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The effects of treating persimmon (Diospyros lotus) seeds with moist-chilling and growth regulators on seeds germination, the subsequent seedling characters and their induced drought tolerance.

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Abstract: *The present experiment aimed at investigating the effects of seed pre-treatment with moist-chilling (mch), GA_3, BA or their combinations on seed germination and subsequent drought resistance of the resulting seedlings. The obtained results indicated that increasing the moist-chilling period from 4 to 8 weeks progressively significantly increased the seeds germination percentages (average from 51.2% to 97.2%) and significantly decreased the time to 50% germination (T50) (average from 47days to 27.3 days). The control one (non-moist-chilled seeds) gave the lowest germination percentage (22.6%) and required the highest T50 (72.6 days). The 4 weeks–chilled seeds soaked in BA gave the most satisfactory results as it yielded high germination percentage (average 85.2%), low T50 (average 31.2 days) and saved about at least 4 weeks which would have otherwise being required for moist-chilling of the seeds. The best vegetative characteristics of 2.5-month-old persimmon seedlings were obtained from seeds treated with 8week-moist-chilling solely or with 4week- moist chilling followed by soaking in 20 ppm BA solution for 16 hr. After exposure the subsequent seedlings to 24 days drought period, the results revealed that increasing the moist-chilling period decreased proline content of the seedlings leaves and roots, while increased their leaf resistance to water vapour (LRWV) (9.9, 11.45 & 19.65 sec/cm for 0, 4, 8 weeks of mch, respectively). Generally, seeds pretreated with BA solely gave seedlings with significantly higher root proline content than those of the other treatments, and hence showing more drought resistance features. After the 24 days of drought, leaves of the seedlings resulting from either 4 or 8-weeks-chilled seeds had significantly higher values of chlorophyll (chl a, b) and total soluble carbohydrates (TSC) than those of their corresponding ones resulting from non-chilled seeds.*
In view of the above findings, the best combination that gave the most satisfactory results was soaking in BA of the seeds which were mois-chilled for 4 weeks.

Keywords**:** *persimmon, seed germination, seedling characteristics, drought tolerance, moist- chilling; Gibberellins, Benzylaminopurine.*

I. Introduction

Persimmon is a deciduous fruit tree adapted to warm temperate and sub-tropical climates Mowat et al. [1]. It has a primary centre in the mountains of central China and a secondary centre in Japan Zeven and Zhukovsky [2]. Recent expansion of persimmon cultivation is mostly in temperate and sub-tropical regions outside the major production areas. The major producers are China, Japan, Brazil, Korea and Italy. Minor producers include Israel, U. S. A., New Zealand, Australia, Spain, Georgia (C. I. S.), Egypt, India and Chile.

Many fruit producing areas are currently threatened by severe drought. This situation will have potent effects on plants such as persimmon trees affected, and will need to be managed by producers to minimize adverse effects. Water is the most powerful regulator of plant growth and development. To understand the physiological and molecular bases of plant responses to mild to moderate water deficits is therefore of utmost importance to modulate the appropriate balance between vegetative and reproductive development, to improve crop water use Blum [3] and to control fruit quality under deficit irrigation Chaves et al. [4].

With more water shortages and drought periods ahead, planting trees and other plants that are drought resistant can be beneficial. Drought resistance requires tree leaves use water efficiently, and continue to grow and make food at relatively low water concentrations. Drought resistance involves characteristics like extensive root systems, thick leaf waxes and bark, good stomata control, and the capacity for leaf cells to function at low water contents. Water stress markedly reduces the amounts of auxins, gibberellins and cytokinins, while it reversibly raises the amounts of ABA Abdalla and El-Khoshiban [5]. Generally, the environmental stresses especially drought stress, give rise to accumulation of soluble carbohydrates, proline and free amino acids as well as antioxidants compounds. These solutes are low molecular weight, highly soluble compounds that are non toxic at high cellular

concentration and protect cellular components from dehydration injury, thus are referred to as osmoprotectants and compatible solutes Reddy et al. [6] and Shao et al. [7].

Various dormancy breaking and germination stimulating treatments have been tried with seeds of many fruit species such as apricot El-Khoreiby & Salem [8], papaya Nagao and Furutani [9], persimmon Taha [10], peach El-Dengawy [11] and loquat Polat [12] and El-Dengawy [13]. In this respect, gibberellic acid and moist-chilling treatments seem the most promising in many woody species Powell [14].

