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# EFFECT OF SOME TREATMENTS ON ANNONA SEED GERMINATION AND GROWTH OF PRODUCING SEEDLINGS.

**Bilates, Ebtisam L.; Amany M. Mostafa and H. A. Abdel - Kareem**
**Tropical Fruit Research Division, Horticulture Research Institute, Agric. Research Center, Giza, Egypt.**

## ABSTRACT

This investigation was carried out at Horticulture Research Institute Orchard. Agriculture Research Center during the two successive seasons of 2005/2006 and 2006/2007 to study the effect of different treatments on germination and seedling growth of *Annona sequamosa* L. (Balady spp.) The treatments used were soaking in water, $GA_3$, fungicide and EM. The obtained results showed that, EM treatments increased germination percentage close to GA3 treatments and fasted rate of germination as compared with untreated treatment (control). Moreover, EM treatments increased stem length and diameter, number of leaves/plant, leaf area and root length, as well as stem, leaf and root fresh and dry weight. Leaf chlorophyll content insignificantly differed between EM and $GA_3$ treatments. Diluted EM treatment was more effective than concentrated one in this concern. Anatomical study of *Annona sequamosa* seed showed that, embryo of fresh extracted seed was not yet complete differentiated and it fully developed when seed stored for long time, so germination of fresh seeds were uneven and irregular. In addition, morphological structure of seed show that water can be enters to seed through hillum.

## INTRODUCTION

Uneven and irregular germination of cherimoya *(Annona cherimola* Mill.) seeds results in difficulties in propagating this fruit tree. This pattern of germination is probably due to the presence of dormancy, a natural protection to give seedlings a better chance of survival, through inducing germination in optimal environmental conditions, and away from the mother plant, so avoiding competition (Bewlay and Black, 1983; Dickie *et al*., 1984 and Smet *et al*., 1999). Dormancy may be seed coat- imposed, where physical barriers prevent germination processes or embryonic, when biochemical characteristics also prevent germination. This dormancy can be broken, depending on the type using different treatments, i.e. scarification, in the case of coat- imposed dormancy, water can be used to wash out germination inhibitors, or else oxidants can be used to inactivate these inhibitors in the case of embryo dormancy (Hayat, 1963; Sanewski, 1991 and Purohit, 1995). Another particular characteristic of Annona seed is the presence of a rudimentary slowly developing embryo which is mostly not yet fully differentiated when fruit are ripe. Development of embryo goes on in the seed after harvesting of the fruits preventing germination before full differentiation of the embryo. However, Annona seeds display prolonged dormancy to embryo immaturity. Seed germination at field conditions takes along time, this makes seedling production and conservation of native populations a very expensive and time-consuming (Rissini, 1973; Baskin and Baskin, 1998 and Smet *et al*., 1999). The physiological dormancy component is broken by low temperature and/or temperature fluctuations proceeding the rainy season.

Subsequent embryo growth and digestion of the endosperm are both likely to be controlled by gibberellins synthesized during the breaking of physiological dormancy (Evaldo *et al*., 2007).

Microorganisms have been successfully used in promoting seed germination of many crops species, and this concept could have an important role in agriculture (Kloepper *et al*., 1986). When microorganisms such as Azoprillum and Pseudomonas have used from natural sources for enhancing germination, the results clearly present a protecting and growth promoting role of these species in the germination process of many cereals. The caused mechanisms of this phenomenon were attributed to the protection of seeds from external stress factors and diseases during the germination process, when seeds and the emerging radicles and plumules are sensitive to the environment (Arsac *et al*., 1990 and Hofte *et al.,* 1991). Microorganisms in the soil play a vital role in nutrients cycling and mediate various processes through their interactions with plant and other soil organisms (Zuberi *et al*., 1991 and Sangakkara and Attanayake, 2001). Application of EM4 to citrus media significantly gave higher number of roots, fresh and dry weight of transplants roots, increased number of shoots, number of leaves and root length (Wibisono *et al.,* 1996).

## MATERIALS AND METHODS

This investigation was carried out in the Horticulture Research Institute Agricultural Research Center, Giza, Egypt during the two successive seasons of 2005/2006 and 2006/2007 to study the effect of water soaking, EM (Effective Microorganisms) and GA3 of two levels on germination of sugar apple (*Annona squamosa* L.) seeds and growth of produced seedlings.

