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# BREAKING SEED DORMANCY IN BLACK MULBERRY (*MORUS NIGRA* L.) BY COLD STRATIFICATION AND EXOGENOUS APPLICATION OF GIBBERELLIC ACID

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Experiments were done to determine the effects of cold stratification (0, 20, 40, 60, 80 or 100 days), application of gibberelic acid ($GA_3$) (0, 250, 500, 1000 or 2000 mg/l) and the combination ($GA_3$ + stratification) on seed germination of black mulberry. Application of 1000 mg/l $GA_3$ proved more effective than any of the other concentrations of $GA_3$ applied. Seeds stratified for 100 days showed 88% germination. The combined treatment of 250 mg/l $GA_3$ and 100 days of stratification yielded 96% germination of seeds. The relationships between $GA_3$ concentration and seed germination (r = 0.93), and between stratification duration and seed germination (r = 0.91) of black mulberry were linear.

**Key words:** Dormancy, black mulberry, germination, gibberellic acid, stratification.

## INTRODUCTION

Seeds are of importance for propagating seedling rootstocks on which to graft or bud varieties, and for obtaining hybrid plants in breeding studies (Westwood, 1995; Hartmann et al., 1997). Whether or not a viable seed germinates and the time at which it does so depend on a number of factors, including factors in the seed's environment (Bewley and Black, 1994). Seed germination is influenced by internal factors controlling dormancy, including phytohormones (e.g., abscisic acid) inducing dormancy, and by seed coat factors (seed coat-enhanced dormancy) (for review: Bewley, 1997). Dry seeds of most temperate trees and shrubs, even though mature, will not germinate and grow until they been imbibed to threshold moisture content under cold conditions (0–5˚C) (cold stratification) (Hartmann et al., 1997). The dormancy of dormant seeds must be broken to induce germination. Various methods are used for this, depending on the plant species and type of dormancy. Chilling plays an important role in providing the stimulus required to overcome dormancy, increase germination, and produce normal seedlings for *Prunus persica* cv. GF305 (Martinez-Gomez and Dicenta, 2001), strawberry tree (Karam and Al-Salem, 2001) and *Prunus avium* (Jensen and Eriksen, 2001). Exogenous growth regulator treatments – gibberellins (usually gibberellic acid $GA_3$ and $GA_{4+7}$) and cytokinins (usually kinetin, benzyladenine) – have been shown to break dormancy in many seed species (Dweikat and Lyrene, 1988; Karam and Al-Salem, 2001; Mehanna et al., 1985).

There have been some studies of the germination ability of *M. alba* seeds (Petkov, 1995), but apparently almost no work has been done on *M. nigra* seeds. The objective of this study was to investigate the effect of stratification, gibberellic acid ($GA_3$) and combination treatments ($GA_3$ + stratification) on germination of black mulberry seeds. The experiments were conducted twice over a 2-year period with consistent results. The data presented here are from the second experimental year.

## MATERIALS AND METHODS

Black mulberry (*Morus nigra* L.) fruits were harvested when ripe in August 2002 from the Egirdir Horticultural Research Institute, Isparta (Turkey). The fruits were soaked in water for 24 h and then the seeds were extracted by hand, washed, dried in the shade for two days, surface-sterilized in 1% aqueous NaOCl solution for 5 min, and then rinsed with distilled water three times. In the stratification procedure (moist chilling),

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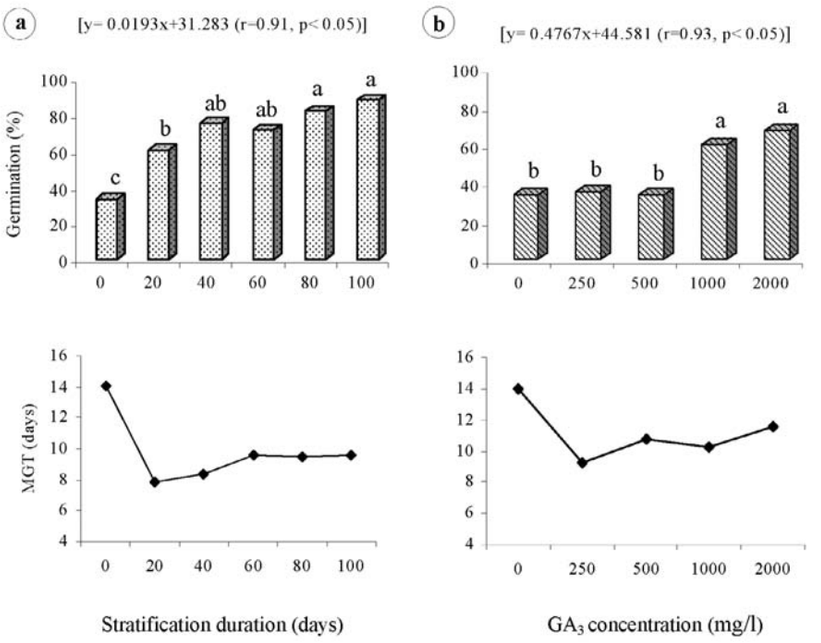