Persimmon seed coat can affect seed germination through inhibiting and mechanically restricting the embryo growth Taha [10], Hayden [15] and Fadhil et al. [16]. Increase cold stratification period up to 10 weeks overcome seed dormancy and promote seed germination of Diospyros lotus Oh et al. [17]. It is known that the persimmon seeds are hard to germinate and needs additional treatments to break dormancy and such germinated seeds are generally used as a rootstock for grafting and producing new trees required for new plantations. Persimmon is also hard to propagate by cuttings or tissue culture. Thus producing rootstocks form seeds for grafting is critically required.

Most research approaches of the subject of dormancy have concentrated on finding ways to dormancy breakage. However, the effects of breaking seed dormancy by moist chilling and growth regulators of Persimmon seeds and their effects on the seedling vegetative characteristics under drought stress has been neglected. Therefore, in this experiment we have primarily aimed at (a) finding out a practical method to promote kaki seed germination and the subsequent seedling growth by means of moist-chilling, application of GA3, BA, or the combination of the two hormones, and (b) to investigate the effect of such treatments on drought tolerance of the subsequent seedlings.

II. Materials And Methods

The present research was carried out on seeds of "Tarabols" Persimmon (Diospyros lotus) during two successive seasons, 2009 and 2010. This study was conducted in the greenhouse (28±2^{O}C and 22±2^{O}C at daylight and night, respectively) and laboratories in Faculty of Science, King Khalid University, Abha, Saudi Arabia. The tested seeds were obtained by extraction from mature fruits which were picked from one tree of 14 years old in a private orchard at Tawfikia village, Damietta Governorate. The extracted seeds were immediately washed with tap water, air dried, divided to 12 groups. Each group was divided into 3 replicates (21 seeds for each) and subjected to one of the following treatments: Soaking into tap water only for16 hr (control, T1); Soaking into a gibberellic acid (GA_3) solution at 200 ppm for16 hr (T2); Soaking into a Benzylaminopurine (BA) solution at 20 ppm for16 hr (T3); Soaking into a mixed solution of 200 ppm GA_3 and 20 ppm BA for16 hr (T4); Four weeks moist-chilling at 5 ± 1^{o}C (T5); Four weeks moist-chilling at 5 ± 1^{o}C followed by soaking into 200 ppm GA_3 solution for 16 hr (T6); Four weeks moist-chilling at 5 ± 1^{o}C followed by soaking into 20 ppm BA solution for 16 hr (T7); Four weeks moist-chilling at 5 ± 1^{o}C followed by soaking into a mixed solution of 200 ppm GA_3 and 20 ppm BA for16 hr (T8); Eight weeks moist-chilling at 5 ± 1^{o}C (T9); Eight weeks moist-chilling at 5 ± 1^{o}C followed by soaking into 200 ppm GA_3 solution for 16 hr (T10); Eight weeks moist-chilling at 5 ± 1^{o}C followed by soaking into 20 ppm BA solution for 16 hr (T11); Eight weeks moist-chilling at 5 ± 1^{o}C followed by soaking into a mixed solution of 200 ppm GA_3 and 20 ppm BA for16 hr (T12).

The treated seeds were sown in first Marsh using nine perforated black polyethylene bags for each treatment (about 4kg weight) contained a medium of peat-moss : sand : clay (1 : 2 : 1 v/v). After sowing the bags were watered regularly and shaded under greenhouse. At 50 days after sowing the subsequent seedlings irrigated with 250 ml tap water for each bag then subjected to 24 days drought period. The following subjects were studied.

2.1. Seed germination behaviour

The germination percentage was calculated starting from 20 days after sowing and so at 10 days-intervals up to 50 days. Time (in days) to obtain 50% germination referred to as T50 Heydecker and Wainwright [18] was also calculated using the following formula:

$$T50 = [(t_2 - t_1) \times 50\% + (p_2 t_1 - p_1 t_2)]/ (p_2 - p_1).$$

Where, t_1 = time at which the germination percentage is less than 50%, t_2 = time at which the germination percentage is more than 50%, and p_1 and p_2 are the measurements of germination percentage occurring at t_1 and t_2, respectively.

2.2. Seedling characteristics

Nine seedlings of 2.5-months old randomly collected from each treatment, after 24 days drought period (3seedlings/replicate) were used for seedling characteristics measurements. These measurements included seedling height (cm), leaf area (cm^2), root length (cm), secondary roots number and dry weight of seedling.

2.3. Drought resistance measurements on the subsequent seedlings

2.3.1. Leaf resistance for water vapor (LRWV) measurement

After 24 days drought period on the subsequent seedlings, the measurements of LRWV were determined from readings (sec cm^{-1}) of a Delta-T, AP4 porometer, 128 Low Road Burwell, Cambridge CB5 0EJ, UK, on the median portion of the youngest fully expanded leaf avoiding the mid rib.

