Improving germination of *Prunus avium* L. seeds by gibberellic acid, potassium nitrate and thiourea

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ABSTRACT: To break dormancy and increase the germination of *Prunus avium* L. (mazzard cherry) seeds, various methods were tested including the removal of the seed coat after cold moist stratification and treatment with GA_3, KNO_3, or thiourea. Treatments with 7,500 ppm KNO_3 after 120 days of stratification were more effective, yielding 64.54% germination of seeds with coat. In seeds without coat, 500 ppm GA_3 treatment after 120 days of stratification gave 79.74% germination; a value increased about 29% compared to control.

Keywords: dormancy; germination; gibberellic acid, potassium nitrate; *Prunus avium* L.; seed; thiourea

Dormancy is a condition in which seeds do not germinate even when the environmental conditions (water, temperature and aeration) are permissive for germination (Nikolaeva 1977; Bewley, Black 1994; Hartmann et al. 1997). Various methods have been used by seed scientists and technologists to break seed dormancy. Stratification plays an important role as a stimulator that helps to break dormancy (Bewley, Black 1994; Agrawal, Dadlani 1995; Hartmann et al. 1997). In order to accelerate this method, it can be combined with some treatments such as chemical applications or mechanical seed coat removal (Mehanna et al. 1985; Martinez-Gomez, Dicenta 2001). Many investigators have studied the effects of exogenous growth regulators on seed germination. Gibberellins eliminated the chilling requirements of peach and apple seeds and increased their germination (Rouskas et al. 1980; Mehanna et al. 1985); neither GA nor BAP affected germination of intact non-chilled plum seeds (Lin, Boe 1972). Among other chemicals, potassium nitrate and thiourea are widely used to break dormancy but their role is not clear (Agrawal, Dadlani 1995). The use of potassium nitrate has been an important seed treatment in seed-testing laboratories for many years without a good explanation for its action mechanism. However, thiourea overcomes certain types of dormancy, such as the seed-coat inhibiting effect of deep embryo-dormant *Prunus* seeds (Hartmann et al. 1997).

Seeds of *Prunus avium* L., also known as Mazzard cherry, are deeply dormant when fully mature and the germination of sweet cherry seeds is dispersed in time, as in the case of other species including many annual species which both have primary and secondary dormancy (Jensen, Eriksen 2001; Finch-Savage et al. 2002). Embryo, endosperm, testa and endocarp all contribute to the delay of germination (Jensen, Eriksen 2001). Although some researches have been carried out on dormancy mechanism of *P. avium* seeds, almost no information is available in literature regarding how such treatments enhance their germination. The objectives of the study were to investigate the effects of cold stratification, seed coat and certain chemical treatments on the germination of *Prunus avium* L. seeds and to provide some practical suggestions.

The seeds of *Prunus avium* L., also known as Mazzard cherry, were used for experiments. *P. avium* seeds were harvested from wild sweet cherry trees in Tokat (Turkey). Seeds were surface sterilized in a 1% aqueous NaOCl solution for 5 min and then rinsed with distilled water three times. For stratification (cold-moist chilling), seeds were soaked in water for 24 h, afterwards they were placed on moist agri-perlit layers in perforated plastic boxes (15 × 10 × 5 cm), and they were kept in a cold storage at 4 ± 1°C for 80, 100 and 120 d. At the end of the stratification periods, seeds were separated into two groups: with coats and without coats. In the group without coats, endocarp (only stone coat) was

