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POSTHARVEST SEED TREATMENTS TO IMPROVE THE PAPAYA SEED GERMINATION AND SEEDLINGS DEVELOPMENT

[TRATAMIENTOS POST-COSECHA DE LA SEMILLA DE PAPAYO PARA MEJORAR LA GERMINACIÓN Y DESARROLLO DE LA PLÁNTULA]

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SUMMARY

Practical technologies are required to preserve the viability of seeds particularly those known to be short-term viable species like *Carica papaya* (papaya). Papaya seeds were imbibed in water or chemical solutions ($CaCl_2$ 10^{-5} M, salicylic acid 10^{-4} M, and gibberellic acid 10^{-5} M) combined with inoculation of bacterial cell suspension to determine their effects on seed germination, plant growth, biomass production and chlorophyll accumulation. Seeds imbibed in water germinated 40 % more than control seeds and the time required to reaching 50 % seed germination was reduced two days in comparison to untreated seeds; however, the untreated seeds generated the largest (9.2 cm) and most vigorous seedlings. When seeds were imbibed in CaSG solution, a significant increase of the growth parameters such as fresh and dry biomass weight was observed. Seeds that were primed in gibberellic acid solution followed by inoculation with a mixture of *Azospirillum brasilense* cell suspension exhibited high seed germination (69 %), plant emergence (47 %) and seedling height (19 %), higher than the control. Differences in chlorophyll accumulation by seedlings were minimal.

Key words: *Carica papaya*; priming; inoculation; seed germination; development; chlorophyll.

RESUMEN

Tecnologías prácticas se requieren para preservar la viabilidad de las semillas en particular cuando su viabilidad no es duradera como la semilla de *Carica papaya* (papaya). Semillas de papaya fueron embebidas en agua o en soluciones de $CaCl_2$ 10^{-5} M, ácido salicílico 10^{-4} M y ácido giberélico 10^{-5} M e inoculadas con una suspensión de células bacterianas para determinar sus efectos en la germinación, desarrollo inicial de las plántulas, acumulación de biomasa y de clorofila. Las semillas embebidas en agua germinaron 40 % más y requirieron de dos días menos para que el 50 % de la semilla germinara en comparación con la semilla sin tratar; sin embargo, la semilla sin tratar generó plántulas de mayor longitud (9.2 cm) y más vigorosas. La semilla embebida en la solución combinada de calcio, ácido giberélico y ácido salicílico generó plántulas de mayor peso (biomasa fresca y seca) superando al control. La semilla embebida en solución de ácido giberélico seguido de la inoculación con células de *Azospirillum brasilense* tuvieron alta germinación (69 %), emergencia (47 %) y generaron plántulas de mayor altura, 19 % más que el testigo. Las diferencias en la acumulación de clorofila fueron mínimas.

Palabras clave: *Carica papaya;* imbibición; inoculación; germinación; desarrollo; clorofila.

INTRODUCTION

Plant development is a programmed process that starts from seed germination to maturity and fruiting. It is mainly modulated by a combination of dormancy, plant cell regulators (Richards *et al*., 2001, Olszewski *et al*., 2002, Peng and Harberd, 2002; Sun and Gubler, 2004; Smalle and Vierstra, 2004) and environment factors such as moist, temperature, oxygen, and light (Toh *et al*., 2008). Identification of triggers of seed germination and seedling growth promotion factors is crucial for the development of technologies to enhance stand establishment (Andrade-Rodriguez *et al*., 2008; De Mello *et al*., 2009; Venier *et al*., 2012). The germination inhibitors present in the papaya seed testa and sarcotesta control its germination (Chow and Lin, 1991; Paz and Vázquez, 1998) and to eliminate them, papaya growers have applied actions such as removing the sarcotesta from seeds, soaking and washing seeds in water (Mirafuentes, 1997) or sun drying (Jiménez 1996). Growth and development in plants is controlled by the selective removal of short-lived regulatory proteins. Degradation of repressor proteins

by the ubiquitin-26S proteasome pathway is a central mechanism of gibberellic acid signal transduction in seed dormancy and germination (Smalle and Vierstra, 2004). ABA signaling, which is essential for seed development and seedling responses to the environment, is also mediated by protein degradation (Rodríguez-Gacio *et al*., 2009). MicroRNAs (miRNAs) are involved in the repression of transcription factors at the mRNA level during seed germination and seedling growth (Nonogaki *et al*., 2008). Emergence of the embryo from seed is repressed by surrounding tissues such as the endosperm and testa (Pinto *et al*., 2007; Sung *et al*., 2008) and this event provide the precise control of seed germination.

