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EFFECTS OF SOAKING TEMPERATURE, STRATIFICATION, POTASSIUM NITRATE AND GIBBERELIC ACID ON SEED GERMINATION OF LOQUAT TREES

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□ The present study was carried out to increase loquat seed germination with treatments consisting of two soaking temperatures ($24 \pm 2^\circ\text{C}$ and $38 \pm 2^\circ\text{C}$), chemical agents [control, 0.5% potassium nitrate (KNO_3) and 250 mgL^{-1} gibberellic acid (GA_3) each for 20 h], and different moist chilling (MC) periods (1, 2, 3 and 4 weeks under $4\text{--}5^\circ\text{C}$). Compared with $24 \pm 2^\circ\text{C}$, soaking at $38 \pm 2^\circ\text{C}$ reduced germination%, mean daily germination (MDG), and mean germination time (MGT), plumule and radicle lengths. Germination percentage, days to 50% emergence, fresh weight and lateral root numbers significantly reduced as MC period increased. KNO_3 and GA_3 had no significant effect on germination percentage, MDG, MGT and lateral root numbers. KNO_3 reduced days to 50% emergence and radicle length, but increased fresh weight compared with control and GA_3 . Finally, our results suggest the soaking at $24 \pm 2^\circ\text{C}$ followed by 0.5% KNO_3 each for 20 h plus 1 week of MC or soaking at $24 \pm 2^\circ\text{C}$ followed by 250 mgL^{-1} GA_3 each for 20 h plus 2 week of MC.

Keywords: potassium nitrate, soaking, loquat, seed germination, moist chilling, gibberellic acid

INTRODUCTION

Loquat (*Eriobotrya japonica* Lindl.) belongs to *Rosaceae* and is a subtropical evergreen fruit tree. The loquat fruit grows in clusters and is oval with a smooth or downy yellow or orange skin. Each fruit contains five ovules, of which three to five mature into large brown seeds. It has high sugar, acid, and pectin contents. Fruits are consumed throughout Asia. The fruit contains citric, malic, succinic, gallic, ellagic and tartaric acids and vitamin C,

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TABLE 1 Nutritional value and vitamins of loquat fruits

Value	Protein (mg)	Sugar (mg)	Vitamin A (mg)	Vitamin C (mg)	Ca (mg)	P (mg)	Mg (mg)
Rate	0.3	8.1	132	2	11	9	9

oxidase, amylase, invertase, corilagin, levulose, sucrose, amygdalin, cryptoxanthin, carotenes, phenyl ethyl alcohol pentosans, and essential oils, and is antitussive and expectorant and is used to treat bronchitis, cough, fever, and nausea (Duke and Ayensu, 1985). The seeds and pulp contain phenolics (Rangkadilok et al., 2005). The seeds contain saponins, amygdalin, emulsin, and hydrocyanic acid. It is bitterish and used to treat ailments related to lungs and stomach such as phlegm, high fever, and nausea (Perry, 1980), gastritis, and vomiting (Lin, 1998). The leaves contain large amounts of saponin, d-sorbitol and ascorbic acid oxidase and are rich in vitamin B and are employed to treat chronic bronchitis and coughs. Fruits can be consumed fresh or processed in several forms and have been traditionally considered to have high medicinal value (Table 1).

Loquats originate from China and are grown mainly in sub-tropical and Mediterranean countries, including Algeria, Cyprus, Egypt, Greece, Israel, Italy, Spain, Tunisia and Turkey (Badenes et al., 2009). In Iran, the loquat is mainly cultivated in the Guilan, Mazandaran, Golestan, Zanzan, Qom and Fars provinces (Anonymous, 2006). Generally, the loquat tree is very well adapted to virtually all soils that have good internal drainage and hence grows equally well in the acid and the alkaline soils (Lin et al., 1999). However, its seed germination, in general, is faced with some problems that lead to low germination percentage and subsequently reduced seedling growth (El-Dengawy, 2005).