**Fig. 1.** Germination percentage and mean germination time (MGT) of black mulberry seeds under different stratification durations (**a**) and $GA_3$ concentrations (**b**). Seeds were incubated at 25˚C in the dark, and germinating seeds were counted daily for 14 days. Bars followed by different letters differ significantly according to Duncan's multiple range test at p = 0.01.

seeds were placed on filter paper moistened with distilled water in 10 cm diameter Petri dishes and stored in a refrigerator at 4 ± 1˚C for 0, 20, 40, 60, 80 or 100 days. In the gibberellic acid treatments, seeds were imbibed in solutions of $GA_3$ (0, 250, 500, 1000 or 2000 mg/l) for 24 h. The other pregermination treatments were arranged as a 4 ($GA_3$ concentration) × 6 (stratification duration) factorial test. Seeds were imbibed with $GA_3$ (250, 500, 1000 or 2000 mg/l) and stratified for 0, 20, 40, 60, 80 or 100 days. Gibberellic acid was purchased from Sigma. In the germination experiments, seeds of all treatments were placed on filter paper moistened with 3% N-(trichloromethyl) thio-4-cyclohexene–1,2-dicarboximide (Captan) to control fungal attack in Petri dishes, and incubated at 25 ± 1˚C in the dark. Moisture was maintained with distilled water. Germination was counted every day for 14 days. A seed with at least a 2 mm long radicle was considered to be germinated. Germination percentage was calculated as the average of four replicates of 50 seeds, and the mean germination time (MGT) was calculated according to the formula in Bewley and Black (1994):

$$MGT = \Sigma(t \cdot n)/\Sigma_n$$

where $t$ is the time in days starting from day 0 to the end of the germination test, and $n$ is the number of seeds completing germination on day $t$.

Statistical analyses were performed with general linear models (GLM) (SPSS ver. 10, SPSS Inc., U.S.A.). Percentage data were subjected to arcsin transformation and ANOVA was performed. Differences among means were analyzed by Duncan's multiple range test at p = 0.01. Germination percentage values were correlated using linear regressions.

## RESULTS AND DISCUSSION

Stratification had a significant effect on seed germination of black mulberry ($p < 0.01$). Non-stratified seeds gave only 33% germination, whereas seeds stratified for 100 days gave 88% germination (Fig. 1a). Increasing the duration of stratification from 0 to 100 days resulted in up to a 164% increase in germination. A linear equation best described the relationship between the cumulative percentage of germination and the duration of stratification ($r = 0.9$, $p < 0.05$) (Fig. 1a). Stratification might act simply to lower the rate of enzymatic reactions taking place in the seed, and might cause differential changes in enzyme concentrations or in enzyme production (Bewley and Black, 1994). Seeds of strawberry tree (*Arbutus andrachne* L.) stratified at 4˚C for 12 weeks had 86% germination (Karam and Al-Salem, 2001). In a study of 18 different shrub