Seed deterioration is generally associated with loss of membrane integrity, biochemical changes, affections in important enzymatic activities, reduction in protein and nucleic acid synthesis, and lesions in DNA molecules (McDonald, 1999), result of adverse physical conditions of storage. The fast deterioration of *C. papaya* seeds prejudices its germination and emergence in the field, therefore growth regulators and enhancers commercially available have been routinely used to stimulate its germination (Bautista-Calles *et al*., 2008).

Seed priming is a technique for improving both seed germination and vigor which involves the imbibition of seeds in water under controlled conditions to allow the initiation of early events of germination, followed by drying the seed back to its initial moisture condition (Jamieson, 2008; Varier *et al*., 2010). Seeds can be imbibed in organic or inorganic solutions (chemopriming) (Nagao *et al*., 1992; Parera and Cantliffe, 1995; Grzesik and Nowak, 1998) as well as inoculated with beneficial microorganisms (biopriming) during or after being primed (Warren and Bennett, 1997; Callan *et al*., 1997). Microbial inoculants, which can promote plant growth and productivity, have internationally been accepted as an alternative source of N-fertilizers (Baset Mia *et al*., 2010). The aim of this study was to evaluate the relevance of applying priming techniques to improve papaya seed germination, growth parameters and plant emergence.

MATERIALS AND METHODS

Three lots of certified papaya seed cv. Maradol with slight differences in germination percentage, were proportioned by Semillas del Caribe, S.A. de C.V. (Guadalajara city, Mexico). The bacterial species *Azospirillum brasilense* strains UAP154 and Sp59 provided by the Universidad Autónoma de Puebla. Calcium chloride ($CaCl_2$), salicylic acid (SA), gibberellic acid (AG_3), and the fungicide Captan® (50%) were commercially available.

Chemopriming procedure

Seeds were imbibed in a solution, such as CaS solution ($CaCl_2$ 10^{-3} M and SA 10^{-4} M), G solution (AG_3 10^{-3} M) or CaSG solution ($CaCl_2$ 10^{-3} M, SA 10^{-4} M and AG_3 10^{-3}M), following the procedure described by Bautista-Calles *et al*. (2008). All these solutions were prepared with distilled water and pH adjusted to 5.8 ± 0.1.

Biopriming procedure

Once the process of imbibition was concluded, dry seeds were mixed with a bacterial cell suspension (100 seeds were mixed with 1.25 mL of a cell suspension containing 10^{-9} cells mL^{-1}, approximately) and allowed to stand by 30 min at room temperature. Seeds were air dried during 30 min at 28 to 30 °C. Lots of 25 seeds were spread on a 22 x 24 cm folded paper towel, moistened with 7 mL distilled water. Paper towels were rolled up, placed in a plastic bag in vertical position and preserved at 28 to 30 °C during 10 days. At the end of this period, total germination was recorded (a seed was considered germinated when the radical protrution was approximately 1 mm). Germinated seeds were placed at one edge of a new paper towel moistened with 7 mL distilled water, rolled up and positioned in vertical position inside a plastic bag with the seeds on the top, to expose the seedlings to the light. Seedlings were allowed to develop during 14 days at 28 to 30 °C, and 16 h light. At the end of this period, stem and root length and weight of fresh and dried plantlets were determined.

Bacterial cell suspensions

Overnight cultures of *Azospirillum* were prepared in 3 mL of King's B medium (Vincent, 1970). Fresh medium (10 mL) was inoculated with 0.1 mL samples of the overnight culture and then incubated with agitation (150 oscillations min^{-1}) during 24 h at 28 to 30 °C. Cultures were harvested and their turbidity adjusted to 0.9 (660 nm, Colleman Junior II spectrophotometer) with sterile water. Samples of 5 mL each of UAP154 and Sp 59 cell suspensions were mixed to obtain the *Azospirillum* mix suspension.

Greenhouse experiment

The greenhouse located in Atoyac de Alvarez, Guerrero, Mexico (400 m, average annual temperature 29 °C) was built with translucent plastic to allow 50 % penetration of daylight. The floor was a mixture of fine gravel and sand in a layer. Black plastic bags (300 mL capacity with five perforations at the bottom) were filled with non-disinfected soil containing 33 % clay, 33 % sand and 33 % slime.

