SEED GERMINATION AND SUBSEQUENT SEEDLING GROWTH OF SCARIFIED SEEDS OF CAROB (Ceratonia siliqua, L.) AS AFFECTED BY DIFFERENT PRE-SOWING TREATMENTS
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ABSTRACT
Scarification and pre-soak treatments with water (25°C, 35°C), Kinetin (10,30 ppm), thiourea (0.015%, 0.030%) and gibberellic acid, GA3 (200, 400 ppm) solutions had a pronounced effects on germination and growth of carob (Ceratonia siliqua, L.) during the two successive seasons of 2000 and 2001. Compared with control germinating seeds, highest final germination percentage was attained in that of pre-soaking scarified seeds in water for 48 hrs at 35°C (85.5 %), Kinetin at 10 ppm (82.1 %), Kinetin at 30 ppm (81.0 %) and thiourea at 0.015% (74.4 %). The values of germination rate index (G.R.I.) were faster in the second season than in the first one. Also, the obtained values of germination velocity coefficient (G.V.C.) revealed that such treatments as T3 (soaking scarified carob seeds into water at 25°C for 48 hrs), T5 (soaking scarified carob seeds into thiourea 0.015%), T7 (soaking scarified carob seeds into thiourea 0.015%), T9 and T10 (soaking scarified carob seeds into GA3 at 200 and 400 ppm) accelerated the germination process than the control. The effect of treatments was extended to the rate of vegetative growth of subsequent seedlings. Since, the strongest seedlings were coincided with those high in their germination parameter values. Moreover, biochemical changes in the seeds during early germination stage gave results partially explained the effect of different treatments on seed germination and subsequence seedling growth.

Keywords: Carob seeds, germination, germination velocity, gibberellic acid, Kinetin, thiourea, sugar and phosphorus contents.

INTRODUCTION
The carob tree (Ceratonia siliqua, L., Fam. Caesalpinaceae) is an important component of the Mediterranean vegetation, especially in marginal and prevailing calcareous soils of that region, thus it is important ecologically and economically (Imam, 1983, Catarins, 1995 and Batlle and Tous, 1997). However, currently the main interest of this tree is seed production for gum extraction, which are used widely in industry as a stabilizer (Catarins, 1995) and the traditional use of their pods as animal and human food (Merwin, 1981). This tree has an important health value as astringent, antidiarrhea, antacids and diuretic (Imam, 1983). In Egypt carob syrup is a popular drink obtained by extracting carob kibbles with water, therefore it can be used as alternative to the other syrups.

Although it is not commonly cultivated in Egypt, it can grow successfully in the northern coastal desert areas, Wally et al. (1975), El-Gazzar et al. (1981) reported that carob seedlings grow in calcareous and acidified soils exhibited greater plant growth than did those of the sandy soil. Also, it grows in many unfavourable environmental conditions. However, the
carob's value extends to be as a drought tolerance, air pollution tolerant and low maintenance tree for street and landscape (Nunes et al., 1989). In addition, carob is a valuable resource in reforestation for prevention of erosion processes in marginal lands, (Correia and Martins-Loucao, 1993). World production of carob is estimated at about 310,000 tons/year; Spain is the leading carob producer, with an average 135,000 tons/year followed by Italy, Portugal, Morocco, and some countries of the Mediterranean region, (MAPA, 1994). Egypt, mainly imports carob fruits from such Mediterranean countries.

The present carob cultivars has been originated from chance seedling and selected from local populations and later commercially established (Batlle and Tous, 1998). Also, Carob rootstocks are still raised from seeds of cultivated or wild trees. The seedling stocks should be budded one- year old after seed germination in the nursery or two- year old from germination after planting it in the permanent orchard. On the other hand, vegetative propagation by cuttings is not yet commercially available (Fadl et al. 1979).

Germination is the process of reactivation of the metabolic machinery of the seed and the emergence of both radical and plumule leading to the production of a seedling. Physiologically, germination begins with the initial stages of biochemical reactivation during enzymatic conversion of complex reserve substances to simple soluble ones; that are readily translocated to the embryonic plant and ends with emergence of the radical (Jann and Amen, 1977, Janik, 1979).