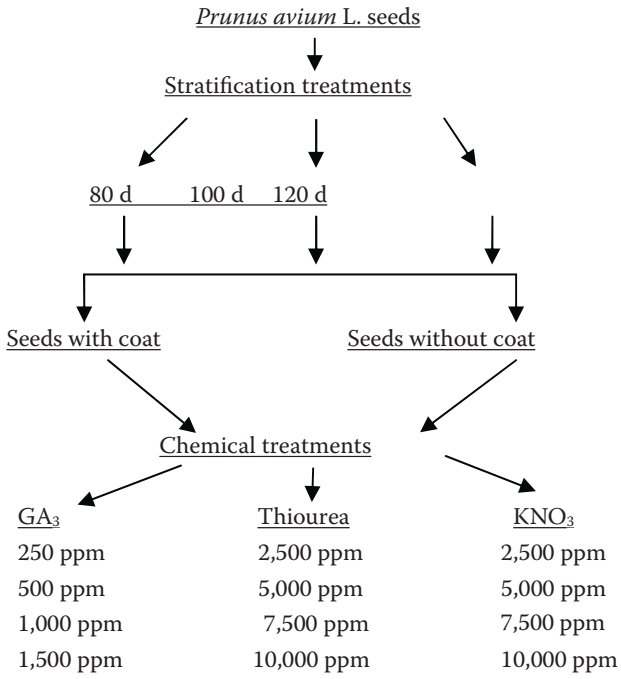

Fig. 1. The schematic diagram of pre-germination treatments

removed manually. For chemical treatments, three chemicals were used to stimulate germination. Each seed group was soaked in solutions of gibberellic acid (GA_3), potassium nitrate (KNO_3) and thiourea [$(NH_2)_2CS$] at different concentrations for 24 hours. A detailed schematic diagram of pre-germination treatments was presented in Fig. 1. Gibberellic acid was purchased from the Sigma Company, KNO_3 and thiourea was obtained from Merck Company.

In germination experiments, seeds of all treatments placed on filter paper moistened with 3% fungicide solution (Captan) in Petri dishes were placed in an incubator at 21 ± 1°C and 70–80% humidity with darkness. Petri dishes were moistened as needed with distilled water to provide humidity. Germination was measured in 3-day intervals during 30 days. All seeds with at least a 5 mm long radicle were considered as germinated. Germination percentage per treatment was calculated as the average of three replicates with 50 seeds. Percentage data of germination were subjected to the angle transformation and the analysis of variance was performed. Pre-ger-

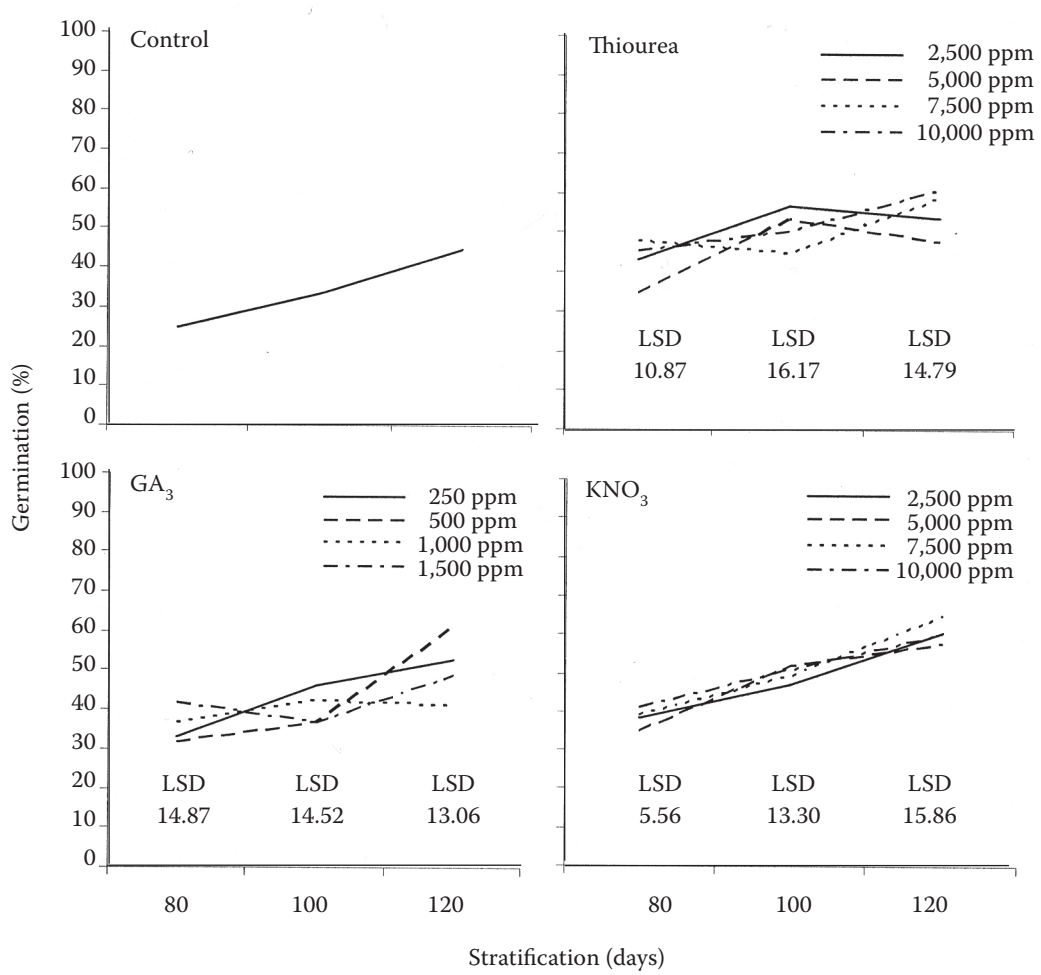

Fig. 2. Effects of GA_3, KNO_3 and thiourea treatments on the germination of 80 to 120 days stratified *P. avium* seeds with coats