Bags were placed on benches 1 m above the ground. Seed was sowed 24 h after being treated. Five seeds were placed 1 cm depth in each pot. Irrigation was provided daily during the first week before and after sowing, and after the first week every 2 to 3 days, depending on climate conditions.

Chlorophyll determination

Samples of stem and leaf (0.5 g fresh tissue) from plants, after 15 days of emergence, were collected to determine, per triplicate, their chlorophyll content according to the procedure of Bruinsma (2009).

Biomass

Died biomass was determine in samples of 10 plantlets (after 15 days of emergence) per replication, after being dehydrated in an oven at 70 ^{o}C until constant weight.

Experimental design

The experimental design used in the combination of the hydro-priming, chemo-priming and bio-priming experiments, as well as treatments taken in the laboratory and in the field, was a completely randomized block design using three replications of 25 seeds per treatment. In the greenhouse, eight replications of 50 seeds each were used. All experiments were analyzed with the statistical package SAS (Statistical Analysis System) 2000 through the analysis of the variance (ANOVA) and average comparison of Tukey (P = 0.05).

RESULTS AND DISCUSSION

In a previous paper, we demonstrated a significant increase, greater than that of the untreated seed, for germination, speed of germination and seedling growth when the papaya seed was imbibed in water and in solutions of calcium, salicylic and gibberellic acids (Bautista-Calles *et al*., 2008). Results presented in this paper deal with the ability of germination and seedling growth parameters exhibited by papaya seed when it was previously exposed to a combined treatment: imbibition in gibberellic acid solution followed by inoculation with the *Azospirillum* mix suspension. Seeds exposed to this combined treatment expressed its highest capacity of germination (69.3 %) in comparison with the untreated seed (30.7 %) (Figure 1). The improvement of seed germination displayed by the treated seed is explained in part, by the presence of gibberellic acid, involved in the dormancy control of the seed, being in consequence an important germination promoter (Groot and Karssen, 1987). GA stimulates the production of the enzyme amylase by the aleurone layer, which breaks down starch into maltose, allowing it to diffuse into the embryo, where it is required to promote the growth of seedlings. The application of GA_3 to scarified seeds significantly promoted germination and decreased the number of days until germination (Nagano *et al*., 2010). In the papaya seed, the combined action of gibberellic acid and potassium nitrate has been found to be advantageous for improving both germination and emergence of seedlings (Nagao and Frutani, 1986; Frutani and Nagao, 1987). The primed seed, in addition, is brought to a stage where the metabolic processes are already initiated (protein synthesis from existing mRNA and DNA and repair of mitochondria and sub-cellular damage), giving it a starting point over the unprimed seed. Upon further imbibition, the primed seed can take off from where it has left completing the remaining steps of germination faster than the untreated seed (Brocklehurst and Dearman, 1983; Heydecker and Coolbear 1997; Derek, 1997; Varier *et al*., 2010). Treated seeds; however, gave rise to a less vigorous seedling as compared with those generated by the untreated seeds (Table 1).

Obtaining a significant increase in the capacity of germination of the papaya seed is an important achievement; however, vigor is another advantageous factor that may be present in any quality seed. The strategy followed to obtain improvements on vigor condition of the seedlings was to expose seeds to the mutual effect of $CaCl_2$, GA_3 and SA, and coupling this treatment with seed inoculation. Today is accepted the statement that Ca^{2+} is a central regulator of plant development and growth (Hepler, 2005). Ca^{2+} plays an important role in controlling membrane structure and function by binding to phospholipids and thus, stabilizing lipid bilayers and providing structural integrity to cellular membranes (Burstrom, 1968), which is particularly important in the germinating seed. Calcium modulates the activity of certain phosphatases and kinase enzymes that participate in the signal transduction during the germination process (Derek, 1997; Harper *et al*., 2004). Research during the last two decades has established that different stresses cause signal-specific changes in cellular Ca^{2+} level, which functions as a messenger in modulating diverse physiological processes that are important for stress adaptation (Kim *et al*., 2009; Redy *et al*., 2011). The process in which this ion participates is large and involves nearly all aspects of plant development (Harper *et al*., 2004; Bothwell and Ng, 2005). A growing body of evidence points to the importance of Ca and Calmodulin in the regulation of the transcriptional process during plant responses to endogenous and exogenous stimuli (Kim *et al*., 2009).