2.3.2. Biochemical measurements

The following biochemical measurements were determined on 2.5-months old seedlings resulted from the tested different germination treatments and subjected to 24 days of drought period.

2.3.2a. Proline determination

Proline concentration in leaves of droughted seedlings was determined following the method of Bates et al. [19]. Leaf samples were harvested at the end of the experiment. A 0.3 g of fresh weight was mixed with 9.0 ml aliquot of 3% (W/V) sulfosalicylic acid in glass tubes covered at the top and boiled in a water bath at 100°C. The mixture was centrifuged at 2000 g for 5min at 25°C. A 200 µl aliquot of the extract was mixed with 800 µl distilled water and 14 ml of the reagent mixture (30ml glacial acetic acid, 20 ml distilled water and 0.5 g of ninhydrin), and boiled at 100°C for 1h. After cooling the mixture, we added 6.0 ml of toluene. The chromophore containing toluene was separated and absorption at 520 nm was read, using toluene as a blank. Proline concentration was calculated using L-proline for the standard curve.

2.3.2b. Total Soluble carbohydrates (TSC) determination

The TSC was extracted from leaves of droughted seedlings according to Kerepesi et al. [20]. Weight of 0.1g of leaf fine dry powder was boiled in 10 ml distilled deionized water under shaking for 45 min and then filtered through qualitative filter paper. An aliquot of this filtrate was used for TSC determination according to Dubois, et al. [21] using D (+)-glucose as standard.

2.3.2c. Chlorophylls determination

Four leaf discs (0.25 cm^2 each) were sampled from the leaves of droughted seedlings avoiding major veins. Chlorophyll was eluted from the discs by submerging them in 2 ml of N,N-dimethylformamide in the dark for at least 72 h. The amount of absorbance was read at 647 nm and 664 nm with UV-vis spectrophotometer (Model UV1601PC, Shimadzu) and used to calculate leaf a and b chlorophyll concentrations according to equations of Moran [22].

2.4. Statistical analysis

The obtained data were statistically analyzed as a factorial experimental design (SAS, [23]) applying the least significant difference (LSD) at 5% for the comparison among the treatment means. Duncan's new multiple range tests and regression analysis was also used.

III. Results

3.1. Seed germination behaviour

For both seasons, the non-moist-chilled seeds treated with BA gave significantly higher germination percentages (table 1) and significantly lower number of days to 50% germination (T50) compared with GA_3 and the control (no growth regulators) treatments. Thus, the germination percentages values for these three treatments were 34.5, 20.3 and 22.6% respectively, while their recorded averages of T50 value were 65.3, 73 and 72.5 days respectively.

Increasing the moist-chilling period significantly increased the germination percentages while significantly decreased the T50. In recognition to this, the germination % values were 22.6, 51.2 and 97.2% and the T50 averages were 72.5, 47 and 27.3 days for 0, 4 and 8 weeks moist-chilling periods respectively.

Application of BA after 4 weeks moist-chilling period gave significantly higher germination percentage and significantly lower T50 compared with solely 4 weeks moist-chilling and other applications of GA_3 or GA_3 and BA combination after the same moist-chilling period.

The highest germination percentages as well as the lowest number of days to 50% germination (T50) were on both seasons associated with the solely 8 week moist-chilling treatment (T9). Additional treatment of the 4 weeks–chilled kaki seeds with BA gave the most satisfactory results. Thus, it yielded high germination percentage, gave comparable T50 data as that of T9 and saved about at least 4 weeks which would have otherwise being required for moist-chilling of the seeds.

3.2. Seedling characteristics

The data of the response of vegetative characteristics namely, seedling height (cm), leaf area (cm^2), root length (cm), number of secondary roots and dry weight (g), in 2.5-month-old kaki seedlings are shown in Table 2. The values for all these parameters were significantly higher under all treatments compared with their control ones.

The leaf area (cm^2), root length (cm), number of secondary roots and the dry weight of the seedlings resulting from seeds pretreated with BA (T3) alone were significantly higher than their corresponding ones resulting from seeds treated with GA_3 (T2) solely. All measured vegetative characteristics of the resulting seedlings were significantly increased by augmentation of the moist-chilling period.