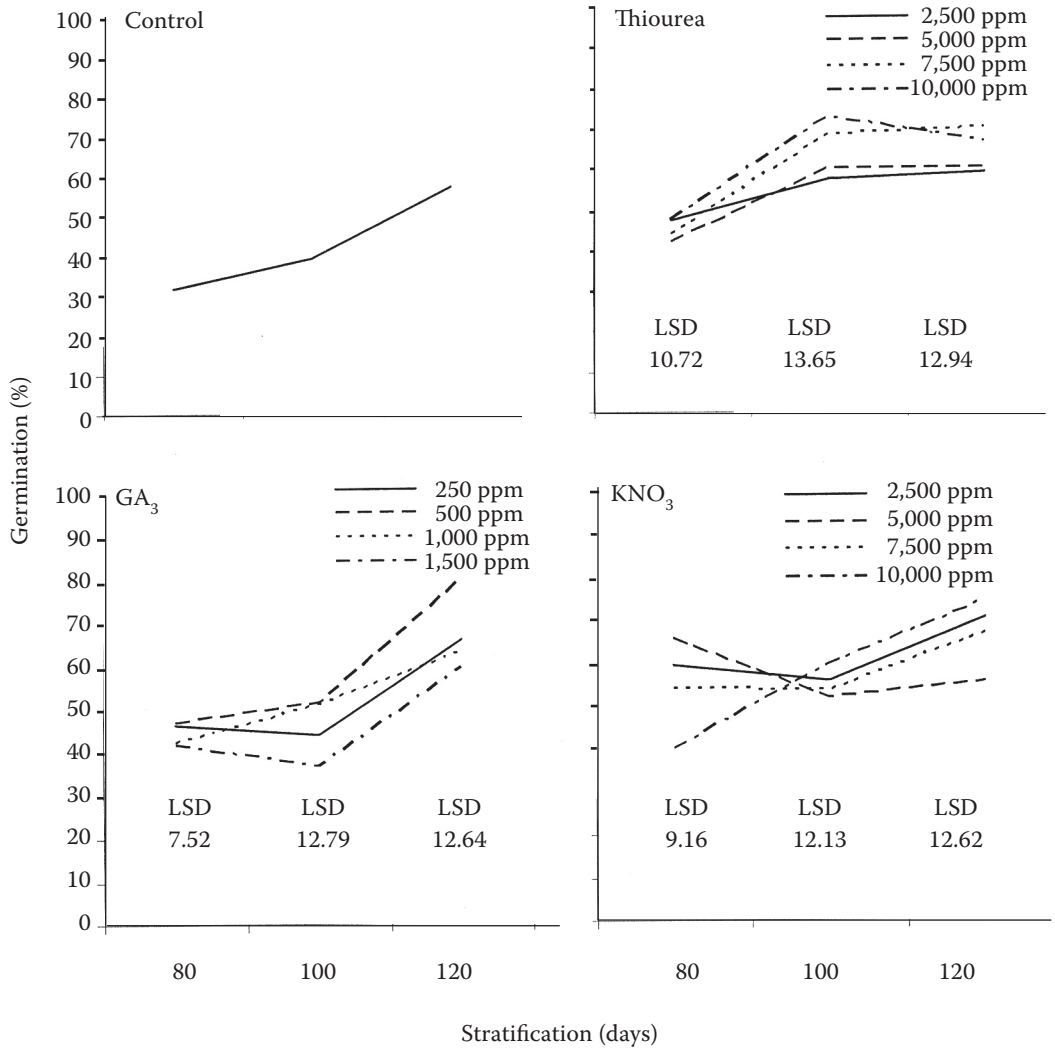

Fig. 3. Effects of GA_3, KNO_3 and thiourea treatments on the germination of 80 to 120 days stratified *P. avium* seeds without coats

mination treatments were arranged as a 3 (stratification duration) × 4 (chemical concentration) factorial. Responses of stratification treatments were evaluated as a control group. Mean differences among means were analyzed by the Least Significant Difference method ($P < 0.01$) using SAS (Statistical Analysis System 1995, SAS Institute, Cary, N.C.) packet program.

Figs. 2 and 3 show the percentage of germination of seeds that were exposed to different treatments leading to breaking dormancy.