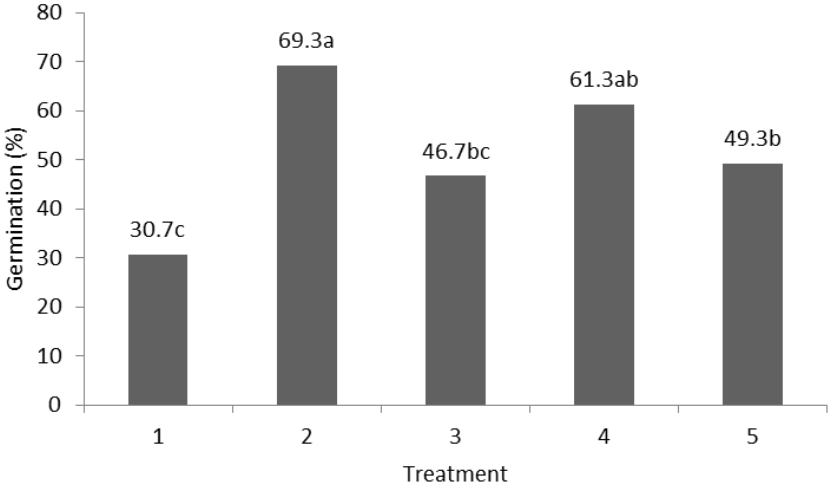

Figure 1. Germination. Papaya seeds were imbibed as indicated and inoculated with the *Azospirillum* mix suspension. 1) untreated; 2) imbibed in G solution and inoculated; 3) imbibed in CaS solution and inoculated; 4) imbibed in CaSG solution; 5) imbibed in CASG solution and inoculated. Different letters on a column represent different results ($P \leq 0.05$).

Disease resistance in Arabidopsis is regulated by multiple signal transduction pathways in which salicylic acid function as key signaling molecule (Clarke *et al*., 2000), acting in both local defense reactions at infection sites and the induction of systemic resistance; therefore, plants developed in the presence of salicylic acid may acquire the systemic resistance condition that might be beneficial to defend themselves, particularly at the stage of early development. Very little information exists on the establishment of defense mechanisms at the stage of seed germination (Rajjou *et al*., 2006). In the root, salicylic acid acts increasing the content of certain enzymes, improving resistance of plant cells themselves, and in leaves accumulating some chloroplast proteins and enzymes capable of degrading the pathogen cell walls (Tarchevsky *et al*., 2010).

Seeds that were imbibed in CASG solution germinated fast (T_{50} = 3 d) and generated seedlings with the highest dry biomass weight; however, when this treatment was coupled with inoculation, a reduction of growth parameters (seedling and root lengths as well as fresh and dry biomass production) and increase of one day in the T_{50} value was observed (Table 1). Thomas *et al*. (2007) demonstrated that papaya seeds inoculated with *Pantoea, Micobacterium,* or *Sphingomonas* spp., led to delayed germination or initial slow seedling growth; however, that slow seedling growth was overcome after 3 months and seedlings inoculated with *Pantoea*, *Microbacterium*, or *Sphingomonas* spp., displayed significantly better root and shoot growth.

Table 1. Seedlings 14 d of development. Seeds were imbibed as indicated and inoculated with a cell suspension of *Azospirillum*. 1) untreated; 2) imbibed in G solution and inoculated; 3) imbibed in CaS solution and inoculated; 4) imbibed in CaSG solution; 5) imbibed in CaSG solution and inoculated. Fresh and dry weight is an average of 10 seedlings

Treatment	Length (cm)			Fresh biomass weight (g)			Dry biomass weight (mg)			T_{50}
	Seedling	Stem	Root	Seedling	Stem	Root	Seedling	Stem	Root	(days)
1	9.2^{a}	2.8^{a}	6.3^{a}	0.8^{ab}	0.5^{b}	0.3^{a}	75.6^{bc}	52.3^{c}	23.3^{a}	7
2	8.2^{a}	2.2^{a}	6.0^{a}	0.6^{bc}	0.4^{b}	0.2^{bc}	62.0^{c}	46.0^{c}	16.0^{bc}	4
3	7.7^{a}	2.4^{a}	5.2^{a}	0.8^{ab}	0.5^{ab}	0.3^{ab}	84.0^{ab}	65.3^{ab}	18.6^{ab}	3
4	7.6^{a}	2.3^{a}	5.3^{a}	1.0^{a}	0.6^{a}	0.4^{a}	94.6^{a}	70.6^{a}	24.0^{a}	3
5	5.1^{b}	2.1^{a}	3.1^{b}	0.6^{c}	0.4^{b}	0.2^{bc}	67.6^{bc}	55.6^{bc}	12.0^{c}	4
Significance	**	ns	**	**	**	**	**	**	**	
DMS	1.9	0.8	1.4	0.2	0.1	0.1	17.0	12.8	5.6	

Different letters in a column represent different results. ** Significance ($P \leq 0.05$).