The effects of the interaction between growth regulators (GR) and the moist-chilling treatments of the seeds on the vegetative characters of the resulting seedlings differed depending on the type of GR used and the moist-chilling duration. Whearas, the 4week-moist-chilling then BA treated seeds produced seedlings had significantly higher vegetative characters than their corresponding ones receiving the same treatment, but after 8 week-moist-chilling period. However, these findings were contrary to those found under GA_3 treatment.

The best vegetative characteristics obtained were those of the 8week- moist-chill solely (T9) followed by application of BA for the seeds pretreated with 4week- moist chilling (T7).

3.3. Drought resistance measurements on the subsequent seedlings

3.3.1. Proline content and Leaf resistance for water vapor (LRWV)

The proline content of the leaves and roots decreased but the leaf diffusive resistance to water vapour increased with increasing the moist-chilling period, especially after 24 days drought period (Table 3). The resulting seedlings from seeds pretreated with GA_3 alone yielded significantly lower leaf and root proline contents compared with the control (no moist-chilling, no growth regulator).

Generally, seeds pretreated with BA solely gave seedlings with significantly higher root proline content than those of the other treatments, and hence showing more drought resistance features. The seedlings emerging from non-moist-chilled seeds treated with GA_3 and BA in combination (T4), showed more drought tolerance features as compared with the seedlings resulting from the corresponding seeds pretreated with GA_3 solely (T2). Thus, the seedling leaf proline content of the former decreased while that of the root increased compared with those of the latter (GA_3 solely).

The same previously mentioned findings regarding the no moist-chilling seeds treated with combination of GA_3 and BA was recorded for the combination of these two GRs when the 4 and 8week moist chilling conditions were considered.

3.3.2. Contents of pigments and total soluble carbohydrates

After exposure to 24 days of drought, the leaves of the seedlings resulting from either 4 or 8-weeks-chilled seeds had significantly higher chlorophyll a and total soluble carbohydrates contents than those of the corresponding ones resulting from non-chilled seeds (Table 4). Shifting the seed chilling period from 4 to 8 weeks resulted in increasing the total soluble carbohydrate content of the seedlings exposed to 24 days of drought.

Treatment of the non-chilled seeds with either GA_3, BA or their combination, resulted in non significant increase of the total soluble carbohydrates content of the 24-day droughted seedlings.

Application of BA to the 4week-chilled seeds, resulted in increasing the leaf total soluble carbohydrates content of the resulting seedlings compared with 4 week moist-chilling alone or in combination with GA_3. The combinations of GA_3 and 4 week-chilling periods, showed no significant differences in the soluble carbohydrates contents of the leaves of the resulting seedlings compared with those of the same chilling period. Treatment of the seeds with the combination of GA_3 and BA improved the contents of the chlorophylls and the total soluble carbohydrates of the resulting seedling leaves after 24 days of drought.

There was a significantly positive correlation (values between 0.62** and 0.70**) between the chlorophylls a & b and the total soluble carbohydrates contents of the leaves of the resulting seedlings subjected to 24 days of drought. Similarly, there was a positive correlation between chlorophylls a and b (values between 0.59** and 0.81**).

IV. Discussion

Seed germination behaviour

In the present study, exogenously applied GA3 alone could not alleviate germination of persimmon seed. This result coincided with those of Sauls and Campbell [24] with 'Waldin' avocado seeds and El-Dengawy [13] with loquat seeds where they found no effect for soaking in 250ppm GA_3 solution on seed germination. The lack of GA_3 effectiveness in stimulating seed germination might be referred to the following possibilities: a negative effect of GA_3 on the level of some enzymes activity (glutamate-oxaloacetate transaminase, pyruvate kinase and

malate dehydrogenase) and consumption of nucleotides in the synthesis of nucleic acid El-Dengawy [11] and/or the production of a proteinaceous germination inhibitor. Gibberellins are reported to have differential response in the germination of tree seeds. The inhibitory effect of abscisic acid (ABA) on germination of the conifer seeds was overcome very successfully by GA_3 alone in comparison with cytokinin (BA) and it's combination with GA_3 Kabar [25]. In general, GA_3 is effective in breaking the non-deep physiological dormancy, but it does not overcome the deep physiological dormancy Baskin and Baskin [26].

