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Effects of Different Pretreatments and Dark-light Conditions on the Seed Germination of Different Mulberry Species

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The research was carried out to determine the seed germination of four different mulberry species. The seeds were exposed to the following treatments prior to germination: scarification with absolute H_2SO_4; H_2SO_4 + 1000 ppm GA_3 application; soaking in water for 24 h; GA_3 application in concentrations of 1000, 2000 and 4000 ppm for 24 h. The seeds sown in petri dishes containing two sheets of filter paper were incubated at 25 ± 1°C in dark and light conditions. Most pretreatments, except in one or two cases, significantly enhanced the seed germination of all mulberry species. Full germination was obtained from all concentrations of GA_3 for white and red mulberries and from 2000 ppm and 4000 ppm concentrations for pendulous mulberry. Treatment of 1000 ppm GA_3 resulted in greater germination in black mulberry among the treatments. Germination did not occur in water-soaked and control seeds of black mulberry at the end of three weeks. The result of this study showed that the darkness had a positive effect on the germination rate as well as germination percentage.

Key Words: Germination, Gibberellic acid, GA_3. Mulberry, Scarification, Seed.

INTRODUCTION

Mulberries are generally propagated by seeds (sexual) or vegetative parts of the plants (asexual). Asexual propagation methods like budding, cutting or layering are commonly used in mulberries, as in other fruit species. Poor rooting of stem cuttings and incompatibility in budding[1] cause problems in vegetative propagation methods, especially in black mulberries. These methods are also complex and require greenhouse facilities. The average rooting from stem cutting sampled in May, September and January was only 7%, regardless of time of the year[2]. Different rootstock-scion combinations can solve the incompatibility problem. However, the propagation of rootstock by seed is more practical as compared to the other vegetative methods in mulberry species.

The rate of seed germination is species-specific and can be highly influenced

by a number of environmental factors. Hartmann and Kester[3] classified the seeds of different species according to the germination requirements. They included mulberries in the group so that the seeds may germinate in 14–28 days with artificial light and at alternating temperatures of 20–30°C. Similarly, the International Seed Testing Association (ISTA)[4] recommends testing non-dormant mulberry seeds on top of the moist blotters for 28 days at diurnally alternating temperatures of 30°C (day) for 8 h and 20°C (night) for 16 h when the seeds were not pretreated. Petkov[5] reported that white mulberry seeds were germinated at 25°C after they had been treated with GA_3, IBA, urea and potassium humate alone or combined. The germination percentage of red mulberry seeds varied from 12 to 50% when sown in fall without any pretreatment[2]. Germination percentage of untreated seeds in the laboratory varies because part of each collection consists of seeds with dormant embryos and impermeable seed coats. Under different light and temperature conditions, higher germinations were obtained varying from 20 to 92%[6, 7]. Several endogenous or exogenous factors can affect the germination of mulberry seeds. Vigour and fertility of seeds are the major requirements for good germination. Some researchers reported that seed vigour was lost parallel to seed aging. Huffman[8] reported that the germination was 89% when the seeds were germinated 4–5 days after harvesting. One week delay after harvest decreased the germination to 73%. Similarly, soaking in water for 48 and 72 h also reduced the germination to 56 and 33%, respectively. Therefore, germinating the seeds in the first three days after harvesting and soaking the seeds in water no more than 24 h prior to germination was recommended. On aging of seeds, a certain percentage will die and not germinate. Naturally, this percentage can also be influenced by the environment of the seeds kept. Although fresh seeds had 90% germination success, the germination of the seeds stored six months at room temperature 25°C and 5°C decreased the germination to 51, 55 and 79%, respectively and to 12, 22 and 65% after a year of storage[9]. Similarly, Dirr and Heuser[10] obtained 75% germination from white mulberry seeds when extracted from freshly collected fruits, cleaned and sown immediately.

The main purpose of pretreatments applied to seeds is avoiding the negative effects of endogenous or exogenous factors inhibiting the germination of seeds. To obtain an optimum germination, the seeds are subjected to pretreatment to remove negative effects prior to sowing, such as: scarification, stratification, soaking in water, pre-chilling and growth regulator applications. Sometimes, these pretreatments can be used together as needed[11]. Ellis *et al.*[12] reported that scarification, pre-chilling and alternating temperatures are successful dormancy-breaking treatments for *M. alba, M. nigra* and *M. rubra*.

In order to meet the growing demand of high-quality grafted mulberry trees, especially black mulberry, good germination, and therefore good quality, are needed for improvement of rootstock-scion grafting success without incompatibility problems. The aim of this study was to obtain the best germinations and determine pretreatments for the seeds of mulberry species.

