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Short communication
In Vitro EVALUATION OF PHOSPHOLIPIDS EFFECT ON GERMINATION OF TOMATO SEEDS *Lycopersicon esculentum* Mill)

Comunicación corta
Evaluación *in vitro* del efecto de fosfolípidos sobre la germinación de semillas de tomate (*Lycopersicon esculentum* Mill)

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ABSTRACT. The effect of a mixture of natural phospholipids applied as substrate on filter paper on *in vitro* germination of tomato seeds of three varieties (Amalia, Campbell 28 and Vita) was evaluated. In all cases, an inducing effect on the radicular growth of seedlings derived from newly germinated seeds, given by lengths of radicles significantly higher in treated groups compared to controls were showed. Althought hypocotyles maxims longitudes were higher in this group with respect to control, no statistical difference in this growth indicator were evidenced. There was not homogeneous behavior in terms of percentages of germination. These results demonstrate the potential use of these compounds as inducers of radicles growth plant and are the first obtained in Cuba on the exogenous application of phospholipids as roots bio-stimulants, opening a new way to study the biological roles of these biomolecules and its exogenous application in economic and social impact crops.

Key words: growth, germination, hypocotyle, plants

RESUMEN. Se evalúo el efecto de una mezcla de fosfolípidos de origen natural, aplicados como sustrato en papel de filtro, sobre la germinación *in vitro* de semillas de tres cultivares de tomate (Amalia, Campbell 28 y Vita). En todos los casos se evidenció un efecto inductor sobre el crecimiento radicular de las plántulas de las semillas recién germinadas, dados por longitudes de radículas significativamente superiores en los grupos tratados con bajas concentraciones con respecto a los controles. Aunque se observaron longitudes máximas de hipocotilos superiores en este grupo con respecto al control, no existieron diferencias estadísticamente significativas en este indicador del crecimiento. En cuanto a los porcentajes de germinación no hubo un comportamiento homogéneo. Los resultados demuestran las potencialidades del uso de estos compuestos como inductores del crecimiento radicular en plantas y constituyen los primeros obtenidos en Cuba sobre la aplicación exógena de fosfolípidos como bioestimulantes de raíces; abriendo un nuevo camino para el estudio de las funciones biológicas de estas biomoléculas, así como de su aplicación exógena en cultivos de impacto económico y social.

Palabras clave: crecimiento, germinación, hipocotilos, plantas

INTRODUCTION

In Cuba, the tomato crop is a priority of the agricultural sector, which constitutes one of the most consumed vegetables by the population and an important source of raw material for the industry as exportable item (1). For some years now, it has been introduced in this culture, the use of biofertilizers and biostimulants of plant growth, highlighting successfully

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brassinosteroids and their analogues (2), the use of rhizosphere bacteria of the genus *Azotobacter* (3), liquid humus (4), among others. However, the availability of these products does not cover all the current demand of producers. For this reason, the search for bioactive products that favor to attaining the required production increases is a priority for research in this sector.

Moreover, recent research suggests the study of phospholipids and lysophospholipids as a new class of growth regulators in plants due to scientific evidence showing that these compounds and enzymes involved in catabolism (phospholipases), plays an important role in the growth and development of plants and in response to these various stresses (abiotic and biotic) to which they are subjected, noting that phospholipases A, C and D, some lysophospholipids and phosphatidic acid are key components of signaling routes of lipids in plant cells that determine cell fate (5).

For some years now, internationally, they have taken basic research boom to elucidate the biological functions of these compounds in plant cells, and their involvement in the development and growth of plants (6), and applied research related to its use in order to improve the yield and quality of agricultural production (7, 8, 9, 10). Notwithstanding this evidence, there is little information available about the effect of exogenously applied phospholipids on germination, growth and plant development (5), so the aim of this study was to evaluate the effect of phospholipids, applied exogenously on the germination of tomato seeds.

MATERIALS AND METHODS

Tomato seeds of three cultivars (Amalia, Campbell 28 and Vita) from the National Institute of Agricultural Sciences (INCA) were used. Aqueous suspensions of phospholipid mixtures purified from natural sources were evaluated with a majority composition of phosphatidylcholine and phosphatidylethanolamine, and presence of others phospholipids such as sphingomyelin, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol and lysophosphatidylcholine, previously characterized by chromatography of thin plate (11).

