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INFLUENCES OF PRE-SOWING TREATMENTS ON THE GERMINATION AND EMERGENCE OF DIFFERENT MULBERRY SPECIES SEEDS

Kazim Gündüz[1✉], Fırat Ege Karaat[2], Fulya Uzunoğlu[3], Kazım Mavi[3]

[1] Malatya Turgut Ozal University, Faculty of Agriculture, Department of Horticulture, 44000, Malatya, Turkey
[2] Adıyaman University, Faculty of Agricultural Sciences and Technologies, Department of Plant Protection, 02040, Adıyaman, Turkey
[3] Mustafa Kemal University, Faculty of Agriculture, Department of Horticulture, 31034, Hatay, Turkey

ABSTRACT

Morus genus includes more than 20 species, some of which are commercially important mulberries with different fruit color and shape. Even though the trees of those species are propagated by rooting of cuttings in practice, mulberry seeds are of importance for breeding studies and rootstock seedling propagation. For that reason, this study was conducted to improve the seed performance of four mulberry species by different pre-sowing treatments including; 3% KNO_3, GA_3 at 500 ppm, organic priming with herbal tea brewed from marigold flowers petals and hydro priming. Results of the parameters of emergence characteristics and seedling vigor were evaluated. According to the results that varied between different species and pre-treatments, since emergence percentage and time, and seedling vigor were improved by the treatments, it was concluded that pre-sowing applications, especially GA_3 and hydro priming, were beneficial in improving the seed performance of mulberry species included in the study.

Key words: emergence, germination, mulberry, priming, species

INTRODUCTION

The genus *Morus* belongs to *Moraceae* family and comprises 24 species, approximately 100 varieties and one subspecies [Ozan et al., 2008]. In all species, *Morus alba* L., (white mulberry), *M. nigra* (black mulberry), *M. rubra* L. (purple or red mulberry) and *M. laevigata* (large mulberry) are the most commonly found and cultivated *Morus* species. For example, in Turkey, 95% of mulberry trees are *M. alba*, 3% is *M. rubra* and 2% is *M. nigra* [Ercisli 2004].

According to Watt [1873], certain forms of *Morus* are originated in India, but according to Vavilov [1926], its origin is China-Japan, including East China, Korea and Japan. Today, *Morus* species are grown in temperate and humid areas that includes Southeastern parts of Asia, Jawa and Sumatra Islands of Indonesia, Oman area located at Southeastern part of Arabia, Caucasia, Iran and West Asia, West Africa and North and South America.

Mulberry (*Morus* spp.) is a fast growing, deciduous, perennial, woody shrub or tree forming deep-growing roots. The plants are monoecious or dioecious, but usually dioecious. Leaf shape can be lobeless or with 1–5 lobes [Das et al. 1994, Benavides 2004, Datta 2004]. The mulberry flower is composed of a large number of flowers arranged very close to each other on the flower bud axis, and the main flower axis is more extended than the side branches [Griggs and Iwakiri 1973]. Mulberry fruit is a collection of fruitlets, each of which consists of flowers on the flower stem. The sepals surrounding the ovary form the mulberry fruit become fleshy. Since together with carpels, cover leaves of the flowers also contribute to the fruitlet formation, mulberry is accepted as false fruit.

✉ kgunduz44@gmail.com

There is a wide range of uses of mulberry fruits. Besides its fruits, other plant parts of mulberry are also evaluated in various forms. For example, mulberry trees are used for landscape design purposes, its leaves are used in tea making, pharmaceuticals, silkworm rearing and cosmetic industry [Ercisli and Orhan 2007].

On recent years, the interest on mulberry fruits has increased together with understanding of their nutritional capacities [Özgen et al. 2009]. Consequently, improving yield and fruit quality by breeding the new high potential cultivars and rootstocks, improving cultivation practices, has gained importance. Even though, commercial seedlings are propagated by rooting of cuttings, seeds are important for propagation of rootstocks to be grafted and budded cultivars on, especially poorly-rooting cultivars, and for growing hybrid plants in breeding programs [Westwood 1995, Hartmann et al. 1997].