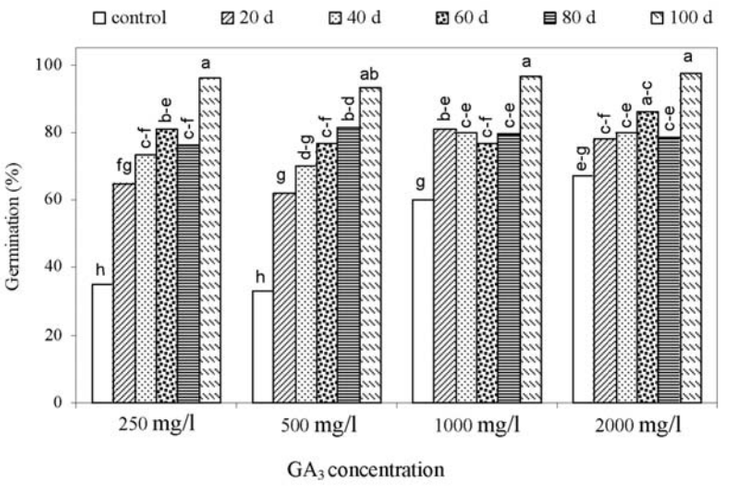


**Fig. 2**. Effect of $GA_3$ and stratification from 0 to 100 days on germination of black mulberry seeds. Seeds were soaked in 250, 500, 1000 or 2000 mg/l solutions of $GA_3$, and then stratified. Seeds were incubated at 25°C in the dark, and germinating seeds were counted daily for 14 days. Bars followed by different letters differ significantly according to Duncan's multiple range test at $p = 0.01$.

species, moist pre-chilling yielded maximum germination (Stidham et al., 1980). Koyuncu and Sesli (2000) reported that 125 days of stratification had a significant effect on the germination percentage of *Juglans regia* L. nuts. These reports and our results show that stratification is successful in breaking seed dormancy, though the duration of treatment may vary with the species.

Pre-treatment with $GA_3$ significantly enhanced germination of non-stratified black mulberry seeds ($p < 0.01$). $GA_3$ treatments at 1000 and 2000 mg/l concentrations yielded the highest germination percentage (60–67%; Fig. 1b). Increasing the concentration of $GA_3$ resulted in an increase in germination percentage. Regressions indicated that the germination rate had a highly significant positive correlation with the $GA_3$ concentrations ($r = 0.93$, $p < 0.05$; Fig. 1b). $GA_3$ has been found to be effective in increasing germination in several species and to break dormancy in dormant seeds. Pre-treatment of blueberry seeds with $GA_{4+7}$ at 100–500 mg/l accelerated germination (Ballington, 1984). *Arbutus andrachne* L. (eastern strawberry tree) seeds treated with 250 mg/l $GA_3$ had 86% germination (Karam and Al-Salem, 2001). It has been reported that germination can be induced by gibberellic acid in *Vaccinium myrtillus* L. (Giba et al., 1993), *Vaccinium corymbosum* L. (Dweikat and Lyrene, 1988) and *Fagus sylvatica* (Nicolás et al., 1996) seeds. These results confirm that $GA_3$ treatment enhances seed germination.

Mean germination time (MGT) decreased with increasing duration of stratification and concentration of $GA_3$ (Fig. 1a,b), indicating that dormancy can be suppressed by moist chilling and exogenous $GA_3$ application. MGT is related to dormancy intensity (Jensen and Eriksen, 2001). The measure of MGT in the present study was measured as the time needed to release dormancy and initiate radicle growth.

The combined treatment with $GA_3$ + stratification had a statistically significant effect on germination (Fig. 2). Seeds treated with 250 mg/l $GA_3$ without stratification gave 35% germination, whereas seeds treated with 250 mg/l $GA_3$ + 100 day stratification gave 96% germination. Increasing the stratification period from 0 to 100 days at 250 mg/l $GA_3$ resulted in up to a 174% increase in germination. A significant ($p < 0.01$) interaction effect of $GA_3$ concentration and duration of stratification was found. Seed dormancy in some species may be due to insufficient development of the embryo, chemical inhibition, or the failure of chemical reactions that make food reserves in the seed available to the developing embryo (Hilhorst and Karssen, 1992; Karam and Al-Salem, 2001). Physiological dormancy in seeds is dependent on the ratio of the levels of abscisic acid (a growth inhibitor) and GA (a growth regulator) (Hilhorst and Karssen, 1992). Serial physiological changes occur in the seed (Hartmann et al., 1997). The chilling process appears to enhance the production of some types of growth-promoting substances such as GA (Powell, 1987). Giba et al. (1993) reported that the inhibitory effect of retardants was overcome by gibberellic acid. Treatment with $GA_3$ + stratification was found to be successful for mahaleb (Gercekcioglu and Cekic, 1999) and plum (Ozvardar and Ozcagiran, 1991). These reports accord with our results showing that $GA_3$ + stratification enhances germination of *M. nigra* seeds.

The results of this experiment confirmed that the black mulberry seeds were in a dormant state. Stratification at 4˚C for 80 to 100 days or 250 mg/l $GA_3$ + 100 days of stratification overcame seed dormancy and increased the germination percentage of black mulberry seeds. Seedlings obtained from stratified or $GA_3$-treated and stratified seeds were transplanted to a 1:1:1 mixture of soil, peat and perlite (v/v). Seedling survival, growth and sprouting were normal.

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