The experimental design consists of evaluating the *in vitro* germination on Petri plate to a control group and three treated groups (three replicates per group). Sowing of 30 seeds per plate was conducted using filter paper as substrate wetted with 5 mL of water (control group) or with suspensions of phospholipid to concentrations 0,1; 1 and 10 mg mL^{-1} (treated groups). Daily observation was made during the seven-day of the test for determining the number of germinated seeds, taking as an indicator of germination when the length of the radicle exceeded 2 mm length. After this time, direct measurement of hypocotyls and radicles lengths of each germinated seed was made to each group as well as a count of number true leaves per seedling. Germination percentage calculation (G) was done at the seventh day (12). The experimental obtained data were processed statistically by variance analysis of simple classification, and averages were compared using the comparison test of Duncan's multiple range to an error probability of 5 % ($p<0,05$) using the SAS 9,0 statistical package.

RESULTS AND DISCUSSION

Results (Tables I, II and III) showed that the tested mixture had no significant effect on germination in the groups treated with low concentrations relative to controls, except for Amalia cultivar to a concentration of 1 mg mL^{-1} . Nevertheless, the groups treated with high concentrations showed inhibition of germination process that differed statistically to controls, showing an inverse relationship between the germination percentage (G) and the phospholipid concentration. These results support the relationship declared by some authors, between phospholipid metabolism and action mechanism of phytohormones (5, 13), influencing physiological processes of plants at low concentrations (14).

On the other hand, the assessed mixture has among its majority components, phosphatidylcholine and phosphatidylethanolamine that are, in turn, the key phospholipid in plant membranes, from which is formed phosphatidic acid constituting the main product of enzymatic degradation of these types phosphatidyl phospholipids. It has been shown that these compounds play a pivotal role in signaling of abscisic acid during seed germination (15), confirming the close relationship, pointed above, among metabolisms, signaling of these compounds and the mechanism of hormone action in plants (5). Although there has been remarkable progress in recent years in understanding the biological functions of the phospholipids in plant cells, signaling of these plants and the relationship to development and growth processes (6, 13, 16) they are not yet fully elucidated, physiological mechanisms and all biological roles of each different type of phospholipids in this cell type. Still less, about the exogenous use advantage of these important compounds, which although promising study, as pointed Cowan, it is still in its early steps (5).

Table I. Results of germination test of Amalia tomato cultivar at seven days

Assessed indicators	Control group	Groups treated with phospholipids		
		0,1 mg mL^{-1}	1 mg mL^{-1}	10 mg mL^{-1}
LR (mm)	23,48 ± 2,15	34,93 ± 2,90*	29,86 ± 2,82	30,54 ±3,85
MLR (mm)	40	60	52	52
LH (mm)	19,67 ± 2,13	17,64 ± 2,13	20,81 ± 2,23	7,92 ± 1,45*
MLH (mm)	36	40	38	16
G (%)	84,38	87,50	67,74*	43,33*

*: p<0,05 (95 %) significant

LR: average length of the radicle, MLR: maximum length of the radicle, LH: Average length of the hypocotyl, MLH: maximum length of hypocotyl, G: percentage of germination

Table II. Results of the seed germination test of tomato cultivar Campbell 28 at seven days

Assessed indicators	Control group	Groups treated with phospholipids		
		0,1 mg mL^{-1}	1 mg mL^{-1}	10 mg mL^{-1}
LR (mm)	34,09 ±4,20	47,26 ± 3,81	46,39± 3,94 *	20,00 ± 10,00
MLR (mm)	70,00	120,00	90,00	30,00
LH (mm)	24,27 ±2,68	28,59 ± 1,99	26,49± 2,31	7,5 ± 2,5*
MLH (mm)	41,00	55,00	55,00	10,00
G (%)	73,33	60,00	55,68	2,25*

*: p<0,05 (95 %) significant

LR: average length of the radicle, MLR: maximum length of the radicle, LH: Average length of the hypocotyl, MLH: maximum length of hypocotyl, G: percentage of germination

Table III. Results of the seed germination test of tomato cultivar Vita at seven days

Assessed indicators	Control group	Groups treated with phospholipids		
		0,1 mg mL^{-1}	1 mg mL^{-1}	10 mg mL^{-1}
LR (mm)	46,37 ± 3,76	67,78 ± 2,80*	68,68 ± 2,77*	43,86 ± 5,85
MLR (mm)	90,00	110,00	105,00	70,00
LH (mm)	34,63 ± 1,64	38,75 ± 1,36	33,21 ± 1,34	15,07 ± 2,48*
MLH (mm)	45,00	55,00	55,00	30,00
G (%)	90,00	87,78	91,11	15,56*