The seed is the structure consisting of a cover, an embryo and some supplemental foods, which enables the embryo to survive the period of time between seed maturation and seedling establishment thereby ensuring the initiation of the next generation. Dormancy is a condition in which seeds do not germinate even when the environmental conditions (i.e., water, temperature and light) are suitable for germination. Research shows that loquat seeds display an endogenous dormancy (El-Dengawy, 2005). Such problems reduce loquat industry development. Although there is potential to use loquat as a rootstock for quince, however, the quince rootstock is usually used in spite of its undesirable characteristics such as shallow root system and the high susceptibility to salinity stress. Therefore, enhancement of loquat seed germination is important in propagation and breeding programs as well as for testing and using germplasms (El-Dengawy, 2005). Breaking seed dormancy is necessary for the completion of germination. Various dormancy breaking and germination stimulating treatments have been tried with seeds

of many fruit species such as papaya (Nagao and Furutani, 1986), persimmon (Taha, 1987), peach (El-Khoreiby and Salem, 1985; El-Dengawy, 1997), and loquat (Polat and Kaska, 1992; Polat, 1997). In this respect, gibberellic acid and moist-chilling treatments seem the most promising in many woody species (Powell, 1987; Rehman and Park, 2000). There is some evidence showing that potassium nitrate can act as a useful means to induce seed germination (Shim et al., 2008; Tzortzakis, 2009). Thus, the main aim of this study was to find a practical way for increasing seed germination and subsequent growth of loquat plants using water soaking under different temperatures, different periods of moist chilling, potassium nitrate (KNO_3) and gibberellic acid (GA_3).

MATERIALS AND METHODS

Fully ripped fruits were picked from trees and transported to the laboratory of Department of Horticultural Science, University of Tehran, Iran, during 2009 growing season. The seeds were taken out of the fruits and washed with tap water for approximately five minutes. Consequently, seeds were surface sterilized using 10% sodium hypochlorite (NaOCl) for 10 minutes, and washed three times with distilled water. Soaking treatments were done using two water temperatures ($24 \pm 2^\circ\text{C}$ and $38 \pm 2^\circ\text{C}$). Chemical treatments consisted of 0.5% potassium nitrate (KNO_3) and 250 mgL^{-1} gibberellic acid each for 20 h. Moist chilling treatments consisted of 1, 2, 3 and 4 weeks under $4\text{--}5^\circ\text{C}$ using plastic bags containing sand. Finally, chemical and moist chilling treatments were chosen as follows:

- 1) Soaking in distilled water at $24 \pm 2^\circ\text{C}$ for 20 h (T1)
- 2) T1 + soaking in 0.5% KNO_3 for 20 h (T2)
- 3) T1 + soaking in 250 mgL^{-1} GA_3 for 20 h (T3)
- 4) Soaking in distilled water at $38 \pm 2^\circ\text{C}$ for 20 h (T control)
- 5) T control+ soaking in 0.5% KNO_3 for 20 h (T4)
- 6) T control+ soaking in 250 mgL^{-1} GA_3 for 20 h (T5)
- 7) T1 + 1 week moist chilling (T6)
- 8) T1 + 2 weeks moist chilling (T7)
- 9) T1 + 3 weeks moist chilling (T8)
- 10) T1 + 4 weeks moist chilling (T9)
- 11) T1 + soaking in 0.5% KNO_3 for 20 h + 1 week moist chilling (T10)
- 12) T1 + soaking in 0.5% KNO_3 for 20 h + 2 weeks moist chilling (T11)
- 13) T1 + soaking in 0.5% KNO_3 for 20 h + 3 weeks moist chilling (T12)
- 14) T1 + soaking in 0.5% KNO_3 for 20 h + 4 weeks moist chilling (T13)
- 15) T1 + soaking in 250 mgL^{-1} GA_3 for 20 h + 1 week moist chilling (T14)
- 16) T1 + soaking in 250 mgL^{-1} GA_3 for 20 h + 2 weeks moist chilling (T15)
- 17) T1 + soaking in 250 mgL^{-1} GA_3 for 20 h + 3 weeks moist chilling (T16)

- 18) T1 + soaking in 250 mgL⁻¹ GA₃ for 20 h + 4 weeks moist chilling (T17)
- 19) T control+ 1 week moist chilling (T18)
- 20) T control + 2 weeks moist chilling (T19)
- 21) T control + 3 weeks moist chilling (T20)
- 22) T control + 4 weeks moist chilling (T21)
- 23) T control + soaking in 0.5% KNO₃ for 20 h + 1 week moist chilling (T22)
- 24) T control + soaking in 0.5% KNO₃ for 20 h + 2 weeks moist chilling (T23)
- 25) T control + soaking in 0.5% KNO₃ for 20 h + 3 weeks moist chilling (T24)
- 26) T control + soaking in 0.5% KNO₃ for 20 h + 4 weeks moist chilling (T25)
- 27) T control + soaking in 250 mgL⁻¹ GA₃ for 20 h + 1 week moist chilling (T26)
- 28) T control + soaking in 250 mgL⁻¹ GA₃ for 20 h + 2 weeks moist chilling (T27)
- 29) T control + soaking in 250 mgL⁻¹ GA₃ for 20 h + 3 weeks moist chilling (T28)
- 30) T control + soaking in 250 mgL⁻¹ GA₃ for 20 h + 4 weeks moist chilling (T29)