Carob seed testa is very hard and impermeable to water and O2 with minute hilum, may require elaborate treatment procedures to accelerate germination process and to maximize germination velocity and rate. Scarification of seed coat by different mechanical or chemical means involving abrasive action, using sulphuric acid, hot water, acetone, potassium nitrate, GA3, kinetins and other substances were being reported by many workers, (Younis et al., 1971, Mitrakos, 1981, Frutos, 1988, Hussein et al., 1993, Russo and Uggenti, 1993, Martins-Loucoa et al., 1996, and Batlle and Tous, 1998).

Carob has been neglected with respect to its cultivation practices, research work and development, therefore, this study was conducted to assess the effect of different treatments on seeds to accelerate germination process and stimulating vegetative growth of the subsequent seedlings, as well as, the associated changes in certain metabolites during germination and growth.

**MATERIALS AND METHODS**

The present work was carried out during 2000 and 2001 seasons to investigate the effect of different treatments on seed germination and subsequent seedling growth of carob.
Time course experiment:
Carob seeds were collected from mature pods, washed with tap water, then distilled water, dipped in 5% sodium hypochlorite solution for 5 seconds, re-rinsed in water and air dried for 4 hrs. The experiment was conducted after seed scarification on both seed sides by abrading their coats by file to weaken it for rendering it permeable to gases and water. The tested treatments were as follows:

- $T_c =$ Scarified seeds only before sowing (control).
- $T_1 =$ Soaking scarified seeds into water at 25°C for 24 hr.
- $T_2 =$ Soaking scarified seeds into water at 35°C for 24 hr.
- $T_3 =$ Soaking scarified seeds into water at 25°C for 48 hr.
- $T_4 =$ Soaking scarified seeds into water at 35°C for 48 hr.
- $T_5 =$ Soaking scarified seeds into Kinetin solution (15 ppm) for 24 hr.
- $T_6 =$ Soaking scarified seeds into Kinetin solution (30 ppm) for 24 hr.
- $T_7 =$ Soaking scarified seeds into Thiourea solution (0.015%) for 24 hr.
- $T_8 =$ Soaking scarified seeds into Thiourea solution (0.03%) for 24 hr.
- $T_9 =$ Soaking scarified seeds into GA$_3$ solution (200 ppm) for 24 hr.
- $T_{10} =$ Soaking scarified seeds into GA$_3$ solution (400 ppm) for 24 hr.

For each treatment, 150 uniform seeds were divided into three replicates, then were planted in nursery mixture sand : peat-moss : clay (1:1:1 v/v) in plastic foam trays, each seed was sown in each hole, covered with washed sand and irrigated by fine sprayer every day until the seeds germinated. One week after sowing date until the end of germination period (35 days), the number of successful germinated seeds were recorded, when a white-hooked protrusion stage was clearly visible on the soil surface; and hypocotyl elevates the cotyledon above the ground (epigeous germination). The randomized complete blocks design with 3 replicates pretreatment was adopted.

Analytical methods:
The following parameters were studied:

I- Germination parameters:

I) Germination percentage (as a final germination percentage; FG%) total number of germinated seeds/initial number of seeds $\times 100$ (cumulative germination percentage).

II) Germination rate index (GRI) were calculated from the following equation:

$$GRI = \frac{\sum T_i N_i}{S}$$

where $T_i$ is the number of days after sowing, $N_i$ is the number of seeds germinated on day $i$, and $S$ is the total number of planted seeds, according to Scott et al. (1984).

III) Germination velocity coefficient (G.V.C) is calculated as:

$$GVC = 100 \left[ \frac{\sum N_i}{\sum N_i T_i} \right]$$
where \( N \) is the number of seeds germinated on day \( i \) and \( T \) is the number of days from sowing, according to Kotowski (1926).

