, D. A., & Pereira, L. (n.d.). Cytotoxicity , Genotoxicity and Antioxidant Activity of Extracts from Capsicum spp. http://doi.org/10.3923/rjmp.2016.265.275

Bewley, J. D., Bradford, K. J., Hilhorst, H. W. M., & Nonogaki, H. (2013). Seeds. New York, NY: Springer New York. http://doi.org/10.1007/978-1-4614-4693-4

Bora, G., Gogoi, H. K., & Handique, P. J. (2014). Effect of silver nitrate and gibberellic acid on in vitro regeneration , flower induction and fruit development in Naga Chilli.

Bosland, P. W., & Votava, E. J. (2012). Peppers: vegetable and spice capsicums. (P. W. Bosland & E. J. Votava, Eds.). Wallingford: CABI. http://doi.org/10.1079/9781845938253.0000

Cano-Vázquez, A., López-Peralta, M. C., Zavaleta-Mancera, H. A., Cruz-Huerta, N., Ramírez-Ramírez, I., Gardea-Béjar, A., & González-Hernández, V. A. (2015). Variación en grados de latencia en semillas entre colectas de chile piquín (Capsicum annuum var. glabriusculum). Botanical Sciences, 93(1), 175. http://doi.org/10.17129/botsci.138

Cortez-Baheza, E., Rivera-Reyes, J. G., Andrio-Enriquez, E., Guevara-González, R. G., Guevara-Olvera, L., Cervantes-Ortiz, F., & Mendoza-Elos, M. (2011). Osmoacondicionamiento de la semilla de chile ancho y su efecto en el vigor. Ecosistemas Y Recursos Agropecuarios, 27(3), 345–349.

Costa, P., Gonçalves, S., Valentão, P., Andrade, P. B., & Romano, A. (2013). Accumulation of phenolic compounds in in vitro cultures and wild plants of Lavandula viridis L'Hér and their antioxidant and anti-cholinesterase potential. Food and Chemical Toxicology, 57, 69–74. http://doi.org/10.1016/j.fct.2013.03.006

Gogoi, S., Acharjee, S., & Devi, J. (2014). In vitro plantlet regeneration of Capsicum chinense Jacq. cv. "Bhut jalakia": hottest chili of northeastern India. In Vitro Cellular & Developmental Biology - Plant, 50(2), 235–241. http://doi.org/10.1007/s11627-013-9569-x

Grozeva, S., Rodeva, V., & Kintzios, S. (2009). Successful Regeneration of Sweet Pepper in an Airlift Bioreactor : Effect of Medium Phase and Genotype. The Open Horticulture Journal, 2, 70–75.

Gupta, R., & Chakrabarty, S. K. (2013). Gibberellic acid in plant. Plant Signaling & Behavior, 8(9), e25504. http://doi.org/10.4161/psb.25504

Ibrahim, E. A. (2016). Seed priming to alleviate salinity stress in germinating seeds. Journal of Plant Physiology, 192, 38–46. http://doi.org/10.1016/j.jplph.2015.12.011

Kehie, M., Kumaria, S., Tandon, P., & Ramchiary, N. (2015). Biotechnological advances on in vitro capsaicinoids biosynthesis in capsicum: a review. Phytochemistry Reviews, 14(2), 189–201. http://doi.org/10.1007/s11101-014-9344-6

Kikuti, A. L. P., Kikuti, H., & Minami, K. (2005). Physiological conditioning in sweet pepper seeds. Revista Ciência Agronômica, 36(2), 243–248.

Kothari, S. L., Joshi, A., Kachhwaha, S., & Ochoa-Alejo, N. (2010). Chilli peppers - A review on tissue culture and transgenesis. Biotechnology Advances, 28(1), 35–48. http://doi.org/10.1016/j.biotechadv.2009.08.005

Kucera, B., Cohn, M. A., & Leubner-Metzger, G. (2005). Plant hormone interactions during seed dormancy release and germination. Seed Science Research, 15(4), 281–307. http://doi.org/10.1079/SSR2005218

Maiti, R., Rajkumar, D., Jagan, M., Pramanik, K., & Vidyasagar, P. (2013). Effect of seed priming on seedling vigour and yield of tomato and chilli. International Journal of Bio-Resource & Stress Management, 4, 119–125.

