# Effect of the Elicitor Hyaluronic acid on Multiplication and Indirect Regeneration of *Curcuma longa* L. Plant

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**ABSTRACT**: The aim of this study was to evaluate the effect of hyaluronic acid (HA) concentrations for multiplication and indirect regeneration of *Curcuma longa* L. plant. Multiplied shoots of *C. longa* L. were used as explant materials and callus for indirect regeneration. The explants cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of HA as0.05, 0.5, 1.5, 3.0 and 15 mg $l^{-1}$ for multiplication and 0.0, 0.16, 0.33, 0.66, 1.00, 2.00 and 5.00 mg $l^{-1}$ for indirect regeneration. Concerning to multiplication, the highest number of shoot (3.77 shoots $explant^{-1}$) was obtained from 3 mg $l^{-1}$ HA. While, the least concentrations of HA (0.16, 0.33 and 0.66 mg $l^{-1}$) enhanced shoot regeneration. On the other hand, the highest concentrations of HA produced higher number of roots and heaviest weight of callus under light condition.

***Keywords:*** *Curcuma longa* L., HA, Hyaluronic Acid, multiplication, indirect regeneration, turmeric

## INTRODUCTION

*Curcuma longa* L. belonging to the family Zingiberaceae, is one of the most important plants that is represented by 120 species (Sumathi, 2014; Chen *et al.,* 2015). This medicinal plant which is widely distributed in the humid tropics, subtropics and some seasonably dry regions of the world (Ravindran *et al.,* 2007; Christenhusz and Byng, 2016). Turmeric (*C. longa* L.) has been an important source of food, spice, dye and medicine for worldwide in the present and for traditional people throughout the ages (Deb and Chakraborty, 2017; Lamo and Rao, 2017). It is extremely nutritious, containing some vitamins such as Betaine, Vitamin A and C, Folate and Choline and rich in some minerals such as Calcium, phosphorus, iron, zinc, magnesium, potassium and sodium (Yadav and Tarun, 2017).

Turmeric contains over than 235 compounds. Traditionally, *C. longa* L. is vegetatively propagated by rhizomes, which is called seed rhizomes. Propagation from seed rhizomes faced several problems for many reasons. For example, rhizomes bits have 1 or 2 buds, takes from 8 to 10 months to fully mature and become dormant over winter, even in tropical climates. Also, about 20 to 30% of whole production was needed for the next season with high cost, low productivity and disease susceptibility of seed rhizomes (Rahman *et al.,* 2004; Goyal *et al.,* 2010; Antoniazzi *et al.,* 2016). Moreover, seed rhizome's flowers don't produce viable seeds because of their natural sterility (triploid 63), mutations over period of the time, low fertility, natural seed set and environmental problems (Sigrist *et al.,* 2011; Cheethaparambil *et al.,* 2014; Raju *et al.,* 2015).

Obtaining an aseptic culture from underground explant is difficult due to high contamination. Rhizomes and leaves are the major parts affected by microbial diseases causing economic and yield losses ranged from 50 to 60 % in turmeric and their control is difficult. Like Zingiberaceae family, turmeric suffers from some disease problems in all turmeric-growing countries depending on environmental condition (Ravindran *et al.,* 2007; Jasim *et al.,* 2014; Sarathi *et al.,* 2014; Ilyas *et al.,* 2016). Unfortunately, *C. longa* L. often presents a challenge to producers since it may be infected with many pathogens either fungi or bacteria and nematodes (Anoop *et al.,* 2014; Ajitomi *et al.,* 2015; Prabhu *et al.,* 2018).

The common plant hormones like Salicylic acid (SA), Hyaluronic acid (HA), Adipic acid and Jasmonic acid (JA) are key signals for defense gene expression and can promote growth in *in vitro* of same ornamental plants (da Silva *et al.,* 2013; Patel and Krishnamurthy, 2013; Morel *et al.,* 2015).Some studies reported that HA serves as a growth regulator (Haque *et al.,* 2016). Also, Sultana *et al.* (2015)and Nahar *et al.* (2011)proved that HA can be used as a plant growth regulator for orchid production through protocorm like bodies.