Seed extracted from mature fruits of sugar apple Balady spp. by rubbing the fruit by hand, then washed, dried and stored until time of sowing. The stored seeds were subjected to the following treatments:

1- Untreated seeds (control).
2- ($T_1$) Soaking in water for 12 h then soaking in GA3 at 1000 ppm. 12h.
3- ($T_2$) Soaking in water for 12 h then soaking in GA3 at 1000 ppm. 24 h.
4-($T_3$) Soaking in water 12 h. then dipped in fungicide (Vetafax capitan) for 10 min.
5- ($T_4$) Soaking in water 12 h.
6-($T_5$) Soaking in diluted EM 10% for 15 min.
7- ($T_6$) Dipping in concentrated EM (1:1) for 1 min.

For this purpose each treatment contained 150 seeds distributed in 3 replicates (50 seeds/ each replicate). Seeds in all treatments were planted in early March during both studied seasons. They were sown in perforated plastic boxes (50 x75 cm) filled with mixture of sand and peat moss (1:1 by volume), then kept under a greenhouse and subjected to the same usual cultural practices used in this concern. After 45 days from sowing and at weekly intervals, the following parameters were recorded in each treatment during both studied seasons:

1- Germination percentage was calculated according to formula, where

$$\text{Germination ( \% )} = \frac{\text{Total number of germinated seeds}}{\text{Total number of planted seeds}}$$

2- Germination rate index also calculated as follows:

$$\text{Germination rate} = \frac{(A)+(A+B)+(A+B+C)\ldots.(\ldots+X)}{N\,(A+B+C+\ldots\ldots\ldots\ldots x)}$$

The length of seedling was recorded at 15 days intervals.
N = number of counts, A+B+C+……X=
Where, A= the first reading, B = after 15 days, C= after one month and so on.
Six months after sowing, samples of 15 plants from each treatment were randomly taken to determinate the following parameters:
1- Average of stem length, diameter (cm) and fresh& dry weight (g).
2- Number of leaves per plant and average leaf area ($cm^2$).
3- Leaf chlorophyll content (mg/100g).
4- Root length (cm) and dry weight (g).

**Seed structure:**

To describe immature and mature embryo of Annona seed as an important factor affecting germination, 10 of fresh and stored seeds were taken and soaked in tap water for 12 h then killed and fixed in FAA (formalin acitic acid) solution for least 48 h. The dehydration was performed in increasing concentration of ethyl alcohol, then clarified in xylol and finally infiltrated with paraffin wax and embedded. Paraffin wax with samples were sectioned by rotary microtome and the sections were stained with Saffranine and fast green then cleared in xylol and mounted in canda balsam (Johansen,1940). In addition, a median section in stored seed was done by using a razor blade to observe the morphological structure of Annona seed.
Statistical analysis of this experiment was complete randomized design and the mean values for both seasons were compared by New LSD as the methods reported by Snedecor and Cochran (1969).

## RESULTS AND DISCUSSION

**Germination percentage and rate:**

The effect of different treatments on percentage and germination rate is presented in Table 1. The obtained results showed that the highest percentage of germination achieved from seeds soaked in water then in GA3 at 1000 ppm. for 24 h. Moreover, EM (1: 1) treatment increased percentage of germination and gave nearly similar. However, the least percentage of germination obtained from untreated (control) and soaking in water only with fungicide seeds. Undiluted EM (1:1) was more effective than diluted (10%) may be due to the possible activation of germination in dormant seeds (Kloepper *et al.*, 1986; Iswandi *et al*., 2001 and Hofte *et al*., 1991).

Concerning germination rate, the presented data show that, EM at diluted concentration (10%) treatments increased rate of germination closed to GA3 treatments while the lowest rate produced from untreated seeds then seeds treated with water only or with water & fungicide, this is clear in both studied seasons. These results are in agreement with the finding of Arsac *et*

*al*. (1990) Hofte *et al*. (1991) and Zuberi *et al*. (1991), who identify the usefulness of naturally-occurring microorganisms and biological products in enhancing germination and early growth of very important crop species of the developing world.