Germination rate and the time are two main items that are important for using the seeds for propagation. These are influenced by a number of factors, which are internal and external factor groups [Bewley and Black 1994]. There have been some efforts made for improving germination performance of different *Morus* species [Gunes and Cekic 2004, Koyuncu 2005], but apparently almost no study has been performed upon *M. laevigata*. Besides, most of the studies have been performed with single species, for that reason the knowledge about comparison of different applications on different species is limited.

Pre-sowing applications are one of the most commonly studied applications in boosting the germination abilities, since it is an easily applicable, time and cost reducing way of application. Even though there have been other successful approaches, high temperature treatment experiment of Kusu and Morohashi [1987] i.e., previous studies on pre-sowing treatments of mulberry seeds, have mainly focused on chemical and hormone priming applications, but apparently almost no work has been done on organic priming of mulberry seeds. Besides, current reports on comparison of different pre-sowing applications are found inadequate for developing the applicable results for practical purposes.

For all those reasons, this study was performed in order to detect the effects of different pre-sowing applications on germination characteristics of mulberry seeds from different *Morus* species. For this purpose, seeds of three white fruited and one purple fruited mulberry species were subjected to hydro, organic and chemical priming treatments. The results on germination characteristics of those seeds were compared with non-treated seeds of those species.

MATERIALS AND METHODS

As a plant material, seeds of the species *Morus rubra*, *Morus laevigata*, *Morus alba* (white-fruited), and *Morus alba* (purple-fruited) were used. The seeds were obtained from fruits collected in full ripening period in 2016 from the trees cultivated under the field conditions (36°54'N, 36°13'E, altitude 198 m) in the Hatay, Turkey. For each species, 2 kg of fruits were randomly collected. Seeds were dried after extraction from the fruits, which were macerated manually.

Together with control line, totally five treatments were applied in the study, which were 3% KNO_3 (10 mL) for 24 h [Demir and Mavi 2004], GA_3 at 500 ppm (10 mL) (Gibberelex, Valent BioSciences) for 24 h, organic and hydro priming treatments. Except from organic and hydro priming, all treatments were moistened on top of filter papers in Petri dishes (80 mm, Isolab Inc.) and exposed to 25°C for 24 h in an incubator.

In order to obtain organic priming material, first of all, flower petals of one marigold flowers species (*Tagetes patula*) were dried under shade at room conditions for 10 days. Dried petals were kept in glass jars until treatment. For the treatment, 4 g of dried petals were brewed with distilled water (1 : 1) and this herbal tea was used as organic priming medium after cooling. Seeds of organic priming treatment were moistened on filter papers with 30 ml of herbal tea in 15 cm Petri dishes. Primed seeds were kept at 25°C for 72 h in the dark [Mavi 2016].

The main idea of including hydro priming treatment in the study was to compare the results with the organic priming applications. For this aim, seeds of hydro priming experiment were kept with water

(10 ml) for 72 h. Temperature, duration, and all other conditions were the same for both organic priming and hydro priming.

In all treatments, Petri dishes were covered with plastic film to prevent moisture loss during the priming treatment. The Petri dishes and incubator were turned off during the treatments. After the treatment, the seeds were rinsed under tap water and used in emergence tests after surface drying.

As part of the study, the parameters of emergence percent (E), time to 50% of emergence (ET_{50}), mean emergence time (MET), early count emergence percent (ECEP), seedling fresh weight (SFW), seedling dry weight (SDW) and seedling vigor index (SVI) were evaluated. Besides, standard germination percent and 100 seed weight were calculated for non-treated seeds of each species included in the study.

Germination tests were conducted in Petri dishes using filter paper moistened with distilled water. The dishes were placed in a seed germinator (ES120 Nüve Cooled Incubator, TR) at 25°C for germination. All the seedlings with the radicle at least 2 mm in length were considered as germinated. The seed germination was recorded for 30 days, and germination percentages were calculated as the average of three replicates of 100 seeds.