HA composed of glucuronic acid and *N*-acetyl glucosamine joined alternately by glycosidic bonds, has biological functions in lower and higher organisms, including in cell adhesion, migration, proliferation and differentiation, regulation of protein secretion, and gene expression (Kogan *et al.,* 2007). HA suppresses disease in cucumber, tomato and pepper (Park *et al.,* 2008) also been shown to improve orchid (*Cymbidium dayanum*) organogenesis *in vitro* (Nahar *et al.,* 2011; Kaewjampa *et al.,* 2012).

HA is polysaccharide compound formed the structural framework for cells and have different agriculture applications. HA are a naturally occurring biopolymer that consists of linear, unbranching, and polyanionic disaccharide units consisting of glucuronic acid. HA serves essential biological functions in lower and higher organisms such as bacteria, plant including humans. For example, it plays a principal role in dynamic processes mediated with extracellular matrix components, cell adhesion, migration, proliferation and differentiation, protein secretion and gene expression. HA is a component of the extra cellular matrixes of some tissue, can modulate growth factor and cytokine secretion, inhibit proteinase hydrolysis and influence several cellular functions such as adhesion, growth, migration, proliferation and differentiation (Habiba *et al.,* 2014; Nahar *et al.,* 2015; Nahar *et al.,* 2017).So, the objective of this study is to evaluate the effects of HA in multiplication and indirect regeneration of turmeric plant.

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## MATERIALS AND METHODS

This work was carried out in the Plant Tissue Culture Laboratory, of Horticulture Department, Faculty of Agriculture, Suez Canal University (SCU), Ismailia Governorate, Egypt, during the period from 2013 to 2017.

**Plant materials**

Multiplied shoots were used asexplant materials for multiplication and callus production from rhizome root explants on MS medium supplemented with 2,4-Dused as explant materials for indirect regeneration.

**Effect of different concentrations of HA on multiplication of *C. longa* L.**

This experiment was designed to investigate the effect of different concentrations of HA as 0.05, 0.5, 1.5, 3.0 and 15 mg $l^{-1}$ which were added to the MS medium. MS medium was supplemented with 3% sucrose, 7.0 g $l^{-1}$ agar and pH was adjusted at 5.7±0.1. This medium was dispersed into glass culture jars (350 ml) where each one contained 40 ml of medium. Jars were autoclaved at 121 ° C at 1.5 Kg/ $cm^2$ for 20 min. The jars were then placed in growth room at 16/8-hour photoperiod with 3000 lux (45 µmol $m^{-2}s^{-1}$) light at 23±2ºC day temperature.

**Data Record for:**

- Multiplication frequency %
- Number of shoots explant$^{-1}$
- Length of longest shoot (cm)
- Number of leaves explant$^{-1}$
- Shoots Fresh weight (g)
- Base diameter (mm)

**Effect of different concentrations of HA on indirect regeneration of *C. longa* L.**

The experiment was designed to determine the optimal concentration of HA at 0.16, 0.33, 0.66, 1, 2 and 5 mg $l^{-1}$ for indirect regeneration. The proliferating calluses were divided into two pieces, one was used for callus maintenance and the other for callus regeneration. MS medium was used and supplemented with 30 g $l^{-1}$ sucrose and 6g $l^{-1}$ agar. The cultures were incubated under 23±2˚C for good callus regeneration, and at 18/ 6h dark/ light photoperiod.