**Table 1. Effect of some treatments on percentage and germination rate during 2005 and 2006, seasons.**

| Treatment | Germination rate (%) | | Average | Germination rate (%) | | Average. |
|---|---|---|---|---|---|---|
| | 2005 | 2006 | | 2005 | 2006 | |
| Control | 14.1 | 13.7 | 13.9 | 0.12 | 0.11 | 0.12 |
| $T_1$ | 67.3 | 66.5 | 66.9 | 0.65 | 0.62 | 0.64 |
| $T_2$ | 73.1 | 74.0 | 73.6 | 0.74 | 0.72 | 0.73 |
| $T_3$ | 31.4 | 32.9 | 32.2 | 0.29 | 0.30 | 0.30 |
| $T_4$ | 31.4 | 32.9 | 32.2 | 0.36 | 0.37 | 0.37 |
| $T_5$ | 38.6 | 34.2 | 36.4 | 0.65 | 0.68 | 0.67 |
| $T_6$ | 61.0 | 60.6 | 60.8 | 0.64 | 0.61 | 0.63 |
| New L.S.D. at 0.05 | 4.17 | 4.24 | | 0.02 | 0.03 | |

**Stem length and diameter, fresh and dry weight**:

The obtained data in Table 2 clearly showed that stem length increased by EM treatments nearly similar with GA3 treatments as compared with untreated one which gave shortest plants. Moreover, the highest plants were produced from seeds treated with GA3 at 1000 ppm for 24 h and those treated with diluted EM (10%) after soaking in water for 12 h. In addition, stem diameter also increased with EM treatments against GA3 treatments and other tested ones and the highest values obtained from both $GA_3$ and diluted EM treatment, this is clear in both studied seasons, respectively. Moreover, Stem fresh and dry weight showed a significant increment with EM treatments as compared with other tested treatments except $GA_3$, diluted EM treatment was superior to concentrated one in this concern. These results go in line with the finding of Zuberi *et al.* 1999 who mentioned that, microorganisms in the soil play a vital role in nutrients cycling and mediate various processes through their interactions with plant and other soil organic.

**Number of leaves, leaf dry weight, leaf area and chlorophyll content:**

The obtained results in Table 3 revealed that GA3 at 1000 ppm for 24 h and diluted EM treatment gave the highest number of leaves per plant followed by EM (1:1) and the lowest numbers were produced from control and fungicide treatments. Accordingly, leaf dry weight increased with EM treatments and the highest values were obtained from GA3 treatment followed by diluted EM (10%) treatment while the lowest values produced from fungicide treatment, however, other tested treatment arranged between them. In addition, GA3 at 1000 ppm for 24 h. gave the widest leaf (18.91 and 19.37 cm.), the nearly same values (17.39 and 17.46 cm.) were produced from diluted EM treatment. Concerning chlorophyll content, the obtained results showed insignificant differences between GA3 and EM treatments while chlorophyll significantly decreased in leaf of control and fungicide treatments, this is clear in both studied seasons. These results coinciding

those revealed by Kloepper *et al*.(1986), Arsac *et al*.(1990), Wibisino *et al*. (1996) and Smet *et al*. (1999).

**Table 2: Effect of some treatments on length, diameter, fresh and dry weight of stem during 2005and 2006, seasons.**

| Treatments | Stem length. (cm). | | Mean | Stem diameter (cm.) | | Mean | Stem FW (g) | | Mean | Stem DW. (g) | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2005 | 2006 | | 2005 | 2006 | | 2005 | 2006 | | 2005 | 2006 | |
| Control | 17.0 | 17.9 | 17.5 | 0.4 | 0.40 | 0.40 | 0.532 | 0.546 | 0.549 | 0.160 | 0.164 | 0.162 |
| $T_1$ | 24.9 | 24.8 | 24.9 | 0.6 | 0.60 | 0.60 | 0.643 | 0.631 | 0.637 | 0.196 | 0.188 | 0.192 |
| $T_2$ | 27.0 | 27.3 | 27.1 | 0.6 | 0.70 | 0.70 | 0.661 | 0.677 | 0.669 | 0.198 | 0.203 | 0.201 |
| $T_3$ | 19.4 | 19.0 | 19.2 | 0.5 | 0.40 | 0.50 | 0.544 | 0.538 | 0.541 | 0.183 | 0.167 | 0.175 |
| $T_4$ | 22.2 | 22.6 | 22.4 | 0.5 | 0.50 | 0.50 | 0.586 | 0.592 | 0.589 | 0.176 | 0.178 | 0.177 |
| $T_5$ | 25.3 | 25.1 | 25.2 | 0.8 | 0.80 | 0.80 | 0.659 | 0.671 | 0.665 | 0.208 | 0.201 | 0.205 |
| $T_6$ | 24.6 | 24.5 | 24.6 | 0.7 | 0.70 | 0.70 | 0.651 | 0.647 | 0.649 | 0.198 | 0.194 | 0.196 |
| New LSD at 0.05 | 4.36 | 4.14 | | 0.06 | 0.08 | | 0.024 | 0.031 | | 0.004 | 0.006 | |