EXPERIMENTAL

The white (*Morus alba*), black (*Morus nigra*), red (*Morus rubra*) and pendulous (*Morus alba var. pendula*) mulberry species seeds were collected from naturally growing populations in Tokat province of Turkey. The mature fruits were squashed to remove water content. The heavier seeds sank to the bottom and the lighter pulp, empty seeds and other extraneous materials floated off the top of water[2, 3, 7]. The seeds were cleaned from sediment and washed several times under tap water, then dried in darkness until seed moisture content became 12–15% varying to different species. Just after the drying process, the following pretreatments were applied to the seeds of the mulberry species; soaking in water for 24 h, scarification (with H_2SO_4 + soaking in water for 24 h), H_2SO_4 + GA_3 1000 ppm application, and 1000 ppm, 2000 ppm and 4000 ppm concentrations of GA_3 applications for 24 h and control with no pretreated seeds.

Two units of absolute (95%) H_2SO_4 (mL) for one unit seed (g) were used for scarification[3]. The black mulberry seeds with a thicker coat were soaked for 1 min and the seeds of another species with a thinner coat were kept for 30 s in H_2SO_4, since prolonged applications of H_2SO_4 damage the embryos in preliminary tests. Following the pretreatments, the seeds were placed on to two sheets of filter paper in sterilized petri dishes. The seeds were watered with adequate sterile water, and the petri dishes were separately incubated at 25 ± 1°C in dark and light conditions[5]. Now each seed was cut transversely into one-third at the end away from the radicle and the seed samples (30 seeds from each species) were treated with 1% of triphenyl tetrazolium chloride (TTC) solution (made up in water) for 24 h in the dark in order to determine the viability. The embryo of seeds stained red was assumed to be live and the non-stained ones dead[3].

Analysis of variance was performed on the data using MSTAT 5.0 (Michigan State University) version and the mean separation was compared by least significant difference (LSD) procedure. For both viability and germination treatments, completely randomized design (CRD), two-factor factorial with three replications were used. Twenty seeds were tested for each replication. Data were transformed using the arc-sine transformation[13] and non-transformed means were presented.

RESULTS AND DISCUSSION

Since only the seeds settled at the bottom of water during the extraction from fruits were used in the viability test, the seeds of all mulberry species showed 100% viability. The effects of different pretreatments on germination of mulberry species are presented in Table-1. The interaction of both light and treatment on seed germination was significant at $P \leq 0.01$ level for white and pendulous, and significant at $P \leq 0.5$ level for red and black mulberry seeds. The best germinations were obtained from GA_3 applications for all species among the pretreatments applied. The seeds of white, red and pendulous mulberries that resemble morphologically showed similar reactions to all pretreatments applied. The seed germination percentages of the three species investigated were ranged from 35 to 100%. All GA_3 concentrations used resulted in 100% germination in light or

dark conditions, except 1000 ppm GA_3 concentration that resulted in 98.3% germination in pendulous mulberry in light. H_2SO_4 application alone or combined with 1000 ppm GA_3 had negative effect on germination. The black mulberry species, on the other hand, showed slightly different reaction than that of the other species. Germination did not occur in both control and water soaked seeds of this species. In contrast to the other species, scarification enhanced the germination of black mulberry seeds (Table-1). The darkness generally had positive effect and the germination percentage increased with darkness for the majority of cases studied.

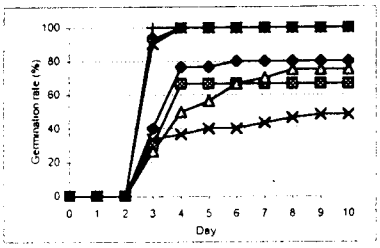
TABLE-1
EFFECT OF PRETREATMENTS ON THE GERMINATION OF DIFFERENT MULBERRY SPECIES (%)

Treatment	Light condition	White mulberry	Red mulberry	Pendulous mulberry	Black mulberry
Control	Light	80.0 bc†	35.0 d*	78.3 de†	0.0 f*
	Darkness	95.0 ab	88.3 b	91.6 a-d	0.0 f
Soaking in water	Light	65.0 cd	65.0 bc	71.6de	0.0 f
	Darkness	96.6 ab	71.6 bc	96.6 a-c	0.0 f
H_2SO_4 + soaking in water	Light	75.0 cd	55.0 cd	65.0 e	51.6e
	Darkness	46.6 d	71.6 bc	85.0 b-e	75.0 cd
H_2SO_4 + GA_3 1000 ppm	Light	48.3 d	71.6 bc	78.3 c-e	88.3 b-d
	Darkness	48.3 d	85.0 b	58.3 e	100 a
GA_3 1000 ppm	Light	100 a	100 a	98.3 ab	100 a
	Darkness	100 a	100 a	100 a	91.6 a-c
GA_3 2000 ppm	Light	100 a	100 a	100 a	91.6 a-c
	Darkness	100 a	100 a	100 a	75.0 de
GA_3 4000 ppm	Light	100 a	100 a	100 a	91.6 a-c
	Darkness	100 a	100 a	100 a	96.6 ab