Treated seeds were kept in plastic bags containing moistened sand in a germinator under $24 \pm 2^\circ\text{C}$ (16-h photoperiod, $700 \mu\text{mol s}^{-1}\text{m}^{-2}$ photon in light period) up to the end of the experiment. The experiment lasted for 25 days. Germination percentage, mean daily germination (MDG) and mean germination time (MGT), the day of 50% emergence, seedling fresh weight, radicle and plumule lengths, and adventitious root number were measured in this experiment. Germination percentage, radicle and plumule lengths, seedling fresh weight and adventitious root number were measured on the 25th day of experiment.

Mean daily germination was assessed using Hartmann et al. (1990) method:

$$\text{MDG} = \sum (N_1T_1 + N_2T_2 + \dots + N_xT_x) / \text{Total number of germinated seeds.}$$

Where N values are the number of seeds germinated within consecutive intervals of time; T values indicate the time between the beginning of the test and the end of a particular interval or measurement.

Mean germination time was calculated based on Schelin et al. (2003) as followed:

$$\text{MGT} = \sum (\text{fini}) / N$$

where f_i is day during germination period (between 0 and 25 day); n_i is the number of germinated seeds per day and N is Sum of germinated seeds.

Day of 50% emergence was calculated based on Heydecker and Wainwright (1976):

$$\text{Day of 50\% emergence} = [(t_2 - t_1) \times 50\% + (p_2 t_1 - p_1 t_2)] / (p_2 - p_1)$$

where t_1 is time at which the germination percentage is less than 50%; t_2 is 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.

Radicle and plumule lengths were measured using a ruler.

The experiment was arranged in a completely randomized design with 30 treatments and three replications, each replication consisted of one plastic bag and 20 seeds in each. Data were analyzed using MSTAT-C software (MSTAT-C, Michigan State University, East Lansing, MI, USA). Means were separated with least significant difference (LSD) at 1% level of probability.

RESULTS AND DISCUSSION

Data analysis related to soaking, moist chilling and chemical treatments are shown in Tables 2, 3, and 4. Results indicated that application of soaking water with different temperatures significantly influence germination percentage, MDG and MGT, days to 50% emergence and fresh weight, radicle and plumule lengths and lateral root numbers. As the data shows, water temperature used for soaking at $38 \pm 2^\circ\text{C}$ strongly reduced all of the above mentioned variables with the exception of MGT and days to 50% emergence, compared with temperature at $24 \pm 2^\circ\text{C}$ (Table 2) that was in agreement with Shin et al. (2006). Soaking in water may change metabolic activity of seeds that influence consequent processes (Basra et al., 2005). Germination percentage, days to 50% emergence, fresh weight and lateral root numbers significantly reduced as moist chilling period increased (Table 3). The highest MDG and the lowest rate of MGT were found by moist chilling treatment for 3 weeks (Table 3). The highest germination percentage, MGT, days to 50% emergence, fresh weight, plumule length and lateral root numbers were found by untreated seeds with moist chilling (Table 3). Moist chilling is a standard procedure used to enhance the germination of dormant seeds. It has been used for various dormant seeds and has been reported successfully to alleviate endogenous dormancy. Loquat seeds display an endogenous dormancy that can be released by moist-chilling treatment for a certain period (El-Dengawy, 2005). It is believed that cold treatment can only work for those seeds that contain both inhibitors and promoters as evidenced by

TABLE 2 Evaluation of different water temperatures used for soaking of loquat seeds

Soaking temperature (°C)	Germination percent	Mean daily germination	Mean germination time	Days to 50% emergence	Fresh weight (g)	Radicle length (mm)	Plumule length (mm)	Lateral root number
24 ± 2	0.81a	12.11a	3.88b	3.54b	0.61a	83.24a	14.98a	23.36a
38 ± 2	0.29b	4.05b	6.69a	6.17a	0.51b	59.22b	12.09b	16.22b

Columns with the same letters are not significantly different according to LSD at 1% probability levels.

TABLE 3 Effect of different periods of moist chilling on seed germination and growth of loquat seeds

Moist chilling at 4–5°C (Week)	Germination percent	Mean daily germination	Mean germination time	Days to 50% emergence	Fresh weight (g)	Radicle length (mm)	Plumule length (mm)	Lateral root number
0 (control)	0.64a	4.49c	9.69a	9.17a	0.58a	73.89b	20.83a	24.61a
1	0.71a	5.26c	7.73b	7.72b	0.61a	91.83a	10.94c	23.33ab
2	0.56b	8.23b	3.36c	3.06c	0.60a	84.44a	11.39c	19.28b
3	0.52b	12.93a	2.20d	2.03c	0.54ab	61.72c	14.83b	20.22b
4	0.31c	9.49b	3.43c	2.31c	0.47b	44.28d	9.67c	11.50c

Columns with the same letters are not significantly different according to LSD at 1% probability levels.