**Table (3) : Effect of some treatments on number of leaves, leaf area, leaf dry weight and leaf chlorophyll content during 2005 and 2006, seasons**

| Treatments | Leaves No. | | Mean | Leaf area (cm2) | | Mean | Leaf DW. (g.) | | Mean | Chlorophyll (mg/100g) | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | 2005 | 2006 | | 2005 | 2006 | |
| | 2005 | 2006 | | 2005 | 2006 | | | | | | | |
| Control | 5.6 | 4.7 | 5,15 | 6.05 | 5.59 | 5.83 | 0.166 | 0.173 | 0.170 | 30.52 | 35.29 | 29.94 |
| $T_1$ | 7.3 | 7.6 | 7.45 | 17.64 | 17.22 | 17.43 | 0.460 | 0.459 | 0.460 | 33.35 | 32.35 | 32.85 |
| $T_2$ | 8.5 | 9.2 | 8.85 | 18.91 | 19.37 | 19.14 | 0.476 | 0.481 | 0.479 | 33.62 | 33.71 | 33.67 |
| $T_3$ | 5.4 | 3,9 | 4.65 | 5.22 | 4.88 | 5.05 | 0.146 | 0.134 | 0.140 | 30.15 | 29.30 | 29.73 |
| $T_4$ | 6.7 | 6.9 | 6.80 | 8.49 | 7.37 | 7.93 | 0.201 | 0.214 | 0.208 | 31.24 | 30.31 | 30.78 |
| $T_5$ | 8.2 | 8.4 | 8.30 | 17.39 | 17.46 | 17.43 | 0.464 | 0.468 | 0.466 | 33.30 | 33.31 | 33.31 |
| $T_6$ | 7.2 | 7.6 | 7.40 | 14.86 | 15.76 | 15.31 | 0.324 | 0.331 | 0.328 | 32.74 | 32.76 | 32.75 |
| New LSD at 0.05 | 1.60 | 1.31 | | 4.27 | 3.69 | | 0.024 | 0.032 | | 2.16 | 2.34 | |

**Root length, root fresh and dry weight**:

The obtained results in Table 4 showed that GA3 treatments significantly increased root length. Moreover EM treatments also increased root length than untreated (control) treatment. On the other hand, EM treatments gave the highest significant values of fresh and dry weight of root followed by GA3 treatments while the lowest values obtained from fungicide and control treatments. In this respect, Wibisono *et al*. (1996) mentioned that, application of EM to citrus media significantly gave higher number of roots, fresh and dry weight of transplants roots, increased number of shoots, number of leaves and root length. Moreover, the concentrated EM solution had a toxic effect on seed germination and seedling growth, and reduced shoot and root dry weight. Diluted solution of EM had a beneficial effect on all measured parameters. The best germination, seedling characteristics and growth occurred with EM applied after dilution of 1: 500 with water Sangakkara and Attanayake (2001).

**Table (4): Effect of some treatments on root length, root fresh and dry weight during 2005 and 2006, seasons.**

| Treatments | Root length. cm. | | Mean | Root FW. (g ) | | Mean | Root DW. (g). | | Mean |
|---|---|---|---|---|---|---|---|---|---|
| | 2005 | 2006 | | 2005 | 2006 | | 2005 | 2006 | |
| Control | 13.4 | 12.9 | 13.2 | 0.166 | 0.154 | 0.160 | 0.116 | 0.108 | 0.112 |
| $T_1$ | 15.9 | 16.3 | 16.1 | 0.173 | 0.204 | 0.189 | 0.121 | 0.134 | 0.132 |
| $T_2$ | 24.6 | 24.1 | 24.4 | 0.387 | 0.389 | 0.388 | 0.271 | 0.272 | 0.272 |
| $T_3$ | 25.4 | 24.9 | 25.2 | 0.410 | 0.394 | 0.402 | 0.287 | 0.276 | 0.282 |
| $T_4$ | 12.6 | 12.8 | 12.7 | 12.7 | 0.157 | 0.152 | 0.102 | 0.110 | 0.106 |
| $T_5$ | 24.5 | 24.3 | 24.4 | 0.416 | 0.477 | 0.469 | 0.323 | 0.334 | 0.329 |
| $T_6$ | 24.1 | 24.0 | 24.1 | 0.415 | 0.413 | 0.414 | 0.291 | 0.289 | 0.290 |
| New LSD at 0.05 | 5.78 | 6.07 | | 0.024 | 0.032 | | 0.017 | 0.021 | |