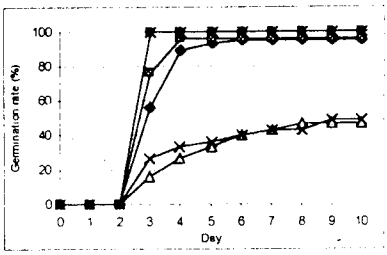
*, † Means with the same letter in a column are not statistically different at 0.05 and 0.01 level of probability, respectively.

The effect of treatments and light-dark conditions on the germination rate of the different species are illustrated in Fig. 1. The black mulberry seeds generally emerged later as compared to the seeds of other species. While the highest germination percentages were obtained in the first four days after sowing from the seeds of white, red and pendulous mulberries, the black mulberry seeds reached the highest germination percentage in 12 to 16 days. Faster seed emerges were observed in darkness, particularly for GA_3 treated seeds of white, red and pendulous mulberries.

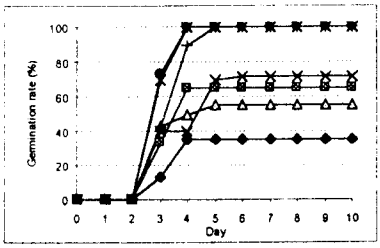
Mulberry species seeds treated with TTC were determined as viable. This was probably due to the method adopted for seed extraction where the only seeds settled at the bottom of water were used as suggested by Lamson[2], Hartmann and Kester[3] and Barbour *et al.*[7]



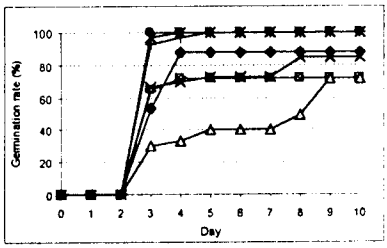
White mulberry-light



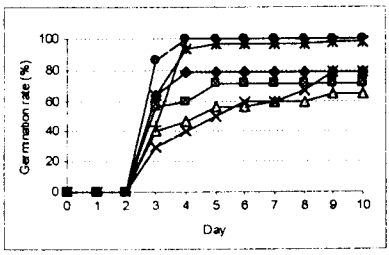
White mulberry-dark



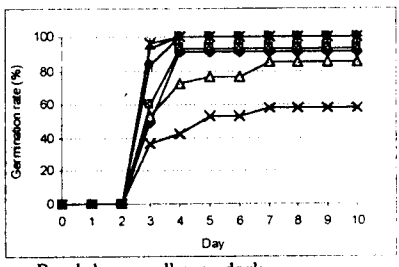
Red mulberry-light



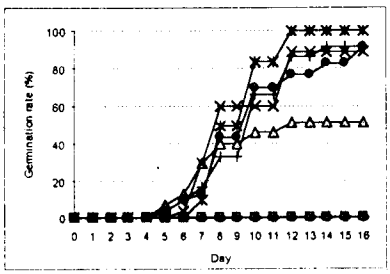
Red mulberry-dark



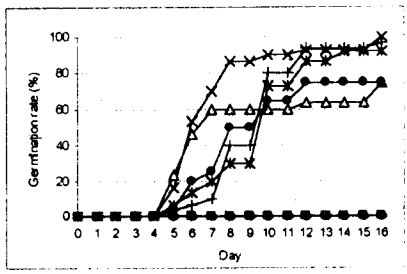
Pendulous mulberry-light



Pendulous mulberry-dark



Black mulberry-light



Black mulberry-dark

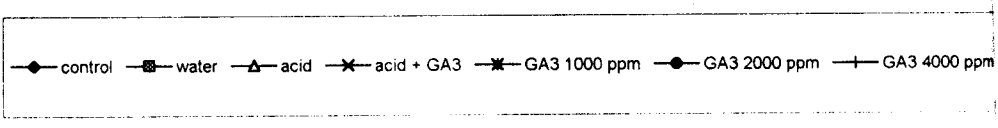


Fig. 1. Germination and germination rates of the seeds treated with different pregermination processes in white, red, pendulous and black mulberries