TABLE 4 Effect of KNO₃ and GA₃ on seed germination and growth of loquat seeds

Chemical treatment	Germination percent	Mean daily germination	Mean germination time	Days to 50% emergence	Fresh weight (g)	Radicle length (mm)	Plumule length (mm)	Lateral root number
Control	0.55a	8.12a	5.25a	5.05ab	0.51b	77.10a	10.97b	19.33a
KNO ₃ (0.5%)	0.55a	7.76a	5.28a	4.27b	0.64a	56.70b	15.57a	20.77a
GA ₃ (250 mg/L)	0.54a	8.16a	5.32a	5.25a	0.54b	79.90a	14.07a	19.27a

Columns with the same letters are not significantly different according to LSD at 1% probability levels.

the fact that the inhibitor: promoter balance is altered by exposing seed to moist chilling (Rehman and Park, 2000). Khan (1977) reported that moist chilling affects metabolic processes such as hormones, i.e., disappearance of ABA and activation of GA and initiation of germination. Moist-chilling may increase the level of the organic phosphates like fructose 2, 6- biphosphate (Bewley and Black, 1994) and nucleotides (El-Dengawy, 1997). Such interpretation is in accordance with the finding of Khan et al. (1968) who found a progressive increase in the synthesis of nucleic acids of pear embryos with the increase in the length of moist-chilling treatment. Potassium nitrate and gibberellic acid had no significant effect on germination percentage, MDG, MGT, and lateral root number compared with control (Table 4). Potassium nitrate reduced days to 50% emergence and radicle length, but increased fresh weight and plumule length compared with control (Table 4). Gibberellic acid increased days to 50% emergence and plumule length, but reduced fresh weight comparing to potassium nitrate (Table 4) that was disagreement with findings of Farhoudi et al. (2007) on Madder seeds. Data showed in Table 5, indicated the interaction effects among all treatments used through the experiment. Results illuminated the highest germination percentage with T10 and T15 that show non-significant differences with T1, T2, T3, T6, T7, T11, T12, T14 and T16, and showed the positive effect of one to three weeks moist chilling plus soaking into distilled water at $24 \pm 2^{\circ}\text{C}$ for 20 h (Table 5). Regarding to both of T10 and T15, it is evident that application of KNO_3 need to lower period of moist chilling compared to treatment included GA_3 as a chemical agent (Table 5). The highest mean daily germination was found with T8, which was statistically similar to T9, T12, T13, T15, T16 and T17 (Table 5). Regarding the mentioned treatments, all treatments needed to 3 to 4 weeks moist chilling. It is clear that application of KNO_3 did not affect the moist chilling effects on MDG compared with treatment without it; however, GA_3 reduced need to moist chilling period from 3 to 2 weeks (Table 5). The highest MGT were observed by T5, T control, T22 and T26 that illustrated the negative effects of soaking into distilled water at $38 \pm 2^{\circ}\text{C}$ for 20 h (Table 5). Regarding to data, it is found that one to four weeks moist chilling reduced MGT regardless to chemical agent used (Table 5). Treatments included T5, T control, T4, T18, T22 and T26 increased the days needed to approach 50% of emergence, which show the effect of soaking into distilled water at $38 \pm 2^{\circ}\text{C}$ for 20 h (Table 5). The highest fresh weight was obtained with T10 that showed non-significant differences with T2, T3, T4, T7, T14, T23 and T24 (Table 5). Evidently, when seed soaked into water at $38 \pm 2^{\circ}\text{C}$ for 20 h, potassium nitrate increased fresh weight compared with GA_3 . The highest radicle and plumule lengths were resulted from T3 (Table 5), showing the positive effects of GA_3 on these variables. Potassium nitrate applied plus soaking into water at $24 \pm 2^{\circ}\text{C}$ for 20 h showed the highest number of lateral roots (Table 5). Regarding to our data, the best treatments were soaking into distilled water at $24 \pm 2^{\circ}\text{C}$ for 20 h followed by

TABLE 5 Interaction between moist chilling and chemical treatments on seed germination