Although the highest germination percentages as well as the lowest number of days to 50% germination (T50) were on both seasons associated with the solely 8 week moist-chilling treatment (T9), but it may be said that T7 (the 4 weeks –chilled seeds soaked in BA) gave the most satisfactory results as it yielded high germination percentage, gave comparable T50 data as that of T9 and saved about at least 4 weeks which would have otherwise being required for moist-chilling of the seeds. Such results might be attributed to the synergistic effect of cold stratification and BA in breaking seed dormancy and stimulating germination. Therefore we suggest that there are different interactions among BA, GA_3 and cold stratification in affecting kaki seeds dormancy and germination. These findings are in line with those of Oh et al. [17] on Diospyros lotus seeds, they reported that the number of days required for 50% germination was less with higher germination temperature, longer stratification (up to 10 weeks), and alternating temperature. Samaan et al.[27] with apricot seeds also indicated that the most effective treatment in augmentation of seed germination and germination velocity was moist-chilling at 5 ± 1oC for 15 days followed by soaking into kinetin solution of 10 ppm for24 hr. They added that embryos of seeds treated by such combination contained the higher values of total soluble phosphorus, soluble organic phosphorus and total reducing sugars as well as a higher number of free amino acids. Therefore, the combination of cytokinin and cold stratification increases growth potential of embryo so that the radical can break through the seed coat resulting in germination. Moreover, the stimulating effect of moist-chilling and cytokinin combination on germination ability of dormant seeds can be attributed to the antagonistic effect of cytokinin on action of inhibitors present in dormant seeds Khan[28]. Similarly, El-Dengawy [13] showed that loquat seeds dormancy may be broken by moist-chilling treatment for 3 weeks at 5 ± 1 °C or 1 week of moist-chilling followed by soaking in 250 ppm GA_3 solution for 20 h. to significantly increase germination percentage. Recently, results of Fadhil, et al.[16] indicated that water soaking of "Lotus" kaki seeds for 72 hr. gave the highest germination percentage, whereas, the lowest was from the seeds soaked for 48 hr. in 300mg.l^{-1} GA_3. Rawat et al.[29] using the seeds of Abies pindrow and Picea smithiana and their overall results showed that soaking seeds in GA_3 (10 mg·L^{-1}) for 24 h, moist chilling for 15 days, and germinating at 10°C produced an effective germination in both the species.

Seedling characteristics

The best vegetative characteristics of 2.5-month-old kaki seedling namely, seedling height, leaf area , root length, number of secondary roots and dry weight, were obtained from seeds pretreated with 8week- moist-chilling solely or 4week- moist chilling followed by application of BA. The trend of kaki seedlings in this experiment was consistent with previous reports regarding the effect on seedling characters of using seed pretreatment with hormones. Ganesh et al.[30] found that seedling establishment of two Green gram pulse varieties resulting from seeds presoaked in various solutions of Indole acetic acid (IAA), Gibberellic acid (GA3) and Indole Butyric Acid (IBA), showed significant increases at 5ppm concentrations in the seedling growth of these two pulse varieties, contrary pre-soaking the seeds in distilled water responded. Kochanovoundá et al.[31] studied the effect of BA, and indole-3-butyric acid (IBA) on adventitious shoot regeneration from dormant persimmon buds and reported that adventitious shoots can be successfully produced in vitro. In another study on persimmon shoot regeneration following hormonal treatments, Tetsumura and Yukinaga [32] obtained a high percentage shoot regeneration confirming the differential reactions of different persimmon cultivars to cultivation media and plant hormones. Furthermore, according to the findings of Amooaghaie [33] with the forage Ferula ovina, moist-chilling treatments induced a great alteration in the level of seed soluble protein and hence seed germination and seedling vigour. This is strengthened by the findings Lin et al.[34] on pear seeds and Mullen, et al. [35] on Loblolly pine seed stratification, germination and post-germinative growth.

Drought resistance measurements on the subsequent seedlings

Proline content and Leaf resistance for water vapor (LRWV)

Generally, seeds pretreated with BA solely gave seedlings with significantly higher root proline content under drought than those of the other treatments, and hence showing more drought resistance features. Similar results were obtained when the GA3 and BA combination was applied to non-moist chilled seeds or to those moist chilled for 4 or 8 weeks, hence reflecting the profound effects of the hormonal combination as regard to proline accumulation. Such increased levels of proline under drought as seen in the present study, agree with those of other workers. Thus, Verbruggen and Hermans [36] stated that proline is considered to act as an osmolyte protecting cells from damage caused by stress conditions. The capacity to accumulate proline has long been

correlated with stress tolerance in some plant species Barnett and Nayor [37] and Szabados and Savoure [38]. Further, in an attempt to study the effect of seed pretreatment with exogenous application of proline, some authors, Ashraf and Foolad [39] and Wahid et al.[40] found that pre-soaking sugar cane cuttings in proline and glycinebetane proved of considerable help in alleviating the adversities of heat and its accompanying drought stress. These data suggested that both these osmolytes, due to their specific membrane protective properties, can be used to improve stress tolerance in many various plant species including the Diospyros.

