

Effect of Pre-Sowing Seeds Soak in Different GA₃ and ZnSO₄ Solutions on Germination and Growth of Cleopatra Mandarin and Rangpur Lime Rootstocks

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ABSTRACT

The present study was conducted during two consecutive 2014 & 2015 experimental seasons on two citrus rootstocks namely: Cleopatra mandarin (*Citrus reshni* L.) and Rangpur lime (*Citrus limonia* L.), to investigate the effect of treating their seeds with some pre-sowing soak solutions. It was aimed to shorten the duration needed for producing standard transplants through accelerating / hastening & raising germination process, as well as seedlings vigour (plant height) and survival percentage of transplanted seedlings, which certainly would be reflected positively in improving their suitability for carrying out budding / grafting. Hence seeds of each citrus rootstock were subjected to soaking for 24 hours in one of either GA₃ or ZnSO₄ solutions (each at 500, 1000, 1500 and 2000 ppm), beside tap water as control for investigating their effect on two germination parameters (total number of germinated seeds & germination percentages) after 6 weeks from sowing (planting) and two growth measurements (average plant height just before transplanting and survival % of translocated seedlings after two weeks). Data obtained during both experimental seasons revealed obviously that all pre-sowing soak treatments increased significantly both germination measurements (total germinated seeds & germination %) and two parameters of induced seedlings (average plant height & survival % of translocated seedlings) as compared to control (soak for 24 hours in tap water) with both citrus rootstocks. However, soaking in the highest concentrated solutions (2000 ppm) of either GA₃ or ZnSO₄ particularly later one was/were statistically the most effective in this concern. In spite of pre-sowing soak of two citrus rootstocks seeds in GA₃ & ZnSO₄ solutions at the least concentration (500 ppm) were statistically the inferior from one hand, but both resulted in a significance increase over control with four investigated measurements. In addition, other GA₃ & ZnSO₄ solutions came significantly in between the aforesaid two extremes with a noticeable tendency showed a relative more efficiency always coupled with the higher concentration of a given chemical solution even within such intermediate category.

Keywords: Cleopatra mandarin, Rangpur lime, GA₃, rootstocks, seeds and ZnSO₄ solutions.

Introduction

Citrus is considered to be one of the world's most common popular and favorite fruits. In Egypt total cultivated citrus area reached 420333.6 Feddans (more than 39% from total fruit area). Citrus production in Egypt increased to 3980151 tonnes in 2012 year, according to FAO statistics, 2015). Thus, Egypt is considered to be one of the ten largest producers of citrus in the world. There by, strenuous efforts have always been exerted to increase the production of citrus through a better understanding of its reaction to environment, mineral nutrition and using the suitable rootstocks. Because of the great role rootstocks could be played not only in absorbance different nutrient elements and water from soil but also its capability to tolerate the unsuitable surrounded environmental conditions like as cold, salinity drought and different spreading diseases (quic decline / tristeza and phytophthora foot rotetc.).

In this respect two citrus rootstocks under study characterized by the following:

1- Rangpur lime is an acid mandarin type, similar in many respects to rough lemon. It induces large vigor trees with higher yields, large fruits size and juice quality slightly better than that on rough lemon. It is also highly tolerant to tristeza and salinity but it is susceptible to phytophthora foot rot .

2- Cleopatra mandarin its performance as rootstock has been established over many years. Tree vigour on Cleopatra mandarin rootstock is very good, fruit quality of the commercial sweet orange and mandarin cultivars as scions on Cleopatra rootstock is equal to those produced on sour orange rootstock, but fruit size is smaller. Cleopatra induces maximum cold hardiness in the scion and trees on this stock are unaffected by tristeza. It is tolerant to phytophthora foot rot and calcareous soil.

Seed germination is a critical step in the nursery procedures. Citrus seeds of all rootstock types characteristically germinate unevenly, resulting in poor seedling uniformity (Andrew and Keith, 1988 and Aubert and Vullin, 1998). Thus, it is highly interested by nursery owners to get higher and earlier germination

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percent associated with rapid growth of the obtained seedlings to fulfill maximal benefit that will be reflected on their profit. Thus, such task was addressed by implementing pre – sowing seed treatment soaks .