Statistical analysis:

Multiplication experiment was designed as factorial experiments in a complete randomized design (Little and Hills, 1978). The obtained data not following normality assumptions, so a transformation data was carried to get normality. Transformed data are an important tool for the proper statistical analysis of biological data. Sometimes, if a measurement variable does not fit a normal distribution and have some of the counts are zeros (some of the experiments), transformation for data should be done. We apply square root for transformation the data according to McDonald (2014). Data were computerized and subjected to statistical analysis using SPSS "version 19" statistical software. The differences between means of treatments were tested using LSD Tests at 0.05 level according to Snedecor and Cochran (1980).

## RESULTS AND DISCUSSION

**Effect of different concentrations of HA on multiplication of *C. longa* L.**

The data shown in the accompanying Table (1) and Fig. (1) indicate that there was a significant difference in number of shoots, length of tallest shoots, number of leaves and shoots fresh weight under different concentrations of HA. Data revealed that the highest number of shoot (3.77 shoots explant$^{-1}$) was obtained from 3 mg $l^{-1}$ HA and the least (1.8 shoots explant$^{-1}$) obtained from lower concentration of HA 0.05 mg $l^{-1}$. In general, HA at 0.5, 1.5 and 3 mg $l^{-1}$ significantly gave the tallest shoots as 14.1, 12.9 and 12.5 cm and number of leaves as 13.9, 12.1 and 13.9 leaves explant$^{-1}$ respectively.

The heaviest shoots weight (4.14 g) was found in the MS medium supplemented with3 mg $l^{-1}$ HA without significant with other concentrations but the least concentration 0.05 mg $l^{-1}$ gave least weight of shoots Table (1). On the other hand, there was no significant differences in base diameter (mm) among different HA concentrations. Besides, the least concentration of HA (0.05 mg $l^{-1}$) give the lower values for all parameters.

The results in the current studies provide, for the first time, some results on the successful micropropagation using the HA for multiplication on *C. longa* L. plant and need more studies. Perhaps case studies could then be used for more in-depth analysis and need for further research.

Elicitors as HA are extrinsic or foreign molecules often associated with plant pests, diseases or synergistic organisms, elicitor molecules can attach to special receptor proteins located on plant cell membranes, it may by control or manipulate or promote plant growth and biosynthesis of active constituents (Coste *et al.,* 2011; Patel and Krishnamurthy, 2013; Singh, 2014) and Nahar *et al* .(2011). In this concern, Habiba *et al.*(2014) found that HA at 1 mg $l^{-1}$produced the highest shoots per explant of *Cymbidium dayanum* and *Dendrobium kingianum* respectively. Whereas, Kaewjampa *et al.* (2012) found that 0.1 mg $l^{-1}$ HA on culture medium was highly potent in PLB (protocorm like bodies)multiplication. Results of Haque *et al.*(2016) showed that BA at 0.1 mg $l^{-1}$ combined with HA at 0.1 mg $l^{-1}$ are the most effective concentration for PLB, shoot induction and maximum fresh weight of *C.insigne*. Also, they found that the maximum shoots number of *C. finlaysonianum were* found to be effective at combination of BA at 0.1 mg $l^{-1}$ and HA at 1 mg $l^{-1}$.

**Table (1):** Effect of HA concentrations on number of shoots, length of longest shoot, number of leaves, shoots fresh weight and base diameter of *C. longa* L

| HA (mg $l^{-1}$) | Multiplication frequency % | No. of shoot explant$^{-1}$ | Length of longest shoot (cm) | No. of leaves explant$^{-1}$ | Shoots fresh weight (g) | Base diameter (mm) |
|---|---|---|---|---|---|---|
| **0.05** | | 1.80 | 10.7 | 6.40 | 2.18 | 3.20 |
| **0.50** | | 2.43 | 14.1 | 13.8 | 2.95 | 3.43 |
| **1.50** | 100 | 3.00 | 12.9 | 12.1 | 3.58 | 3.86 |
| **3.00** | | 3.77 | 12.5 | 13.9 | 4.14 | 4.15 |
| **15.0** | | 3.00 | 12.1 | 8.00 | 3.61 | 4.00 |
| **LSD 5%** | | **1.14** | **2.03** | **4.19** | **1.62** | **NS** |