Treatment	Germination %	Mean daily germination	Mean germination time	Days to 50% emerg.	Fresh weight (g)	Radicle length (mm)	Plumule length (mm)	Lateral root number
T1	0.90ab	5.70e-g	9.13b-d	9.16a-d	0.56b-h	100.30a-c	14.33b-g	28.67a-d
T2	0.90ab	7.06d-g	6.83c-g	6.16d-g	0.70a-d	108.70ab	23.33ab	34.67a
T3	0.83ab	5.83e-g	8.00c-e	7.50b-e	0.70a-d	125.00a	27.33a	32.00a-c
T control	0.56cd	3.36fg	11.43ab	10.50ab	0.43f-h	24.33hi	20.00a-d	13.67e-h
T4	0.36d-h	3.03fg	9.90a-d	9.50a-d	0.66a-e	28.33i-i	22.67a-c	21.67a-f
T5	0.30e-i	1.93g	12.83a	12.17a	0.40gh	56.67d-h	17.33a-e	17.00d-h
T6	0.93ab	7.26d-g	4.53f-l	3.50fk	0.56b-h	105.30a-c	9.00e-g	21.67a-f
T7	0.90ab	12.30b-e	2.73i-l	2.16h-k	0.70a-d	124.70a	18.33a-e	32.33ab
T8	0.73bc	21.30a	1.80j-l	1.83i-k	0.50d-h	76.67b-e	9.33e-g	17.00d-h
T9	0.46d-f	13.93a-d	1.63kl	1.50jk	0.46e-h	43.33f-i	5.33g	16.00d-h
T10	1.00a	7.23d-g	6.63d-h	6.83c-f	0.86a	94.67a-c	20.33a-d	32.33ab
T11	0.83ab	12.30b-e	2.23j-l	1.50jk	0.60b-g	50.00e-i	16.00b-f	16.00d-h
T12	0.86ab	14.33a-d	2.13j-l	1.50jk	0.63b-f	74.67c-f	20.00a-d	23.33a-f
T13	0.50de	19.47ab	1.40l	1.50jk	0.43f-h	20.67i	5.66g	6.66gh
T14	0.93ab	7.30d-g	5.66e-i	5.50e-h	0.73a-c	108.70ab	13.33b-g	32.67ab
T15	0.96a	14.20a-d	2.26j-l	1.50jk	0.53e-h	84.67b-d	9.33e-g	19.67b-g
T16	0.86ab	16.70a-c	1.73j-l	1.50jk	0.56b-h	73.33c-f	14.67b-g	25.00a-e
T17	0.50de	16.70a-c	1.40l	1.50jk	0.56b-h	58.00d-g	18.33a-e	12.33e-h
T18	0.40d-g	3.00fg	10.00a-c	10.17a-c	0.56b-h	79.33b-e	8.33e-g	19.67b-g
T19	0.20h-i	3.46fg	4.36f-l	5.16e-i	0.50d-h	88.00b-d	5.66g	15.67d-h
T20	0.30e-i	8.96d-g	2.03j-l	2.50h-k	0.40gh	43.33f-i	6.66fg	10.67f-h
T21	0.10i	1.86g	4.83e-k	4.00e-k	0.36h	85.67b-d	12.67c-g	18.00d-g
T22	0.53cd	3.80fg	9.73a-d	10.17a-c	0.53c-h	83.33b-d	9.66e-g	19.00c-g
T23	0.26f-i	4.53fg	3.50g-l	3.16g-k	0.76ab	57.00d-h	10.67d-g	17.00d-h
T24	0.16h-i	6.06e-g	3.43h-l	1.83i-k	0.66a-e	30.00h-i	21.00a-c	32.67ab
T25	0.10i	1.80g	7.00c-f	0.50k	0.53c-h	19.67i	6.33fg	4.33h
T26	0.43d-f	2.93fg	9.83a-d	10.17a-c	0.40gh	79.67b-e	5.00g	14.67e-h
T27	0.16hi	2.56g	5.06e-j	4.83e-j	0.53c-h	102.30a-c	8.33e-g	15.00e-h
T28	0.20h-i	10.23c-f	2.06j-k	3.00g-k	0.46e-h	72.33c-f	17.33a-e	12.67e-h
T29	0.20h-i	3.20fg	4.33f-l	4.83e-j	0.46e-h	38.33h-i	9.66e-g	11.67f-h

Columns with the same letters are not significantly different according to LSD at 1% probability levels.

soaking into 0.5% KNO₃ for 20 h plus 1 week moist chilling (T10) or soaking into distilled water at 24 ± 2°C for 20 h followed by soaking into 250 mgL⁻¹ GA₃ for 20 h plus 2 weeks moist chilling to attain the highest germination of loquat seeds.

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