Citrus trees as most fruit trees, are propagated by budding rather than grafting on seedling rootstocks. Choosing a suitable rootstock for commercial use is a very important and difficult decision because rootstocks greatly vary in their adaptability to environmental conditions, i.e. soil type, irrigation source, climateetc., their tolerance to various pests and diseases, and markedly affect scion performance (Castle, 1982).

Materials and Methods

The present study was conducted during two consecutive experimental seasons (2014 & 2015 years) to investigate the effect of some pre-sowing soak treatments of two citrus rootstocks, namely Cleopatra mandarin (*Citrus reshni* L.) and Rangpur lime (*Citrus limonia* L.). This study aimed to shorten the duration needed for producing standard transplants and improving their suitability for budding / grafting through stimulating / activating seeds germination and accelerating seedlings growth development for both citrus rootstocks hoped to be realized by investigated treatments in 1st & 2nd experiments, respectively. Both experiments were carried out in the green house of pomology belong Horticulture Department to the Experiment Station of Faculty of Agriculture, Benha University, at Moshtohor, Toukh region, Kalubia Governorate, Egypt.

Effect of pre-sowing seeds soak in different GA₃ and ZnSO₄ solutions on germination and growth of Cleopatra mandarin and Rangpur lime rootstocks:

Required seeds for both citrus rootstocks were obtained by extracting from healthy mature fruits picked on mid February 2014 & 2015 years from adult tree grown in the nursery of Faculty of Agriculture, Benha University, at Moshtohor, Toukh region, Kalubia Governorate during 1st & 2nd seasons, respectively.

Extracted seeds were washed and air dried. One day, just before subjecting seeds to the differential pre-sowing treatment, they were soaked for 24 hours in Carpdazim fungicide solution at 50% concentration. Hence seeds of each citrus rootstock were subjected to soaking for 24 hours in one of either GA₃ or ZnSO₄ solutions (each at 500, 1000, 1500 and 2000 ppm), besides tap water as control as follows:

- 1- Soaking for 24 hours in tap water (Control).
- 2- Soaking for 24 hours in 500 ppm GA₃ solution.
- 3- Soaking for 24 hours in 1000 ppm GA₃ solution.
- 4- Soaking for 24 hours in 1500 ppm GA₃ solution.
- 5- Soaking for 24 hours in 2000 ppm GA₃ solution.
- 6- Soaking for 24 hours in 500 ppm ZnSO₄ solution.
- 7- Soaking for 24 hours in 1000 ppm ZnSO₄ solution.
- 8- Soaking for 24 hours in 1500 ppm ZnSO₄ solution.
- 9- Soaking for 24 hours in 2000 ppm ZnSO₄ solution.

Experiment layout:

At late February 2014 and 2015 years seeds of each citrus rootstock were separately subjected to the abovementioned nine pre-sowing treatments just before planting in germination boxes during 1st & 2nd experimental seasons, respectively. Herein, the complete randomized block design with five replications was employed for arranging the investigated nine pre-sowing soak treatments. Whereas each replicate was represented by 150 seeds, planted in an individual germination box previously filled with planting medium consisting of sand + clay mixture at equal proportion (v:v).

The effect of the differential investigated pre-sowing soak treatments were evaluated through the response of the following germination and vegetative growth measurements:

1- Seeds germination:

During each experimental season two seeds germination measurements i.e., number & percentage of germinated seeds per every replicate (an individual germination box) were periodically counted at one-week interval starting from such week during which the earliest emergence of germinated seedlings took place and continued until an or some investigated pre-sowing soak treatment/s exhibited approximately 60.0% germination percentage or more, regardless of such treatment/s. Then an average value of either number or percentage of germinated seeds per each investigated pre-sowing soak treatment was estimated as an average of its five replicates during every experimental season.

2- Average plant height (cm.):

On July 16th and 18th during 2014 & 2015 years' respectively the average plant (seedling) height in cm. was counted for ten seedlings per each replicate (an individual germination box), then an average of plant height per every investigated pre-sowing soak treatment was estimated (as an average of its five replicates).