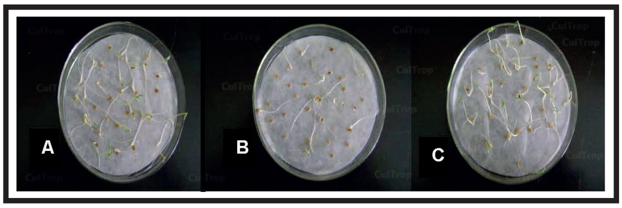
*: p<0,05 (95 %) significant

LR: average length of the radicle, MLR: maximum length of the radicle, LH: Average length of the hypocotyl, MLH: maximum length of hypocotyl, G: percentage of germination

These results corroborate other ones reported in the literature demonstrating the relationship among levels of phospholipid membranes tomato seeds, with the quality and viability of these, without affecting directly the germination rate, obtaining information, including losses feasibility related to decrease of phosphatidylcholine levels, which forms the majority phospholipids type in this kind of seed (17) and the mixture evaluated. Moreover, the phospholipids in the living systems (factible seeds) become sources of organic phosphorus, by the phospholipases C and D action, with direct involvement in all stages of development, specially in the initial stage of seed germination and seedling formation (18, 19, 20).

The results of the radicle lengths (LR) as a growth indicator of newly germinated seedlings showed, in all cultivars, a statistically significant increase in groups treated with low concentrations relative to controls (except cultivar Amalia to a concentration of 1 mg mL^{-1}), while the highest assessed concentration showed no involvement of the indicator relative growth controls. Other authors have shown the involvement of phosphatidylcholine with root growth, confirming a mutation *XIPOTL1* gene encoding S-adenosyl-L-methionine: phosphoethanolamine N-methyltransferase which is a critical enzyme in the biosynthesis of phosphatidylcholine, resulting in affecting the growth of primary roots and epidermal cells, due to cell death induced by decrease in the content of phosphatidylcholine (16, 21). Moreover, the evaluated mixture containing phospholipids that are substrates of phospholipases, specifically phospholipase A_2 whose main function has been associated with the regulation of the release of linoleic acid to the jasmonic acid biosynthesis, and its involvement in germination process, seedling growth, auxin-mediated growth such as cell growth and response to stress (5), confirming the potential of these phospholipid compounds as inducers root seedling growth (16). However, no differences were observed in the hypocotyl lengths (LH) or in the presence of true leaves at this level of concentration versus control.

Another interesting finding constitutes proof that high concentrations of phospholipids (10 mg mL^{-1}) caused an inhibitory effect on germination and seedling growth lower, given LH lower values statistically compared to other treated groups and controls, and the lack of true leaves (figure).



A: Low phospholipid concentrations (0,1 mg mL^{-1}). B: High concentrations of phospholipids (10 mg mL^{-1}). C: Control

Germination of tomato cultivar Amalia at seven days

Besides these phosphatidylphospholipids, which are substrates of phospholipase enzymes families (A, C, D) with direct involvement in cell fate and development and growth processes (6), in the evaluated mixture other interconvertible phospholipid components are present in other precursors and important second messengers in the germinative processes, and thus it constitutes a challenge in our research to elucidate the individual effect and the action mechanism of each type of the phospholipids, synergism or antagonism of evaluated mixture and the inhibitory effect on the germination and evidenced growth to high levels of phospholipids.

These results, although constitute preliminary evidences of biostimulating effect of the phospholipid mixture applied exogenously, demonstrate the importance of knowing the levels required to achieve the optimum effect and evaluation importance in different stages of plant development.

Generally, there have been recent advances in the knowledge of the metabolism and functions of endogenous phospholipids and their catabolites, showing that phospholipids and phospholipases have an important role in normal plant development and in response to abiotic and biotic stress. In comparison, there is little information available on the use of exogenous phospholipids. New evidences suggest that these compounds applied exogenously exert profound effects on plant growth and our results provide elements about the possibilities of these substances use as plant growth regulators.

CONCLUSIONS

With these results it can conclude that the phospholipid assessed mixture has an inductive effect on root growth of seedlings of newly germinated seeds, when it is applied as a substrate at low concentrations. Similarly there was no significant effect on germination percentage and no homogeneous behavior in this indicator in studied cultivars.Results demonstrate the potential use of these compounds as plant growth inducers.

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