Means with the same letters in the same column are not significantly different according to least significant difference test (LSD) 5%

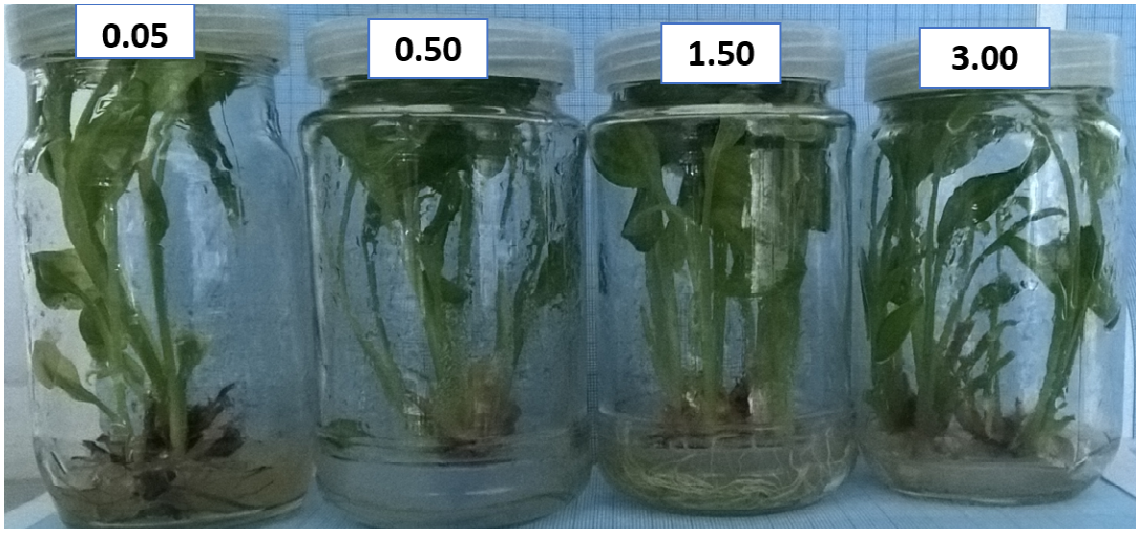


**Figure (1):** Multiplies shoots of *C. longa* L. under HA concentrations (0.05, 0.50, 1.50, and 3.00 mg $l^{-1}$)

**Effects of HA concentrations on indirect regeneration of *C. longa* L.**

All HA treatments had a positive effect on callus formation and weight gain significantly from the control treatment (transformed data). The maximum fresh weight of callus was obtained by adding 2 mg $l^{-1}$ HA to the medium. On the other hand, the low concentrations of HA up to 1 mg $l^{-1}$ encouraged the formation of plantlets from callus (Table 2) and (fig. 2).

In general, such this work on turmeric was not done before according to the available researchand previous studies were on the Orchid Cymbidium only. Another successful result has been obtained by Kaewjampa *et al.*(2012)and Nahar *et al.*(2011). They investigated the effect of two biopolymer HA and Chitosan H, and BA supplemented with modified MS on organogenesis in protocorm like bodies (PLBs) of a *Cymbidium*. They found that the addition of 0.1 mg $l^{-1}$HA to culture medium was highly efficient for PLB multiplication. Both 10 mg $l^{-1}$HA and 1.0 mg $l^{-1}$BA significantly increased the number of shoots in cultures. Information gained from this study shows that HA which is new additive for plant tissue culture and act as growth regulator for hybrid *Cymbidium* micropropagation.