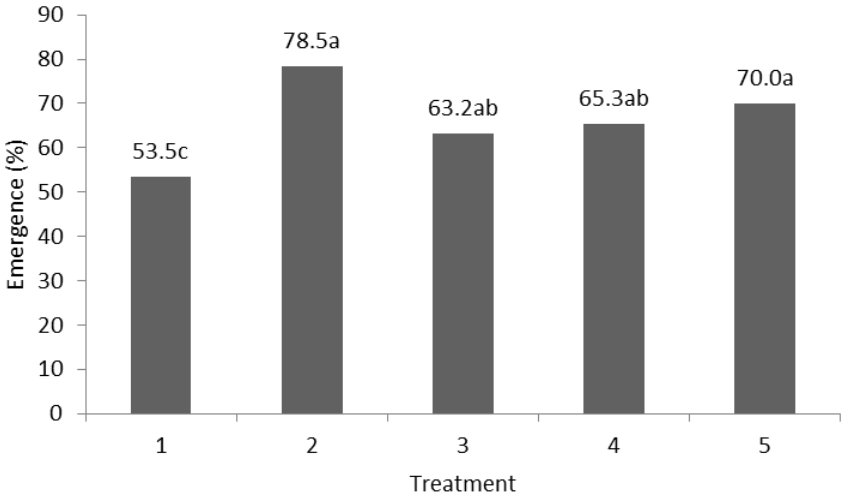

Figure 2. Seedling emergence in the greenhouse. Papayo seeds were imbibed as indicated and inoculated with a cell mixture of *Azospirillum*. 1) untreated; 2) imbibed in G solution and inoculated; 3) imbibed in CaS solution and inoculated; 4) imbibed in CaSG solution; 5) imbibed in CaSG solution and inoculated. Different letters on a column represent different results ($P \leq 0.05$).

Seeds that were exposed to a combined treatment, imbibed in G solution and inoculated, exhibited the greatest emergence (Figure 2) seedling height (Figure 3) and accumulated the highest amount of chlorophyll as well (Figure 4). Gibberellic acid is essential for multiple processes of plant development, such as seed germination, stem elongation, and floral development (Richards *et al.*, 2001; Olszewski *et al*., 2002; Peng and Harberd, 2002; Sun and Gubler, 2004; Cao *et al.*, 2006). Emergence of seeds that were imbibed in CASG solution and inoculated, were in second place followed by the seeds imbibed in CASG solution alone.

The presence of Gibberellic acid and the plant growth promoting microorganisms improved germination (Figure 1) and emergence (Figure 2). Some microorganisms are able to induce the systemic resistance in host plants to a broad variety of fungal, bacterial, and viral pathogens (Kaymak *et al*., 2008; Aliye *et al*., 2008; Jogaiah *et al.*, 2010); the presence of these plant growth promoting microorganisms seems to persist during plant lifetime, since they colonize active growth zones of roots and, for this reason, their beneficial effects will be effective until harvest.