Manonmani, V., Junaithal, M. A., & Jayanthi, M. (2014). Halo Priming of Seeds. Research Journal of Seed Science, 7(1), 1–13. http://doi.org/10.3923/rjss.2014.1.13

Manzur, J. P., Penella, C., & Rodríguez-Burruezo, A. (2013). Effect of the genotype, developmental stage and medium composition on the in vitro culture efficiency of immature zygotic embryos from genus Capsicum. Scientia Horticulturae, 161, 181–187. http://doi.org/10.1016/j.scienta.2013.06.036

Matkowski, A. (2008). Plant in vitro culture for the production of antioxidants - A review. Biotechnology Advances, 26(6), 548–560. http://doi.org/10.1016/j.biotechadv.2008.07.001

Nascimento, W. M. (2005). Condicionamento osmótico de sementes de hortaliças visando a germinação em condições de temperaturas baixas. Horticultura Brasileira, 23(2), 211–214. http://doi.org/10.1590/S0102-05362005000200010

Orlinska, M., & Nowaczyk, P. (2015). In vitro plant regeneration of 4 Capsicum spp. Genotypes using different explant types. Turkish Journal of Biology, 39(1), 60–68. http://doi.org/10.3906/biy-1403-89

Paparella, S., Araújo, S. S., Rossi, G., Wijayasinghe, M., Carbonera, D., & Balestrazzi, A. (2015). Seed priming: state of the art and new perspectives. Plant Cell Reports, 34(8), 1281–1293. http://doi.org/10.1007/s00299-015-1784-y

Popinigis, F. (1985). Fisiologia de sementes. Brasília: Agriplan.

Purohit, S. D. (2013). Introduction to plant cell, tissue and organ culture. Delhi: PHI Learning Private Limited.

Raj, R., Glint, V., & Babu, K. (2015). In vitro plant regeneration in Capsicum chinense Jacq. (Naga Chili). Journal of Applied Biology and Biotechnology, 3(1), 030–033. http://doi.org/10.7324/JABB.2015.3106

Roy, J., & Banerjee, N. (2001). Cultural requirements for in vitro seed germination, protocorm growth and seedling development of Geodorum densiflorum (Lam.) Schltr. Indian Journal of Experimental Biology, 39(10), 1041–1047.

Shahzad, A., & Sahai, A. (2014). Genotype-dependent responses during in vitro seed germination and establishment of Balanites aegyptiaca (L.) Del. – an endangered agroforestry species. Forest Science and Technology, 10(3), 130–135. http://doi.org/10.1080/21580103.2014.884984

Singh, H., Jassal, R. K., Kang, J. S., Sandhu, S. S., Kang, H., & Grewal, K. (2015). Seed priming techniques in field crops - A review. Agricultural Reviews, 36(4), 251–264. http://doi.org/10.18805/ag.v36i4.6662

Smith, P. T., & Cobb, B. G. (1991). Accelerated Germination of Pepper Seed by Priming with Salt Solutions and Water. HortScience, 26(4), 417–419.

Valadez-Bustos, M. G., Aguado-Santacruz, G. A., Carrillo-Castañeda, G., Aguilar-Rincón, V. H., Espitia-Rangel, E., Montes-Hernández, S., & Robledo-Paz, A. (2009). In vitro propagation and agronomic performance of regenerated chili pepper (Capsicum spp.) plants from commercially important genotypes. In Vitro Cellular & Developmental Biology - Plant, 45(6), 650–658. http://doi.org/10.1007/s11627-009-9193-y

Verma, S., Dhiman, K., & Srivastava, D. K. (2013). Efficient in vitro regeneration from cotyledon explants in bell pepper (Capsicum annuum L. Cv. California wonder), 4(3), 391–396.

Vipranarayana, S., Prasad, T. N. V. K. V., & Damodharam, T. (2012). In vitro seed germination and induction of enhanced shoot multiplication in Pterocarpus santalinus Linn . f : An endemic medicinal plant of Seshachalam Hills, Tirumala. International Journal of Pure and Applied Sciences and Technology, 9(2), 118–126.

Wilson, S. A., & Roberts, S. C. (2012). Recent advances towards development and commercialization of plant cell culture processes for the synthesis of biomolecules. Plant Biotechnology Journal, 10(3), 249–268. http://doi.org/10.1111/j.1467-7652.2011.00664.x

Zimmer, A. R., Leonardi, B., Miron, D., Schapoval, E., Oliveira, J. R. de, & Gosmann, G. (2012). Antioxidant and anti-inflammatory properties of Capsicum baccatum: From traditional use to scientific approach. Journal of Ethnopharmacology, 139(1), 228–233. http://doi.org/10.1016/j.jep.2011.11.005.