The plant hormones cytokinins comprise a class of growth regulators involved in the stress response and antagonize many physiological processes induced by water stress, mainly those mediated by abscisic acid (ABA). Well known is the reversal of ABA-induced stomatal closure by cytokinins Incoll et al. [41], Rulcova and Pospisilova [42] and Werner and Schmulling [43].

Contents of pigments and total soluble carbohydrates

After exposure to 24 days of drought, it was evident that the effects of GA3, BA or their combination on seedling chlorophylls and TSC parameters were more pronounced on seedlings resulting from moist- chilled seeds compared with those of the corresponding ones resulting from non-chilled seeds. Drought inhibits the photosynthesis of plants and causes changes of chlorophyll contents, damage the photosynthetic apparatus and decreases the activities of Calvin cycle enzymes Monakhova and Chernyadev [44]. It also gives rise to accumulation of various low molecular weight substances namely, soluble carbohydrates, proline and free amino acids as well as antioxidants compounds. These osmoprotectants and compatible solutes play important roles in protecting cellular components from dehydration injury Reddy et al. [6] and Shao et al. [7].

Since we imposed severe drought in this research, the decrease in TSC concentration and the chlorophylls contents in the present study may be explained by the adverse effect of drought in damaging the photosynthetic apparatus, and decreasing the activities of Calvin cycle enzymes Monakhova and Chernyadev [44] and hence the drop in TSC production. So, treatment of the seeds with the combination of GA_3 and BA resulted a further improved the contents of the chlorophylls and the total soluble carbohydrates of the resulting seedling leaves after 24 days of drought. This is substantiated by the significantly positive correlation (values between 0.62** and 0.70**) between the chlorophylls (a & b) and the total soluble carbohydrates contents of the leaves of the resulting seedlings subjected to 24 days of drought. Similarly, there was a positive correlation between chlorophylls a and b (values between 0.59** and 0.81**).

V. Conclusion

As with some other species, Diospyros responds well to seed-moist chilling or BA and GA3 hormonal treatments. This was evident from the response of germination, T50%, and the acquired drought tolerance of the resulting seedlings. It was also known that any improvement in drought resistance would make a plant more adapted to saline soil. False efficacy is likely to be gained by reliance solely on germination testing; therefore, testing should be extended to the level of physiological aspects of seedling drought tolerance mechanisms. Further work is needed to study the feasibility of using persimmon pretreated seedlings as compatible combination in the process of grafting scions onto seedling rootstocks. Partitioning of TSC and its translocation between the scion and rootstock needs to be investigated together with the rate of healing at the graft union area. Perhaps the choice of Diospyros lotus as rootstock would enhance the translocation of photosynthates and thereby improve the productivity and longevity of persimmon trees.

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Table (1): Effect of GA_3, BA and three moist-chilling periods either alone or in combination on persimmon seeds germination.

Treatments			Seed germination%, days from sowing									
MCh		Growth	20 days		30 days		40 days		50 days		T50	
(weeks)		Regulators	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
(T1)	0	0 (Control)	00.0e	00.0d	00.0g	00.0f	17.5f	15.9f	23.8h	21.4f	73.6a	71.5a
(T2)	0	200 ppm GA_3	00.0e	00.0d	03.0g	04.8f	14.3f	16.7ef	19.1h	21.4f	74.3a	71.6a
(T3)	0	20 ppm BA	00.0e	00.0d	11.3def	09.5def	19.0f	23.8e	33.3fg	35.7e	66.4b	64.2b
(T4)	0	GA_3 + BA	00.0e	00.0d	14.3de	19.1cd	28.6e	33.3d	38.1f	40.5e	61.7b	55.1c
(T5)	4	0	09.5c	12.7b	34.9c	33.3b	44.4d	47.6c	49.2e	53.2d	49.1c	44.9d
(T6)	4	200 ppm GA_3	00.0e	00.0d	09.5ef	17.9cde	40.5d	45.2c	69.1d	70.2c	43.0c	41.2d
(T7)	4	20 ppm BA	05.6cd	11.1bc	48.1b	53.7a	77.8c	81.5b	87.0b	83.3b	32.2de	30.1e
(T8)	4	GA_3 + BA	00.0e	04.8cd	19.8d	24.6bc	44.4d	49.2c	69.1d	70.2c	41.1d	40.2d
(T9)	8	0	14.3b	14.3b	57.1a	65.9a	95.2a	90.5a	100.0a	94.4a	27.6e	27.0e
(T10)	8	200 ppm GA_3	28.6a	23.8a	64.9a	66.7a	85.7b	80.1b	85.7bc	81.0b	26.9e	26.1e
(T11)	8	20 ppm BA	04.8de	09.5bc	47.6b	57.1a	76.2c	81.0b	90.5ab	85.7ab	30.1e	29.0e
(T12)	8	GA_3 + BA	14.3b	09.5bc	47.6b	52.4a	71.4c	75.0b	76.2cd	81.0b	30.6e	30.2e
F-test			***	***	***	***	***	***	***	***	***	***
LSD:		MCh	2.23	3.02	4.19	6.58	3.25	3.49	4.82	4.57	4.58	3.49
		GR	2.57	3.48	4.83	7.59	3.76	4.03	5.57	5.27	5.28	4.03
		MCh*GR	4.23	6.16	8.28	13.54	6.66	7.30	9.72	8.76	9.28	6.79