In the emergence experiments, the seeds were sown in plastic trays (container number, 12 × 16; volume 15 cc) filled with peat (Potground P, 70 L, Klasmann, Germany), and placed under room conditions (minimum of 21°C and maximum temperature of 27°C).

Seedling emergence was recorded after the hypocotyls had risen above the surface of the growing media. The seedling emergence was recorded for 40 days, and the emergence percentages were calculated as the average of three replicates of 50 seeds.

Time to 50% (T_{50}) of emergence was calculated from the grade line formula:

$$T_{50} = ti + \left[\frac{\frac{(N+1)}{2} - ni}{nj - ni}\right](tj - ti)$$

where: *N* is the final number of seeds emerged, and *ni* and *nj* are total number of seeds emerged by adjacent counts at time *ti* and *tj*, ($ni < (N+1)/2 < nj$) [Coolbear et al. 1984].

The mean emergence time (MET) was calculated according to Orchard [1977]:

$$\text{MET} = \Sigma\,(tn)/\Sigma n$$

where: *t* is the time in days from 0 to the end of the emergence test, and *n* is the number of emerged seeds on the day *t*. Early count emergence percentage (ECEP) represents the rate of seeds emerged at 13^{th} day of emergence tests.

For the evaluation of seedling performance, seedling fresh weight (SFW), seedling dry weight (SDW) and seedling vigor index (SVI) were calculated. SFW represents the weight of seedlings cut when they had 2–4 true leaves. After weighting, those seedlings were dried in a dry oven at 65°C until they reached constant weight. The weight of this dried seedlings represented SDW. Seedling vigor index (SVI) was calculated according to the following formula:

$$\text{Seedling vigor index} = \text{Emergence percentage} \times \text{Seedling dry weight}$$

As part of the statistical analyses, the percentage values, standard germination, emergence and early count emergence percentage, were transformed by the angle transformation before variance analysis. Differences among mean results of the treatments were analyzed by Duncan's test ($P < 0.05$). IBM SPSS Statistics 24 software was used for statistical analyses.

RESULTS AND DISCUSSION

Standard germination rates and 100 seed weights of the species were given in Table 1. Germination rates of the species varied between 9.3% (*M. rubra*) and 66.5% (*M. alba*, purple). Weights of 100 seeds varied between 0.16 (*M. alba*, white) and 0.21 (*M. rubra*). Song et al. [2016] reported the germination rate of *Morus bombycis* Koidz. between 20% and 30%. Shafiei and Basavaiah [2017] used three different sized seeds in their study on a *Morus* species, of which name was not stated, and 100 seed weight of smallest seeds were reported as 0.27 g, heavier than the seeds used in this study.

For all evaluated parameters of pre-sowing treatment experiments, mean squares of two way interactions, their significance levels and main effects were shown in Table 2. According to ANOVA results, all parameters were significantly affected by cultivar and treatment, cultivar × treatment interactions. Results showed that effects of different treatments on E, ET_{50}, MET, ECEP, SFW, SDW and SVI in all species statistically varied at the significance level of 0.01 or 0.05.

Emergence percentages of treated mulberry species seed calculated by angle transformation and mean emergence time calculation results were given in Table 3. Besides, results of emergence percentages of each count made on 7^{th}, 13^{th}, 16^{th} and 23^{th} days after sowing were illustrated in Figure 1. According to the results, pre-sowing treatments significantly affected the emergence of *M. alba* seeds. The highest emergence percentage (52%) was obtained from priming with GA_3 in *M. alba* (white) seeds, whereas in *M. alba* (purple) seeds, hydro priming gave the highest percentage (90%). Pre-sowing treatments included in the study, reduced MET in general, but the effects varied between species. While decreases were not significant in *M. rubra* and *M. laevigata*, they were in both *M. alba* species. Similarly, Koyuncu [2005] reported GA_3 pre-treatments as enhancing the germination rate and reducing mean germination time due to its dormancy breaking effect, according to the study conducted with *M. nigra* seeds. Similar effects were also observed on seeds of *Ficus carica* L. [Caliskan et al. 2012].