3- Survival percentage of transplanted seedlings:

After the average plant height of developed seedlings by every pre-sowing soak treatment had been done i.e. on July 16th & 18th of 2014 & 2015 years during 1st & 2nd experimental seasons, respectively, forty seedlings from each pre-sowing treatment were carefully selected and transplanted individually in polyethylene bags previously filled with specific weight (5Kg/each) of growing medium consisting of (sandy & clay at 1:1 by volume) whereas each replicate was represented by eight transplants. Two weeks later survival % of transplanted seedlings for every citrus rootstock as influenced by the different pre-sowing soak treatments was Periodically estimated for every treatment as an average of its own 5 replicates.

Statistical analysis:

All data obtained during both experimental seasons were subjected to analysis of variance and significant difference among means were determined according to Snedecor and Coshran (1982). In addition significant differences among means of various treatments were compared using the established new L.S.D- values at 5% level according to Waller and Duncan (1969).

Results and Discussion

In this regard some measurements of either seeds germination (total number of germinated seedlings & germination %) or seedlings growth (seedling height & survival % of translocated seedlings) of two citrus rootstocks seeds (Cleopatra mandarin & Rangpur lime) in response to pre-sowing soak in some GA₃ and ZnSO₄ solutions were investigated during 2014 & 2015 seasons. Data obtained during both 2014 & 2015 experimental seasons for Cleopatra mandarin & Rangpur lime rootstocks are presented in Tables (1) & (2), respectively.

Germination measurements:

Total number of germinated seedlings:

Table (1) and (2) display obviously that soaking seeds of two citrus rootstocks for 24 hours in different GA₃ and ZnSO₄ solutions just before sowing increased significantly the total number of germinated seeds during both experimental seasons. However pre-sowing soak in the highest GA₃ & ZnSO₄ concentration surpassed significantly other investigated concentrations, regardless of citrus rootstock and investigated chemical substrate. Such trend was true during both seasons in spite of the highest ZnSO₄ concentration (2000 ppm) tended relatively to be more effective than the analogous one of GA₃ particularly with Rangpur lime during 2nd season, whereas difference was significant. On the other side, the least concentration of both GA₃ & ZnSO₄ solutions (at 500 ppm) showed significantly the lightest increase over control with seeds of two citrus rootstocks during two experimental seasons. In addition, other pre-sowing soak solutions (1000 & 1500 ppm) of either GA₃ or ZnSO₄ were in between the aforesaid two extremes. Taking in to consideration that differences between four pre-sowing soak treatments (GA₃ and ZnSO₄ at either 1000 or 1500 ppm) were so slight to reach level of significance with both citrus rootstocks during two seasons. These results are in harmony with those obtained by Misra *et al.*, (1982), Brown *et al.*, (1983), Yousif *et al.*, (1989), Singh *et al.*, (1989), Gupta (1992), Agha *et al.*, (1990), Moustafa and AI - Zidgali (1994) and El - Baowab (2002).

Germination percentage:

Concerning the germination percentage of two citrus rootstock seeds after six weeks from planting as influenced by their soaking for 24 hours in different GA₃ & ZnSO₄ solutions just before sowing, data obtained during both seasons are presented in Tables (1) and (2) for Cleopatra mandarin and Rangpur lime rootstocks, respectively. It is quite evident that the response followed typically the some trend previously discussed with total number of germinated seeds. Herein all GA₃ & ZnSO₄ solutions resulted in a significant increase over the tap water soaked seeds (control) during both seasons for both citrus rootstocks. The highest GA₃ & ZnSO₄ concentrated solution (2000 ppm) were statistically the superior while their lowest concentration (500 ppm) was the inferior. In addition other GA₃ & ZnSO₄ solutions were in between the aforesaid two extremes. These results

are confirmed by those obtained by Singh *et al.*, (1979), Abou-Rawash *et al.*, (1980), El- Said (1980), Choudhari and Chakrawar (1981) and (1982), Moustafa and AI - Zidgali (1994), Babaeva *et al.*, (1999), Dunn, (2002) and Broadley *et al.*, (2007).

Table 1: Effect of pre-sowing seeds soak in different GA₃ and ZnSO₄ solutions on total number & percentages of germinated seedlings after 6 weeks, average seedling height and survival percentage of translocated Cleopatra mandarin seedlings during 2014 and 2015 seasons.