Values within each column followed by the same letter are not statistically different at 5% level. ***, Significant at level $p = 0.001$. MCh, moist-chilling, T50, time (in days) to obtain 50% germination. T, treatments. S1, season 2009. S2, season 2010.

Table (2): Response of vegetative characteristics in 2.5-month-old persimmon seedlings (resulted from different germination treatments) subjected to 24 days of drought period.

Treatments			Seedling characteristics									
MCh		Growth	Seedling height (cm)		Leaf area (cm^2)		Root length (cm)		Secondary roots No.		Dry weight (g)	
(week)		regulators	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
(T1)	0	0 (Control)	14.8i	13.9g	141 de	136f	11.0g	10.6e	15.3g	13.5d	1.09g	1.10f
(T2)	0	200 ppm GA_3	17.3gh	17.8f	123 f	121g	13.8ef	13.1cd	17.2ef	16.4c	1.20f	1.10f
(T3)	0	20 ppm BA	17.6fgh	18.5f	188 cd	191bc	15.3bc	16.0a	17.7e	19.3b	1.38d	1.47d
(T4)	0	GA_3 + BA	17.2h	18.2f	187cd	179d	13.0f	12.0d	15.7fg	15.0c	1.48cd	1.42d
(T5)	4	0	18.2ef	18.8ef	169d	159e	13.7ef	15.3ab	20.7c	18.7b	1.41d	1.23e
(T6)	4	200 ppm GA_3	18.0fg	19.5bde	172 d	180cd	14.0def	13.0cd	18.7de	15.0c	1.31e	1.53d
(T7)	4	20 ppm BA	19.5cd	20.8bc	214ab	212a	16.3ab	14.7ab	27.3a	20.7b	1.88a	1.82b
(T8)	4	GA_3 + BA	19.7bc	20.2cd	221a	217a	13.3f	15.6a	26.7ab	24.3a	1.59b	1.49d
(T9	8	0	22.3a	22.8a	193c	192bc	17.0a	15.8a	25.3b	24.0a	1.86a	2.01a
(T10)	8	200 ppm GA_3	18.8de	20.0cd	198bc	200b	15.7bc	12.3d	20.0cd	20.7b	1.40d	1.80b
(T11)	8	20 ppm BA	19.0cd	20.0cd	172 d	164e	14.7cde	14.0bc	17.3ef	18.7b	1.55bc	1.68c
(T12)	8	GA_3 + BA	20.35b	21.5b	193c	195b	15.0cd	15.7a	20.0c	15.7c	1.41d	1.67c
F-test			***	***	***	***	***	***	***	***	***	***
LSD:		MCh	0.38	0.48	8.7	5.3	0.52	0.68	0.83	0.93	0.05	0.08
		GR	0.43	0.55	10.1	6.1	0.60	0.79	0.95	1.07	0.05	0.07
		MCh*GR	0.76	0.99	18.0	10.8	0.99	1.43	1.60	1.86	0.09	0.12

Values within each column followed by the same letter are not statistically different at 5% level. ***, Significant at level $p = 0.001$. MCh, moist-chilling. T, treatments. S1, season 2009. S2, Season 2010.

Table (3): Response of proline content and leaf resistance to transpiration in persimmon seedling (resulted from different germination treatments) after 12 and 24 days drought period.