**Seed anatomy and structure**:

This part of investigation aim to describe the state of *Annona squamosa* seed embryo in fresh and stored seed beside the morphological structure as an important factor affecting seed germination. In this concern and from the microscopic examination of longitudinal section in fresh seed, it is clearly that, embryo of fresh extracted seed is mostly not yet fully differentiated and there were nine or ten cell layers in the front of embryo located at the micropylet end of the endosperm (Fig., 1). So, germination of such seeds is very difficult. In addition, longitudinal section in stored seed clearly show that, the embryo became fully developing (Fig., 2) and seeds in this respect germinated well. As for the morphological structure, the longitudinal median section of *Annona squamosa* seed show the dark brown testa consists of an impermeable woody sclernchyma layer, meaning that water can only enter the seed at the hilum, through the micropyle. The embryo is very small and rudimentary located with its radicle directed towards the micropyle. The endosperm is white in color and consists of two clearly distinguishable parts, the inner central unlobed endosperm and the outer lobed endosperm (the endosperm which has a surface that is irregular in shape). Protrusions of the tegmen, a membrane-like cover of the endosperm can be observed as lamellas between the lobed endosperm (Fig., 3). These observations are in agreement with those of Hayte, 1963; Rizzini (1973), Purohit (1995), Baskin and Baskin (1998), Smet *et al.* (1999) and Evaldo et *al*. (2007).

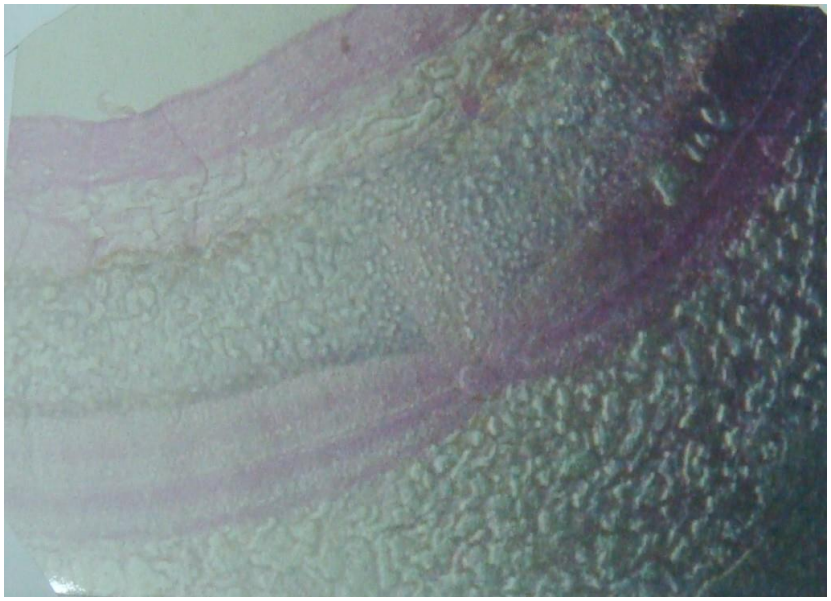

**Fig.(1). L S in *Annona squamosa* fresh seed ( x 250)The embryo not yet fully developing.**
**Nine or ten cell layers in front of embryo**