2- Seedling vigor:

After the germination had ceased and seedling produced two leaves plus to the cotyledon leaves, plants were transplanted with the complete block of soil into perforated black polyethylene bags contained a medium of peat-moss : sand : clay (1:2:1 v/v) and placed under nursery conditions. After three months of transplanting, growth vigor of the subsequent seedlings was measured in 9 seedlings collected from each treatment (3 seedlings / replicate). The measurements of seedling length, root length, seedling top dry weight, leaves area per plant were recorded.

3- Biochemical changes in the treated seeds:

Biochemical changes in carob seeds at early stages of germination were determined. Oven dried samples at 70°C of soaked seeds representing each treatment, and dry seeds as a reference evidence explaining the attained changes which ground to a fine powder and kept in a dessicator to be used for determination of phosphorus and sugars.

3-1 Determination of phosphorus:

Extraction method of phosphorus contents by using 8% (w/v) TCA solution and inorganic and total phosphorus contents were spectrophotometrically determined using the method of Hasaneen (1981). Organic phosphorus content was calculated by subtracting the amount of inorganic phosphorus from the total phosphorus readings (Humphries, 1956).

3-2 Determination of sugars:

The method outlined by Younis (1963) and El-Saht (1980) was used for extraction of sugar contents. The direct reducing values (D.R.V.) was determined spectrophotometrically using the method of Naguib (1964). According to Dubois et al. (1956) total reducing value (T.R.V.) was determined spectrophotometrically. The estimation was expressed as mg glucose equivalent / g of seed dry weight. Sucrose was estimated by subtracting the D.R.V. value from the T.R.V. value.

The full data of the differently treated groups of seedlings were statistically analysed and comparison among means was carried out by calculating the L.S.D. at 5% (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Scarified carob seeds soaked either in water for 48 hrs at 35°C (T4), in Kinetin solutions at 15&30 ppm (T5 & T6) into GA3 at 400 ppm, (T10) or in thiourea 0.015%, (T7) resulted in the highest percentages of final seed germination, germination rate and also germination velocity than the control
values after 30 days from sowing date (F.G.P. were 85.5%, 82.15%, 81.0%, 75.2% and 74.4% respectively). Moreover, the applied treatments led to earlier germination of seeds and produced seedlings of stronger vegetative growth (Tables, 1&2).

The obtained results agreed with those obtained by Spyropoulos and Lambiris (1980) who mentioned that water stressed carob seeds by PEG 4000 were clearly retarded the germination, and this retardation was closely correlated to galactomannan depletion, where the latter being inhibited with lower external water potential. Also, endosperm α-galactosidase was inhibited. Frutos (1988) who scarified carob seeds with sulphuric acid at different concentrations for 30 minutes, obtained the best germination percentage 99.1% by using H₂SO₄ (80%). Also, Hussein et al. (1993), Russo and Uggentic (1993) and Martins-Loucoa et al. (1996) reported that the best treatments for scarification carob seeds were sulphuric acid (80%) for 20 minutes, and warm water (40°C) for 48 hrs. The starting process is commonly considered to be imbibitions of water into the seeds, (Koller et al., 1962, and Nagao et al., 1980) which facilitated by scarification of seed coat chemically or mechanically. In addition to quiescent seed requirements for germination in addition to the availability of water, favorable temperature and sufficient oxygen supply in the soil, which in the range from minimum to optimum will enhance the germination process (Koller, 1972, Mitrakos, 1981 and Obroucheva, 1999).