Table 1. Standard germination and seed weight of mulberry species

Species	Standard germination (%)	100 seed weight (g)
Morus rubra	9.3 ±1.1	0.21
Morus laevigata	32.7 ±2.2	0.24
Morus alba (white)	11.7 ±6.8	0.16
Morus alba (purple)	66.5 ±1.8	0.17

Table 2. Analysis of variance for the mean squares of emergence (%), time to 50% of emergence (ET_{50}), mean emergence time (MET), early count emergence percentage (ECEP), seedling fresh weight (SFW), seedling dry weight (SDW), seedling vigor index (SVI) of *Morus* species as affected by species and treatments

Source	df	E (%)	MET (day)	ET_{50} (day)	ECEP (%)	SFW (mg)	SDW (mg)	SVI
Species (S)	3	5070.46**	76.14**	115.37**	10810.56**	46573.71**	162.99**	10052473.31**
Treatment (T)	4	90.06*	19.23**	13.22**	1516.14**	13917.04**	100.89**	974404.43**
S × T	12	95.86**	3.47*	7.15**	352.86**	3932.86**	43.15*	545121.81**
Error	40	19.98	1.60	1.06	31.08	1083.48	18.42	164074.53
CV (%)		9.76	9.02	8.59	10.94	13.63	15.16	17.30