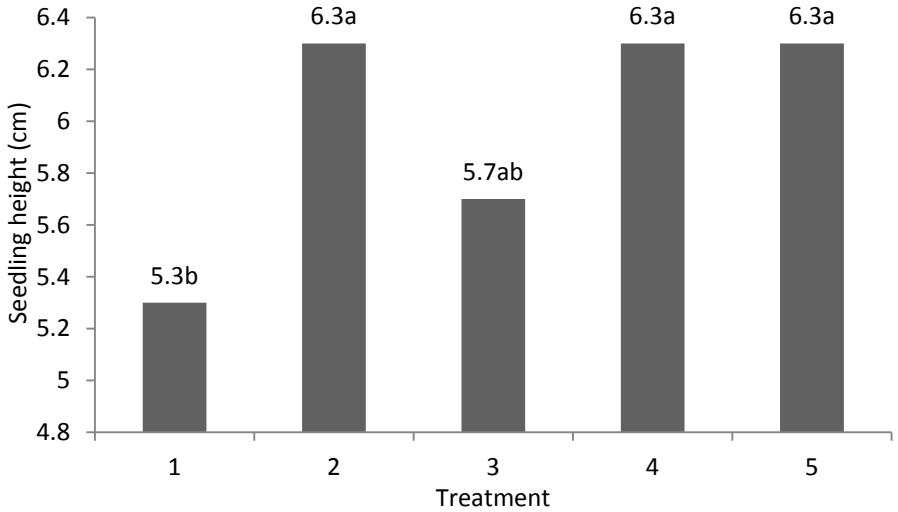

Figure 3. Seedling height. Papayo seeds were imbibed as indicated and inoculated with a cell mixture of *Azospirillum*. 1) untreated; 2) imbibed in G solution and inoculated; 3) imbibed in CaS solution and inoculated; 4) imbibed in CaSG solution; 5) imbibed in CaSG solution and inoculated. Different letters on a column represent different results ($P \leq 0.05$).

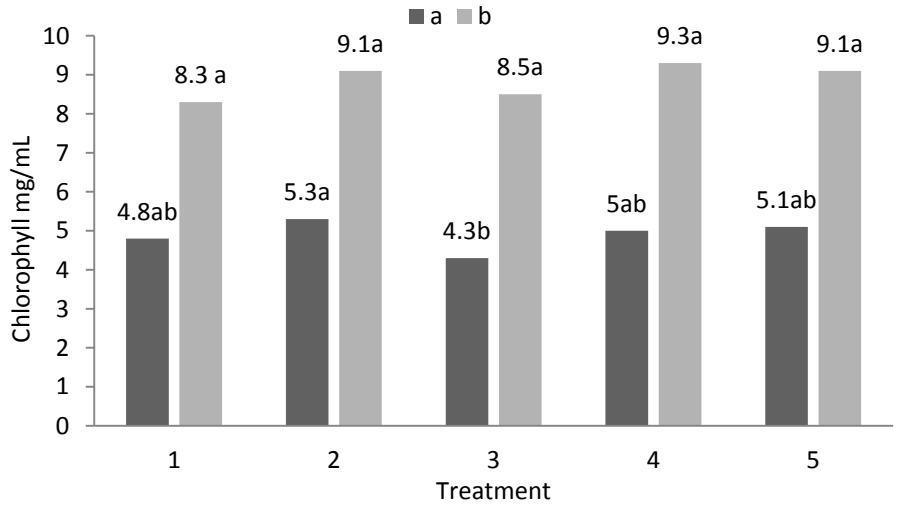

Figure 4. Chlorophyll determination after 15 days of emergence. Total (DMS = 1.71), chlorophyll a (DMS = 0.83) and chlorophyll b (DMS = 1.44). Seeds were imbibed as indicated and inoculated with a cell mixture of *Azospirillum*. 1) untreated, 2) imbibed in G solution and inoculated, 3) imbibed in CaS solution and inoculated, 4) imbibed in CaSG solution, 5) imbibed in CaSG solution and inoculated. Different letters on a column represent different results ($P \leq 0.05$).

The use of microorganisms to improve crop development and yield is a technically simple process, though conceptually difficult to explain; however, results obtained when plants are inoculated with bacterial cells or mycorrhizal fungi are generous and this could become an everyday technique in the field. 'Maradol' papaya plants inoculated with arbuscular mycorrhizal fungi *Glomus mosseae* and *Entrophospora colombiana* exhibited increases in the number of fruits and yield by 41.9 and 105.2 % for *G. mosseae* and 22.1 and 44.1 % for *E. colombiana*, respectively, compared to control plants (Vázquez-Hernández *et al*., 2011). The gap between production potential of the crop and the current average production could be attributed to different factors, being diseases very significant. In consequence, seeds could be treated with chemical compounds and microorganisms to protect them and improve the development of papaya plants.

Seeds treated with Gibberellic acid solution followed by the inoculation with the cell mixture of *Azospirillum* exhibited the best response and therefore is the recommended procedure to achieve the best germination and emergence, and has great possibilities of being adopted by papaya growers.

CONCLUSIONS

Seeds that were exposed to a combined treatment, imbibed in G solution and inoculated a cell mixture of *Azospirillum* exhibited the maximum germination, the greatest emergence, seedling height, and accumulated the highest chlorophyll amount as well. This treatment, which is economic and practical, can be attractive to enhance papaya seed germination capability.

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