Also, Soaking scarified Carob seeds in different concentrations of GA₃ and Alar improved germination and stimulated seedling growth of carob and cotton seeds. (Khafagy and Moussa, 1981, Hussein et al., 1993). Furthermore, (Moustafa and Al-Zidgali, 1995) reported that preliminary soaking in water for 24 hr induced germination, and germination was more rapid by soaking the seeds of citrus rootstock in warm water at 57°C for 10 min before sowing. They added that warm water may activate imbibition and stimulate synthesis of new mRNA and de-novo enzymes which needed to break-down stored material in the seeds, and mobilize the building blocks of soluble sugars and amino acids to the suitable sites. Samaan et al. (2000d) indicated that apricot seeds pre-sowing treated with moist-chilling at 5±1°C for 15 and 30 days followed by soaking in tap water for 24 hr produced the highest percentage of germination and viability, as well as, germination velocity. In the case of carob seeds water-impermeable coats, also may be restrict the diffusion of gases, resulting in an O₂ supply insufficient for the high respiratory activity of the embryo.
**Table (1): Effect of different physiological treatments on final germination percentage, Germination rate index and germination velocity coefficient of carob seeds (Seasons 2000 & 2001).**

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<td>F.G.%</td>
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<td>38.8</td>
<td>6.73</td>
<td>7.64</td>
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<td>5.80</td>
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<td>8.85</td>
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<td>5.36</td>
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<td>12.75</td>
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<td>73.30</td>
<td>71.30</td>
<td>13.97</td>
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<td>79.00</td>
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<td>10.76</td>
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Cytokinins secretion by the embryonic axis and its controlling effects of events in the cotyledons seems to be a general phenomenon in dicotyledonous seeds. The work of Khan (1971) and Saaman et al. (2000b) showed clearly that kinetin at 5 & 10 ppm either alone or in combination with two–mosit–chilling periods (15 & 30 days) increased apricot seed germination and viability percentages, accelerated germination process and produced seedling of apricot accompanied with strong vegetative growth.

Thus, cytokinins may have a role similar to GA<sub>3</sub>. GA<sub>3</sub> affect metabolic events occurring in the embryo, and endosperm at two possible modes, one on α– amylase synthesis via an effect on DNA, second on the release of such enzyme into the endosperm , as increase the activity of phosphomonoesterase, ATPase, phytase and other enzymes. Subsequently it was shown that very rapid responses in release of soluble sugars and phosphatase activity could be included by GA<sub>3</sub> and that this effect could be mimicked by cyclic AMP (Mayer & Poljakoff-Mayber, 1975). Also, the level of many enzymes increases as a result of GA<sub>3</sub> treatment of seeds during germination. Among those involved may be mentioned enzymes of the glyoxylate cycle and those of pentose phosphate cycle ,as well as, leading to an increase in the energy charge (Mayer & Poljakoff-Mayber, 1980).
It clear from tables 1 and 2 that thiourea stimulates germination while its internal concentration is still comparatively low, whereas the subsequent inhibition of seedling growth occurs when the internal concentration has risen appreciably. Hu and Couvillon (1990) and Samaan et al. (1997) confirmed the use of thiourea in soaking seeds of peach and nectarine for overcoming dormancy and increasing seed germination, where seeds were germinated rapidly and uniformly. Also, Samaan et al. (2000,c) reported that the use of thiourea especially at lower concentration increased apricot seed germination and accelerated germination process. It is possible that thiourea exerts its effect by changing the level or activity of some growth promoters or inhibitors. Thiourea also may acts by affecting the respiratory mechanism, possibly by rapidly channeling all respiration in the direction of energy yielding processes (El-Saht, 1985).

**Biochemical changes during germination as affected by different treatments:**

The early phase of germination of carob seeds associated approximately within the first 6 days after sowing. During this phase and after 48 hrs. from sowing dates samples were taken for analyses of reducing sugars and sucrose in the treated seeds and also in the dry ones.

It is clear from table 3 that higher values of reducing sugars and non-reducing ones were observed in carob seeds after soaking for 48 hr depending on the treatment applied as compared with the dry seeds. These results are in harmony with those obtained by different workers, Spyropoulos and Lambiris (1980) who concluded that higher sugar content during the early stage of germination in the endosperm in carob seeds were mainly due to higher galactose and mannose contents. The degradation of storage materials in carob seeds often occur as a result of enzyme activation which convert the stored materials to suitable forms usable for respiration and for new syntheses. (Mayer and Poljakoff-Mayber, 1980). Also, Hussein et al. (1993) revealed that total soluble sugar content was higher; at the early stage of germination in the \( \text{GA}_3 \)-treated seeds as compared with the control. \( \text{GA}_3 \)-treated seeds were also the highest in their germination percentage and velocity. Their results, indicated also that before sowing, total soluble sugars increased significantly in seeds treated with either acetone or \( \text{H}_2\text{SO}_4 \) singly or in combination with distilled water, compared with untreated seeds which still low in sugar content.