Presowing treatments (soaking for 24 hours in)	First season 2014				Second season 2015			
	Total. No. of seedlings	Germination (%)	Seedling height (cm)	Survival (%)	Total. No. of seedlings	Germination (%)	Seedling height (cm)	Survival (%)
T ₁ - Tap water (Control)	41.4 e	27.6 e	7.20 d	71.52 f	42.60 e	28.40 e	7.40 d	70.86 f
T ₂ - GA ₃ at 500 ppm	72 d	48 d	9.94 bc	76.16 e	76.20 d	50.80 d	9.80 c	74.90 e
T ₃ - GA ₃ at 1000 ppm	84 bc	56 bc	10.40 bc	75.38 e	84.60 cd	56.40 cd	9.60 c	75.86 e
T ₄ - GA ₃ at 1500 ppm	86.4 bc	57.6 bc	11.00 bc	80.52 d	85.20 cd	56.80 cd	11.00 bc	80.68 d
T ₅ - GA ₃ at 2000 ppm	100.8 a	67.2 a	12.60 a	87.72 b	100.8 ab	67.20 ab	12.20 ab	86.92 b
T ₆ - ZnSO ₄ at 500 ppm	79.2 cd	52.8 cd	9.66 c	73.00 f	76.20 d	50.80 d	9.94 c	70.32 f
T ₇ - ZnSO ₄ at 1000 ppm	90.6 b	60.4 b	10.40 bc	80.88 d	93.00 abc	62.00 abc	9.80 c	80.66 d
T ₈ - ZnSO ₄ at 1500 ppm	88.2 bc	58.8 bc	11.40 ab	83.88 c	91.20 bc	60.80 bc	10.60 c	83.50 c
T ₉ - ZnSO ₄ at 2000 ppm	101.4 a	67.6 a	12.80 a	95.06 a	102.6 a	68.40 a	13.00 a	95.04 a

Means followed by the same letter / s within each column during every season are not significantly different at 5% level

Table 2: Effect of pre-sowing seeds soak in different GA₃ and ZnSO₄ solutions on total number & percentages of germinated seedlings after 6 weeks, average seedling height and survival percentage of translocated Rangpur lime seedlings during 2014 and 2015 seasons.

Presowing treatments (soaking for 24 hours in)	First season 2014				Second season 2015			
	Total. No. of seedlings	Germination (%)	Seedling height (cm)	Survival (%)	Total. No. of seedlings	Germination (%)	Seedling height (cm)	Survival (%)
T ₁ - Tap water (Control)	51.00 d	34.00 d	8.28 f	72.90 e	51.60 e	34.40 e	7.40 d	72.76 e
T ₂ - GA ₃ at 500 ppm	78.00 c	52.00 c	10.54 d	76.18 de	85.20 d	56.80 d	10.60 bc	76.82 de
T ₃ - GA ₃ at 1000 ppm	95.40 b	63.60 b	11.20 cd	78.08 d	91.20 cd	60.80 cd	10.80 bc	78.86 d
T ₄ - GA ₃ at 1500 ppm	95.40 b	63.60 b	12.00 bc	82.06 cd	90.60 cd	60.40 cd	11.40 b	81.68 cd
T ₅ - GA ₃ at 2000 ppm	114.0 a	76.00 a	13.20 ab	89.64 b	106.20 b	70.80 b	13.40 a	91.04 b
T ₆ - ZnSO ₄ at 500 ppm	92.40 b	61.60 b	10.46 d	77.28 d	85.80 d	57.20 d	9.94 c	78.00 de
T ₇ - ZnSO ₄ at 1000 ppm	99.60 b	66.40 b	11.00 cd	78.94 d	97.20 c	64.80 c	10.60 bc	79.32 d
T ₈ - ZnSO ₄ at 1500 ppm	99.00 b	66.00 b	12.40 abc	85.36 bc	97.20 c	64.80 c	11.40 b	86.28 bc
T ₉ - ZnSO ₄ at 2000 ppm	119.40 a	79.60 a	13.80 a	96.70 a	118.80 a	79.20 a	13.20 a	97.00 a

Means followed by the same letter / s within each column during every season are not significantly different at 5% level

Some seedlings growth parameters:

In this respect average plant height and survival percentage after two weeks from their translocation were two growth measurements investigated in response to the differential pre-sowing seeds soak treatments GA₃ & ZnSO₄ solutions each at 500, 1000, 1500, and 2000 ppm.