The majority of pretreatments applied to the mulberry species seeds investigated, significantly enhanced germination. In some cases, especially for white and pendulous mulberry, scarification of seed with H_2SO_4 had negative effect, and resulted in lower germination than that of untreated seed. This may be due to the corrosive effect of H_2SO_4 on the thin seed coat, damaging the embryo consequently. Therefore, scarification of thin seed coats in white, red and pendulous mulberry is not recommended before germination. On the other hand, H_2SO_4 treatment increased the germination percentage of black mulberry seeds which have thicker coats from no germination to 51.66% in light and 75.00% in dark. Compared to the previous studies on the germination of mulberry seeds[2, 6, 8, 9, 14], higher germination percentages were obtained in this study reaching to 100% for some pretreatments. GA_3 pretreatments were more effective on germination than other pretreatments used. Among the GA_3 concentrations, 1000 ppm alone or combined with H_2SO_4 was more effective in black mulberry. However, concentration of GA_3 did not show any significant differences for the other three mulberry species investigated. GA_3 pretreatments had a positive effect on the germination rate as well as the enhanced germination percentage by initiating the germination by the second day of sowing. Huffman[8] and Thomas[9] obtained the best germination from fresh seeds taken from just-harvested fruits. Black mulberry seeds did not germinate without any pretreatments. This shows the existence of an obligate seed dormancy after harvesting and should be broken up with pretreatments. On the other hand, the other species studied did not exhibit a certain dormancy, but germination can be enhanced with pretreatments. The seeds of black mulberry soaked in water for 24 h prior to germination was not sufficient enough to break up dormancy. The reason is, either water treatment is not effective alone or the duration of water application needs to be prolonged. The black mulberry seeds with thicker coat had lower germination percentage as compared to the other species with thinner seed coat.

Darkness also enhanced the germination rate of black mulberry seeds treated with H_2SO_4 and combination of H_2SO_4 + 1000 ppm GA_3 (Fig. 1). The results indicated that both internal and external factors cause dormancy. The lower germination of black mulberry seeds treated with H_2SO_4 reveals that some internal factors also cause dormancy. While the seeds applied with GA_3 showed higher germination, the scarified seeds couldn't reach the same germination rate. Without a solid conclusion the results indicate that darkness can enhance both germination percentage and rate in many cases. Consequently, treatment of 1000 ppm GA_3 is recommended as a useful pretreatment prior to the germination of all mulberry species evaluated in this study.

REFERENCES

1. A. Unal, R. Ozcagiran and S. Hepaksoy, A Research on the Rooting of Hard-wood Cuttings Black and Red Mulberry, Turkey 1st National Horticulture Congress, Izmir, pp. 267–270 (1992).
2. I.N. Lamson, *Morus rubra* L. (Red Mulberry), in: Russell M. Burns and Barbara H. Honkala (Technical Coordinators), Silvics of North America, Vol. 2, Hardwoods Agric. Handbook 654, U.S. Department of Agriculture, Forest Service, Washington, DC, pp. 470–473 (1990).
3. H.T. Hartmann and D.E. Kester, Plant Propagation, Principles and Practices, Prentice-Hall, Inc., Englewood Cliffs, New Jersey, p. 662 (1975).
4. ISTA, International Rules for Testing Seeds, Rules 1999, *Seed Science Technology*, **27** (Suppl.), 333 (1999).
5. Z. Petkov, *Rasteniev"dni Nauki*, **32**, 149 (1995).
6. R.A. Read and R.L. Barnes, *Morus* L. Mulberry, in: C.S. Schopmeyer (Technical Coordinator), Seeds of Woody Plants in the U.S., U.S. Department of Agriculture, Agricultural Handbook 450, Washington, DC, pp. 544–547 (1974).
7. J.R. Barbour,, R.A. Read and R.L. Barnes, *Morus* L. (Mulberry), Woody Plant Seed Manual (online: http://wpsm.net), pp. 1–10 (2002).
8. G.R. Huffman, Forest and Conservation Nursery Association Meeting, General Technical Report, Portland (1996).
9. T.D. Thomas, Storage, Viability and Effect of Some Growth Regulators on Viability in Mulberry (*Morns alba* L.), XVI International Botanical Congress, (1999).
10. M.A. Dirr and C.W. Heuser, The Reference Manual of Woody Plant Propagation: From Seed to Tissue Culture, G.A. Varsity Press, Athens, p. 239 (1987).
11. J.K.A. Bleasdale, Plant Physiology in Relation to Horticulture, Macmillan Educ. Ltd. (1984).
12. R. Ellis, T.D. Hong and E.H. Roberts, Handbook of Seed Technology for Genebank, Vol. II, Compedium of Specific Germination Information and Test Recommendations, International Board for Plant Genetic Resources, Rome (1985)
13. A.K. Gomez and A. Gomez, Statistical Procedures for Agricultural Research, 2nd Edn., John Wiley & Sons, Inc. (1984).
14. Y. Huo, Mulberry Cultivation and Utilization in China, FAO Electronic Conference on Mulberry for Animal Production (2000).

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