In the present study, increase in sugar content of carob seeds treated with \( \text{GA}_3 \), kinetin or thiourea may result from the increased permeability of seed coat which allow to rise water content inside the seed and concomitant increase in hydrolytic enzyme activities which acts on the reserved poly-saccharides in the endosperm and embryo axis (see table 3). Samaan et al. (2000a) reported that \( \text{GA}_3 \) treatments of apricot seeds either alone or combined with moist-chilling presowing treatments caused an obvious increase in total reducing sugars compared to those of the control. Also, Samaan et al. (2000 b) stated that an increase in the values of total reducing sugars, direct reducing sugars was attained in apricot seeds treated with kinetin at different concentrations, along with thiourea and moist–chilling presowing treatments (Samaan et al., 2000 c).
The changes in phosphorus content of carob seeds treated with different pre-sowing treatments are presented in table 4. As compared to the initial value of dry seeds, pertinent contents of either total phosphorus, organic phosphorus or inorganic phosphorus were observed. Phosphorus play an extremely important role in a variety of reactions in germination process. (Mayer and Poljakoff-Mayber, 1975). They reported that the exogenous application of GA₃ markedly stimulate the rate of P₃² – total phosphate incorporated into RNA of embryonic axis. Samaan et al.(2000,a), mentioned that GA₃ application of apricot seeds caused a marked increase in total soluble phosphorus with special emphases to soluble organic phosphorus compared to those of the control. Also, Samaan et al. (2000 b), came to similar results by using kinetin and thiourea on apricot seeds.

On the basis of the forecited results, the improvement of germination process and subsequent seedling growth vigor can be attributed to the pre-treatment used advised; such scarification of carob seeds mechanically as a safety procedure, which facilitate the penetration of water into the dry seeds, that considered a limiting factor in carob seed germination. In addition, GA₃, Kinetin, thiourea, and warm water at 35°C, resulted in minimizing the costs of producing carob transplants, and enhancing their vegetative growth to obtain strong seedlings, which can be used as a rootstock for budding the superior cultivars on it (Samaan et al., 2000 a, b & c).
Table (4): Comparative determination of phosphorus content in scarified carob seed under different pre-sowing treatments, mg phosphorus per g dry seed dry weight. (Seasons 2000 & 2001).

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<td>12.45</td>
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<td>6.80</td>
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<td>L.S.D. 5%</td>
<td>0.96</td>
<td>0.96</td>
<td>-----</td>
<td>0.74</td>
<td>0.77</td>
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<td>0.96</td>
<td>1.12</td>
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إنّات بذور الخروب المخدوشة و نمو الشتلات الناتجة منها تحت تأثير معاملات ما قبل البذار

السيد البدوي طه الباز
قسم الفاكهة - كلية الزراعة جامعة المنصورة

اجري هذا البحث خلال موسمين متتاليين 2000/2001 ميلادية لمقارنة تأثير بذور نقع الخروب المخدوشة القشرة والمنقوعة بعدة طرق تشمل معاملات طبيعية مثل نقع البذرة في الماء على درجات حرارة 25° و 35° س لمدة 24 ساعة و48 ساعة، واستخدام محايلين من الكينتين بتركيز 10 و30 جزء في المليون، وثنائيروبية بتركيز 0.015% و0.030% وحمض الجيرليك بتركيز 200 جزء في المليون و400 جزء في المليون لمدة 24 ساعة.