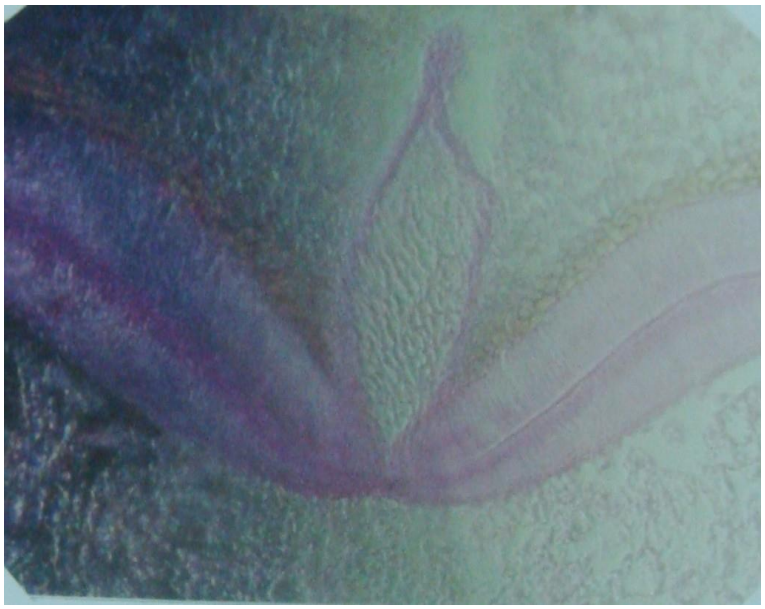

**Fig. (2). L S in stored seed ( x 250 )Fully developing embryo.**

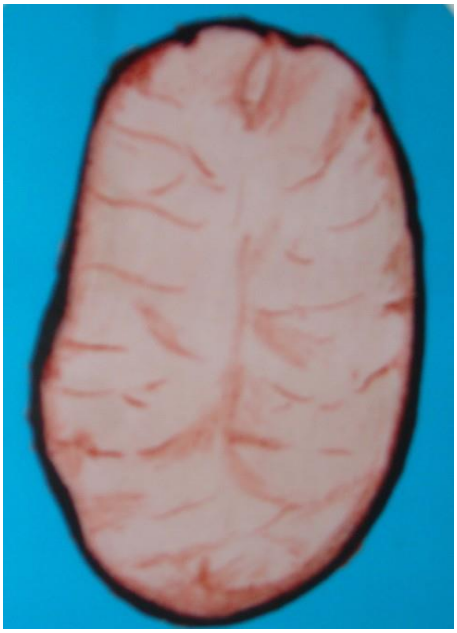

**Fig. (3). Median section of *Annona squamosa* seed ( x 24 )**
**1- hilum 2- micropyle 3- embryo 4- tegmen 5- testa**
**6- lamella 7- unlobed cellular endosperm 8- lobed cellular endosperm**
**9- perichalasal zone.**

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## تأثير بعض المعاملات على إنبات بذور القشطة ونمو الشتلات الناتجة.

**أبتسام لبيب بيلاطس ، أماني احمد مصطفى و حسن على عبد الكريم**

**قسم بحوث الفاكهة الاستوائية، معهد بحوث البساتين، مركز البحوث الزراعية، الجيزة، مصر.**

أجريت هذه التجربة بمعهد بحوث البساتين ، مركز البحوث الزراعية خلال موسمى ٢٠٠٥ ، ٢٠٠٦ لدراسة تأثير بعض المعاملات المختلفة على إنبات البذور ونمو الشتلات فى القشطة البلدى مثل النقع فى الماء ومبيد فطرى وحمض الجبريلين وكذلك الـ EM وقد أوضحت النتائج المتحصل عليها على أن ال EM قد أعطت نسبة إنبات متساوية تقريبا مع معاملات حمض الجبريلين كما أدت الى زيادة فى معدل الإنبات وذلك مقارنة بالكنترول.

كما أدت المعاملة بال EM الى زيادة طول وقطر النباتات الناتجة وزيادة عدد الأوراق ومساحتها وطول الجذور. وبناء عليه زيادة فى الوزن الطازج والجاف لكل من الساق والأوراق والجذور ، وكانت المعاملة بال EM المخففة بنسبة ١:١٠٠ أفضل من استخدام ال EM المركزة. كما أوضحت الدراسة التشريحية لبذور القشطة البلدى أن الجنين لا يكون مكتمل النمو فى البذور الطازجة عند استخراجها مباشرة من الثمار ويكتمل نمو الجنين بتخزين البذور لفترة طويلة وبذلك يقل الإنبات عند زراعة البذور بعد الاستخراج مباشرة. كما أوضحت الدراسة المورفولوجية تركيب بذرة القشطة وان الماء لا يدخل الى البذور إلا من فتحة النقير فقط.