**Significant at $P < 0.01$; *Significant at $P < 0.05$

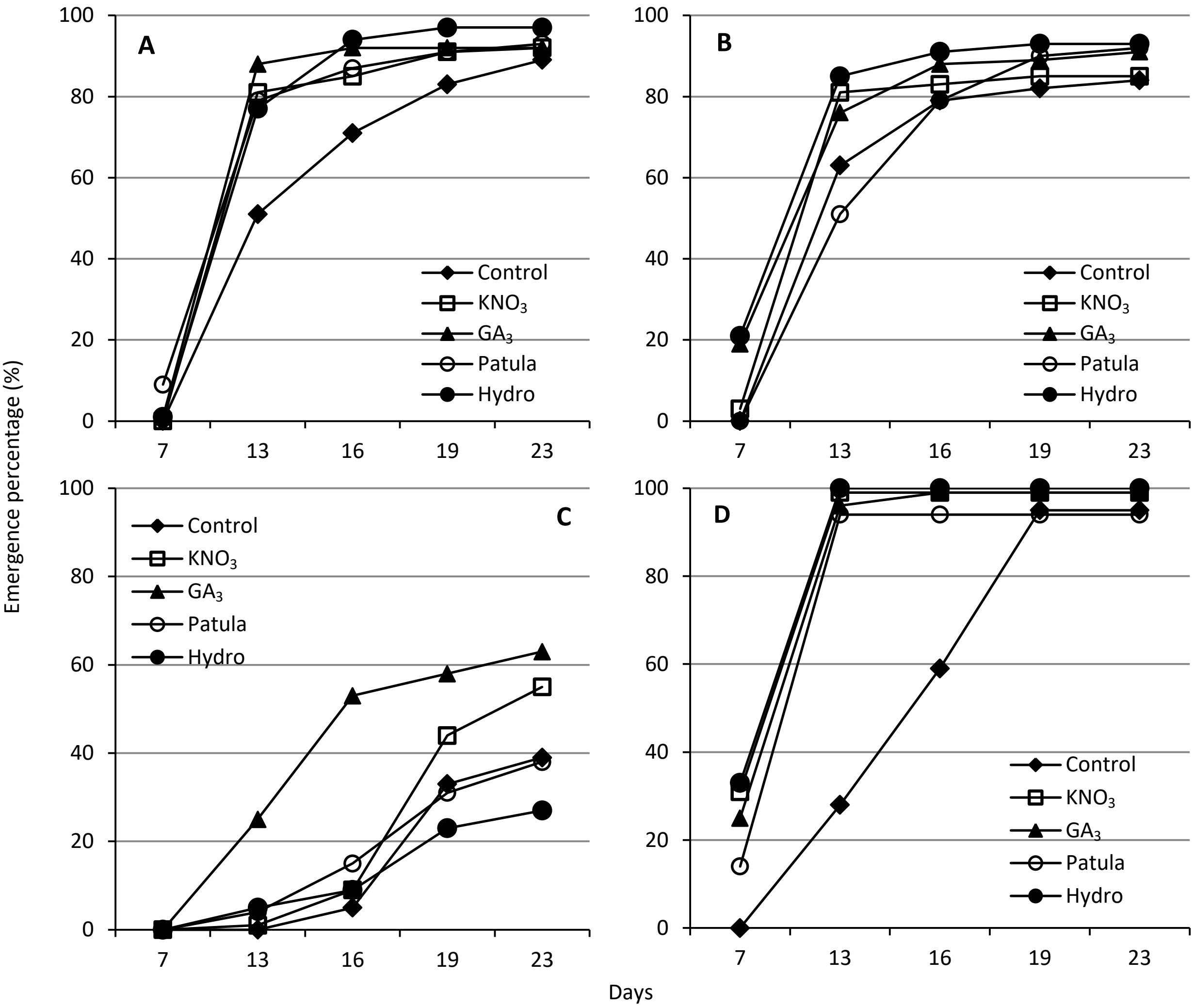

Fig. 1. Cumulative seedling emergence of primed and control *Morus* species seeds. A: *Morus rubra*, B: *Morus leavigata*, C: *Morus alba* (white), D: *Morus alba* (purple)

Table 3. Emergence percentage (transformed) (E) and mean emergence time (MET) and in five treatments of mulberry species seeds

Treatment	E (%)				MET (day)			
	M. rubra	*M. laevigata*	*M. alba* (white)	*M. alba* (purple)	*M. rubra*	*M. laevigata*	*M. alba* (white)	*M. alba* (purple)
Control	71	67	39 bc	80 ab	14.5	14.0 a	19.2	16.3 a
KNO_3	77	68	48 ab	85 ab	13.7	13.0 b	16.2	11.1 b
GA_3	74	73	52 a	85 ab	13.2	11.3 c	15.5	10.3 b
Patula	76	74	38 bc	77 b	13.2	14.6 a	17.4	12.1 b
Hydro	82	75	31 c	90 a	13.6	12.2 bc	18.1	11.0 b

Values in the same column that are followed by different letters are significantly different ($P < 0.05$) using Duncan's comparison test

Table 4. Early count emergence percentage (ECEP) (13^{th}) and time to 50% of emergence (ET_{50}) in five treatments of mulberry species seeds

Treatment	ECEP (13^{th})				ET_{50}			
	M. rubra	*M. laevigata*	*M. alba* (white)	*M. alba* (purple)	*M. rubra*	*M. laevigata*	*M. alba* (white)	*M. alba* (purple)
Control	45 b	52 b	0 c	31 b	12.5	11.2 b	16.2 a	14.7 a
KNO_3	65 a	65 a	5 c	85 a	11.4	10.1 c	17.5 a	8.5 b
GA_3	70 a	61 a	30 a	81 a	11.0	9.8 c	13.5 b	9.1 b
Patula	63 a	46 b	11 b	77 a	10.4	12.5 a	16.4 a	9.5 b
Hydro	62 a	68 a	12 b	90 a	10.8	9.3 c	16.8 a	8.4 b

Values in the same column that are followed by different letters are significantly different ($P < 0.05$) using Duncan's comparison test

Table 5. Seedling fresh weight (SFW) and seedling dry weight (SDW) in five treatments of mulberry species seeds

Treatment	SFW (mg)				SDW (mg)			
	M. rubra	*M. laevigata*	*M. alba* (white)	*M. alba* (purple)	*M. rubra*	*M. laevigata*	*M. alba* (white)	*M. alba* (purple)
Control	247.3 b	248.3 b	138.7 c	132.0 c	26.0 b	28.0	18.3 b	20.7 b
KNO_3	305.3 ab	279.3 ab	250.7 a	276.0 a	31.3 ab	32.7	23.3 b	31.0 a
GA_3	258.3 b	265.3 b	243.0 ab	191.3 b	30.7 ab	30.3	34.6 a	22.3 ab
Patula	337.3 a	267.7 b	180.3 bc	139.7 c	33.7 a	29.0	24.3 b	28.0 ab
Hydro	336.3 a	342.0 a	212.3 ab	178.0 bc	35.0 a	34.3	22.7 b	30.0 ab