**Table (2):** Effects of HA (HA) concentrations on callus fresh weight and callus induction frequency in *C. longa* regeneration (Based on transformed data)

| HA (mg $l^{-1}$) | Callus FW (g) | Regeneration |
|---|---|---|
| **0** | 0.71 | NR** |
| **0.16** | 1.77 | plantlets |
| **0.33** | 1.66 | plantlets |
| **0.66** | 1.60 | plantlets |
| **1** | 2.00 | plantlets |
| **2** | **2.31** | roots + callus |
| **5** | 2.01 | roots + callus |
| **LSD 5%** | **0.92** | |

*Means with the same letters in the same column are not significantly different according to least significant difference test (LSD) 5%.
**NR= No response

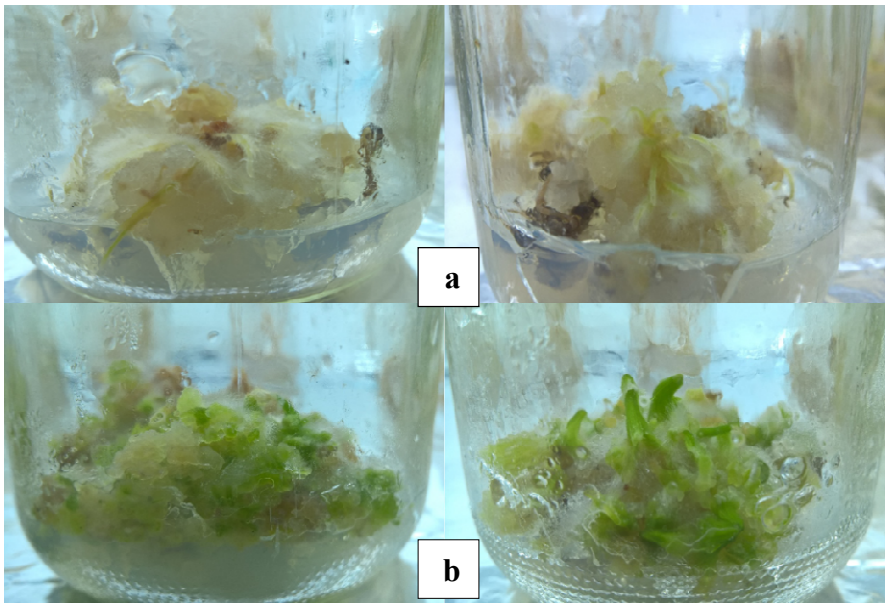


**Figure (2):** Direct regeneration from callus of *C. longa* L. on medium containing HA concentrations (a) regenerated roots from callus (b) regenerated leaves, roots and shoots from callus

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# تأثير حمض الهيالورونيك على التضاعف وتشكل للكالوس في نبات الكركم

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الهدف من هذه الدراسة هو تقييم تأثير تركيزات حمض الهيالورونيك HA على تضاعف وتشكل للكالوسفي نبات الكركم *Curcuma longa* L. معمليا. تم استخدام الأفرع المتضاعفة كمصدر للمنفصل النباتي في تجربة التضاعف كما تم استخدام الكالوس لتجربة التشكل.المنفصلات النباتية تم زراعتها على بيئة موراشيج وسكوج مضافا اليها تركيزات مختلفة من هرمون HA (0.05 و0.5 و1.5 و3.0 و15 ملجم/لتر) لتجربة التضاعف و التركيزات (0.0 و0.16 و0.33 و0.66و1.00 و 2.00 و 5.00 ملجم/لتر) لتجربة التشكل. فيما يتعلق بتجربة التضاعف أظهرت النتائج ان أكبر عدد للفروع المتضاعفة (3.77 فرع/قمة نامية) تم الحصول عليه بواسطة (3.0 ملجم/لتر) من HA. بينما في تجربة التشكل كانت التركيزات المنخفضة من هرمون HA(0.16 – 0.33 – 0.66 ملجم/لتر) أدت الى تكون أفرع جديدة على الكالوس. كما ان التركيزات المرتفعة من HA أدت لتكوين الجذور على الكالوس وانتجت زيادة معنوية للوزن الطازج في الإضاءة.