وقد أظهرت النتائج المتحصل عليها زيادة معنوية في نسبة الإنباتات ومعدلها وسرعتها في غرب المعاملات مقارنة بالمعاملة (الكنترول) وقد أدت المعاملات إلى تحسين معدل النمو الخضري في البذور المختلطة في البدار الدائمة مماثلة في قياسات النمو الخضري المختلفة وقد كانت نتائج التحليل اليوبيكيميائي مقارنة بالبذور الجافة مكوفة ومقررة لارتفاع نسبة الإنباتات وتحسين سرعتها و ذلك باستخدام مقياس تحمل مخزون السكريات الثلاثية (سكروز) إلى سكريات أحادية مختلطة مخلطة علي التنشيط الحادث و كذلك زيادة محترق الفسفر الغير عضوي كدنيل علي التنشيط وتكوين ونقل الطاقة اللازمة للعملية الفسيولوجية المختلفة المصاحبة للإنباتات و يمكن الاستفادة من هذا البحث تطبيقياً بإتباع الوسائل الأمنة لخدش قصيرة البذرة ونقعها في الماء لمدة 48 ساعة على درجة 35°س.
Table (2): Comparative measurements of different characters associated with seedling growth vigor resulting from scarified carob seeds as affected by pre-sowing treatments (Seasons 2000 & 2001).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Seedling length (cm)</th>
<th>Root length (cm)</th>
<th>Seedling dry weight (g) / seedling</th>
<th>Leaves area (cm²) / seedling</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀</td>
<td>24.4</td>
<td>17.1</td>
<td>20.75</td>
<td>23.3</td>
</tr>
<tr>
<td>T₁</td>
<td>25.3</td>
<td>19.0</td>
<td>22.15</td>
<td>24.0</td>
</tr>
<tr>
<td>T₂</td>
<td>34.6</td>
<td>27.1</td>
<td>30.85</td>
<td>40.9</td>
</tr>
<tr>
<td>T₃</td>
<td>23.8</td>
<td>21.3</td>
<td>22.55</td>
<td>40.2</td>
</tr>
<tr>
<td>T₄</td>
<td>41.8</td>
<td>31.1</td>
<td>36.45</td>
<td>32.4</td>
</tr>
<tr>
<td>T₅</td>
<td>21.4</td>
<td>20.4</td>
<td>20.90</td>
<td>36.1</td>
</tr>
<tr>
<td>T₆</td>
<td>27.6</td>
<td>18.7</td>
<td>23.16</td>
<td>37.0</td>
</tr>
<tr>
<td>T₇</td>
<td>28.2</td>
<td>18.7</td>
<td>23.45</td>
<td>25.0</td>
</tr>
<tr>
<td>T₈</td>
<td>17.6</td>
<td>13.3</td>
<td>15.45</td>
<td>30.8</td>
</tr>
<tr>
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<td>25.3</td>
<td>22.1</td>
<td>23.70</td>
<td>42.3</td>
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<tr>
<td>T₁₀</td>
<td>26.33</td>
<td>22.9</td>
<td>24.61</td>
<td>36.50</td>
</tr>
</tbody>
</table>

L.S.D. 5% | 9.92  | 5.07  | ----- | 13.85 | 17.54 | ----- | 0.80  | 1.21  | ----- | 132.7 | 96.44 | ----- |

T₀ = Scarified seeds only before sowing (control).
T₁ = Soaking scarified seeds into water at 25°C for 24 hr.
T₂ = Soaking scarified seeds into water at 35°C for 24 hr.
T₃ = Soaking scarified seeds into water at 25°C for 48 hr.
T₄ = Soaking scarified seeds into Kinetin solution 15 ppm for 24 hr.
T₅ = Soaking scarified seeds into Kinetin solution 30 ppm for 24 hr.
T₆ = Soaking scarified seeds into Thiourea solution 0.015% for 24 hr.
T₇ = Soaking scarified seeds into Thiourea solution 0.03% for 24 hr.
T₈ = Soaking scarified seeds into GA₃ solution 200 ppm for 24 hr.
T₉ = Soaking scarified seeds into GA₃ solution 400 ppm for 24 hr.