Treatments			Proline (mg/g dry weight)				Leaf resistance for water vapor (sec cm^{-1})			
MCh		Growth	Leaf proline		Root-Stem proline		after 12 days drought		after 24days drought	
(weeks)		Regulators	S1	S2	S1	S2	S1	S2	S1	S2
(T1)	0	0 (control)	1.58a	1.52a	4.61c	4.60c	3.55g	3.29h	10.93g	8.86i
(T2)	0	200 ppm GA_3	1.31c	1.25c	2.62f	2.49g	5.42e	5.49cd	9.28h	8.63i
(T3)	0	20 ppm BA	1.45b	1.41b	6.90a	6.78a	6.42c	6.99b	13.55d	11.20de
(T4)	0	GA_3 + BA	1.15e	1.10d	3.06e	2.80f	6.01d	5.58c	11.3fg	10.09g
(T5)	4	0	1.42bc	1.39b	2.06g	2.04h	7.50a	6.55a	12.1ef	10 .80ef
(T6)	4	200 ppm GA_3	0.79ef	0.77f	3.20e	3.12e	3.85f	3.67g	10.81g	9.38h
(T7)	4	20 ppm BA	0.83ef	0.80f	1.94g	1.87h	6.58bc	5.60c	13.64d	11.45d
(T8)	4	GA_3 + BA	0.71f	0.70g	1.29h	1.30i	6.73b	6.10b	14.60c	12.54c
(T9)	8	0	0.93e	0.90e	1.01h	0.97j	5.43e	5.07f	20.45a	18.84a
(T10)	8	200 ppm GA_3	0.71f	0.70g	3.98d	3.89d	6.11d	5.4cde	12.18e	10.39fg
(T11)	8	20 ppm BA	0.92e	0.90e	4.69bc	4.56c	5.40e	5.20ef	11.6ef	10.53fg
(T12)	8	GA_3 + BA	0.81ef	0.79f	5.09b	5.05b	5.94d	5.3def	15.6bc	13.46b
F-test			***	***	***	***	***	***	***	***
LSD:		MCH	0.07	0.03	0.19	0.09	0.08	0.07	0.42	0.28
		GR	0.08	0.03	0.21	0.11	0.09	0.08	0.49	0.33
		MCH*GR	0.12	0.05	0.38	0.15	0.16	0.13	0.69	0.49

Values within each column followed by the same letter are not statistically different at 5% level. ***, Significant at level $p = 0.001$. MCh, moist-chilling. T, treatments. S1, season 2009. S2, Season 2010.

Table (4): Response of pigments and total soluble carbohydrates contents in leaves of persimmon seedlings (resulted from different germination treatments) after 24 days drought period.

Treatments			Pigments content (µg/cm^2 of leaf)				Total soluble carbohydrates	
	MCh	Growth	Chlorophyll (a)		Chlorophyll (b)		(mg/g dry weight of leaf)	
	(weeks)	Regulators	S1	S2	S1	S2	S1	S2
(T1)	0	0 (Control)	6.5f	7.7e	3.5e	5.0ef	65.5ef	67.2ef
(T2	0	200 ppm GA_3	7.9e	8.5de	5.4c	5.2de	67.2def	64.4ef
(T3)	0	20 ppm BA	7.4e	9.3d	3.9e	4.1g	67.3def	65.6ef
(T4)	0	GA_3 + BA	8.8d	10.5c	5.0cd	5.5cd	69.4de	67.4e
(T5)	4	0	11.3b	12.1ab	6.4ab	6.1ab	80.1c	76.1d
(T6)	4	200 ppm GA_3	9.1d	10.6c	4.9cd	5.0ef	84.5c	81.5c
(T7)	4	20 ppm BA	12.0ab	11.4bc	6.9a	6.2ab	97.2b	95.1b
(T8)	4	GA_3 + BA	12.6a	12.5ab	6.3b	5.8bc	114.9a	112.5a
(T9)	8	0	12.3a	13.1a	6.5ab	6.4a	100.5b	97.1b
(T10)	8	200 ppm GA_3	12.8a	12.5ab	6.3b	5.8bc	82.4b	81.7c
(T11)	8	20 ppm BA	12.4a	11.7bc	5.3c	4.7f	71.4d	69.5e
(T12)	8	GA_3 + BA	10.1c	10.7c	4.7d	5.2de	64.5f	66.5ef
F-test			***	***	***	***	***	***
LSD:	MCh		0.41	0.58	0.34	0.27	2.10	1.96
	GR		0.48	0.67	0.39	0.31	2.42	2.26
	MCh*GR		0.77	1.19	0.65	0.55	4.01	4.09

Values within each column followed by the same letter are not statistically different at 5% level. ***, Significant at level $p = 0.001$. MCh, moist-chilling. T, treatments. S1, season 2009. S2, Season 2010.