Average plant height:

Tables (1) and (2), reveal obviously that two citrus rootstocks followed approximately the same trend in their response to the differential investigated pre-sowing soak treatments during both seasons of study. Anyhow,

all GA₃ & ZnSO₄ pre-sowing soak solutions increased significantly the average seedling height over the analogous ones of tap water soaked seeds (control). The tallest seedling of both citrus rootstocks were significantly always in concomitant to such seedlings of the pre-sowing seeds soaked in 2000 ppm solution of either GA₃ or ZnSO₄ during 2014 & 2015 experimental seasons. Moreover produced seedlings by pre-sowing soak in 1500 ppm GA₃ or ZnSO₄ ranked statistically second, descendingly followed by those of GA₃ / ZnSO₄ soaked seeds at 1000 and 500 ppm concentrations. However differences between three 1500, 1000 and 500 ppm GA₃ and ZnSO₄ solutions in most cases didn't reach level of significance as compared each other's, regardless of citrus rootstock during both seasons. Such trend was true during two seasons with very few exceptions particularly with comparing pre-sowing seeds soak of both citrus rootstocks in 1500 ppm ZnSO₄ to the analogous ones of soaking in 500 ppm ZnSO₄ during, 1st season only. The present results go general in line with those previously found by Suzuki and Konakahara (1985), Salem *et al.*, (1989), Gupta (1992), Babaeva *et al.*, (1999), Dunn, (2002) and Broadley *et al.*, (2007).

Survival percentage of translocated seedlings:

In this respect survival percentage of translocated seedlings after two weeks as influenced by the differential pre-sowing seeds soak treatments was concerned and data obtained during both seasons for Cleopatra mandarin and Rangpur lime are presented in Tables (1) and (2), respectively. It is quite clear to be noticed that the highest ZnSO₄ concentration resulted significantly in the highest survival % during both seasons i.e., (95.06 & 95.04 %) and (96.70 & 97.00 %) for Cleopatra mandarin and Rangpur lime rootstocks, respectively. Moreover pre-sowing soak in 2000 ppm GA₃ solution came statistically 2nd for both citrus rootstocks during two seasons of study. The superiority of the highest concentration (2000 ppm) of ZnSO₄ over the analogous one of GA₃ was also reflected on the following two lower concentrations (1500 & 1000 ppm) of both GA₃ & ZnSO₄ pertaining their influence on survival % of translocated seedlings induced after pre-sowing soak in the investigated GA₃ & ZnSO₄ solutions. Anyhow, pre-sowing soak in 1500 ppm ZnSO₄ solution ranked statistically 3rd, descendingly followed by soaking in 1500 ppm GA₃ & 1000 ppm ZnSO₄ treatments as both came 4th during two seasons for Cleopatra mandarin. However with Rangpur lime the efficiency of ZnSO₄ than GA₃ in increasing survival % could be clearly noticed even with 2000 ppm GA₃ solution which showed statistically the same value of survival % exhibited by pre-sowing soak in 1500 ppm ZnSO₄ solution. The least significant increase in survival % of translocated seedlings over control (pre-sowing soak in tap water) was generally coupled with pre-sowing soak seeds in 1000 ppm GA₃ with both rootstocks. However, pre-sowing soak seeds in the lightest ZnSO₄ solution (500 ppm) was the unique exception which didn't significantly differ than control (tap water) with both citrus rootstocks.

The shift in trend of response for the survival % of translocated seedlings may be attributed to the variance in the biological and physiological roles could be played by either GA₃ or Zn pertaining shoot growth and roots development. These results are confirmed by those obtained by Makarem (1978), Hassaballa and Mougheith (1979), Gravina and Vidal (1989), Mehanna and Samira (1989), Gupta (1992), Leonel and Rodrigues (1999), Bayerly (2000), El - Baowab (2002) and Singh *et al.*, (2003).

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