Values in the same column that are followed by different letters are significantly different ($P < 0.05$) using Duncan's comparison test

Table 6. Seedling vigor indexes (SVI) in five treatments of mulberry species seeds

Treatment	SVI			
	M. rubra	*M. laevigata*	*M. alba* (white)	*M. alba* (purple)
Control	2334.7 b	2362.7	726 c	1981.3 b
KNO_3	2878.7 ab	2668.0	1270 b	3055.3 a
GA_3	2814.0 ab	2731.3	2166.7 a	2204.7 ab
Patula	3141.3 a	2800.0	904.7 bc	2624 ab
Hydro	3400.0 a	3176.0	589.3 c	3000 a

Values in the same column that are followed by different letters are significantly different ($P < 0.05$) using Duncan's comparison test

In order to evaluate the effects of treatments on reducing emergence time, which is of importance in obtaining the uniform seedlings, MET, ECEP and ET_{50} parameters were counted and the results of those parameters were presented on Tab. 3 (MET) and Table 4 (ECEP and ET_{50}). Results indicated that pre-sowing treatments significantly increased ECEP. With all species, GA_3 and hydro primed seeds displayed significantly higher values compared to control seeds. Except from *M. rubra*, ET_{50} values were significantly reduced by pre-sowing treatments in all species, and GA_3 priming was the only pre-sowing treatment that significantly reduced ET_{50} to control in all species. Similar to our results, previous studies on various *Morus* species declared that seed treatments could stimulate the MET and ET_{50}

[Burton and Bazzaz 1991, Gunes and Cekic 2004, Koyuncu 2005].

In order to evaluate plant vigor characteristics of seedlings obtained from treated and non-treated seeds, fresh and dry weights of the seedlings and seedling vigor indices were calculated and the results were expressed in Table 5 and 6. The effects of treatments varied between the species. For example, when the highest SFW value was obtained from organic priming (337.3 mg) treatment in *M. rubra*, it was obtained from hydro priming (342.0 mg) in *M. laevigata* and KNO_3 (250.7, 279.0 mg, respectively) in both *M. alba* species. Similarly, while hydro priming treatment resulted with the highest SDW value in *M. rubra* (35.0 mg) and *M. laevigata* (34.3 mg) seeds, GA_3 (34.6 mg) and KNO_3 (31.0 mg) were leading pre-treatments in *M. alba* (white) and *M. alba* (purple) species, respectively.

When the results of seedling weight and vigor index values were compared, it was observed that treated seeds consisted vigorous seedlings rather than non-treated ones. Hence, fresh and dry weights, and vigor indices of the seedlings obtained from treated seeds were relatively higher. In *M. rubra* (3400.0) and *M. laevigata* (3176.0) species, hydro priming gave the highest SVI values, whereas GA_3 (2166.7) and KNO_3 (3055.3) gave the highest SVI values in *M. alba* (white) and *M. alba* (purple) species, respectively.

CONCLUSIONS

Results of this study, which were conducted to determine the effects of different pre-sowing applications on seeds of different mulberry species indicated that together with varying effects of pre-treatments between species and parameters, when compared with non-treated seeds, emergence percentage and time, and seedling vigor were improved by the priming treatments included in the study. Even though germination rates of the species except from *M. alba* (purple) were found low in standard germination tests, germination occurred in all species indicating that there was no primer (physiological) dormancy in that species. As a result of overall evaluations, it was concluded that pre-sowing applications, especially GA_3 and hydro priming, were beneficial in improving the seed performance of the mulberry species included in the study. In future studies, the effects of high temperature applications on relatively low emergence rates of *M. alba* (white) and the effects of light and temperature on that secondary dormancy should be determined.

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