Germination of the control treatment (stratified seeds) showed the variation among stratification duration of seeds both with and without coat. The increase of the stratification duration from 80 d to 120 d resulted in an increase in germination percentage. Germination rates of seeds with and without coat stratified for 120 days were 44.51% and 56.90%, respectively.

The treatment with exogenous GA_3 stimulated the percentage of germination of all seed groups. Differences among responses of GA_3 concentrations were statistically significant ($P < 0.01$). In seeds with coat and without coat, the highest germination results were obtained from 120 days stratified and treated with 500 ppm GA_3; values close to 60.85% and 79.74%, respectively (Figs. 2 and 3). Gibberellins showed to increase germination in several species (Carrera et al. 1988; Giba et al. 1993; Karam, Al-Salem 2001) and to overcome physiological dormancy in seeds with dormant embryos (Hartmann et al. 1997). Seed dormancy may be caused by an inadequate development of embryo and/or an existence of chemical inhibitors (Karam, Al-Salem 2001). In addition, physiological dormancy in seeds is closely related to the proportion between inhibitors (especially ABA) and growth regulators (especially gibberellins) (Hartmann et al. 1997).

Stratification in cold instantly stimulates the structural GA synthesis (Powell 1987). Mehanna et al. (1985) reported that internal GA percentage is at a high level, but the proportion of ABA is at a low level in dormant seeds. While GA in the structure increases the enzymatic activity, it slows the ABA activity. In this study, in order to remove dormancy in *P. avium* seeds, exogenous GA_3 application has been successful in breaking dormancy with 500 ppm for seeds with coat and without coat. In compliance with our results, Gerçekçioğlu and Çekiç (1999) and Carrera et al. (1988) determined that the best result in mahalep seeds were obtained from stratification + GA_3 applications.

Compared to the control group, the thiourea treatment improved the germination of *P. avium* seeds, and the effect of thiourea on germination was statistically significant ($p < 0.01$). The highest germination rates were observed with 120 days stratification + 10,000 ppm thiourea for seeds with coat and 100 days stratification + 10,000 ppm thiourea for seeds without coat (Figs. 2 and 3). Thiourea overcomes certain types of dormancy, such as deep embryo-dormant *Prunus* seeds (Hartmann et al. 1997). Similar results regarding the effects of thiourea on the germination were recorded in some other species (Stidham et al. 1980; Agrawal, Dadlani 1995). This stimulative effect of thiourea on seeds germination can be attributed to a reduction of the preventive effect of seed coat and its cytokinin activity in overcoming inhibition.

Treatments of KNO_3 had positive effects on the germination of seeds with and without coat, as well. Soaking in 7,500 ppm and 10,000 ppm KNO_3 gave the most significant germination rates: 64.54% for seeds with coat and 74.24% for seeds without coat, respectively. Moreover, all concentrations tested in this research increased the germination of mazzard cherry seeds. Use of KNO_3 has been an important seed treatment in seed-testing laboratories for many years without a good explanation for its action (Hartmann et al. 1997). Potassium nitrate was found to be effective in breaking dormancy of many species (Agrawal, Dadlani 1995). Yet, Stidham et al. (1980) reported that the use of KNO_3 in combination with prechill had a beneficial effect on seed germination of 18 shrub species.

In order to increase germination in fruits with hard seeds, like in *P. avium* fruits, different pre-germination treatments have been used. If the germination of the seeds is not homogeneous, researchers can use combinations of one or more treatments with cold-moist stratification to break seed dormancy. In the current study the seeds of *P. avium* were confirmed to be in a dormant state; impermeability of seed coat to water or gases is an important factor which may cause seed dormancy. Overall results indicated that the removal of seed coat after stratification and chemical treatments positively effect the germination of *P. avium* seeds. In seeds with coat, 500 ppm GA_3, 10,000 ppm thiourea or 7,500 ppm KNO_3 treatments after 120 d stratification can potentially enhance seed germination of mazzard cherry. We think that an adaptation and practical application of these findings in nurseries might also have an economic effect.

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Zlepšování klíčivosti semen *Prunus avium* pomocí kyseliny giberelové, dusičnanu draselného a tiomočoviny

ABSTRAKT: S cílem přerušení dormance a zvýšení klíčivosti semen byly u *Prunus avium* L. (ptáčnice) zkoušeny různé postupy včetně jejich vylušťování (odstraňování pecky) po chladové a vlhkostní stratifikaci a po ošetření kyselinou giberelovou (GA_3), KNO_3 a tiomočovinou. Nejúčinnější bylo ošetření dusičnanem draselným o koncentraci 7 500 ppm, aplikované po stratifikaci s dobou trvání 120 dnů, při kterém bylo dosaženo u nevyluštěných semen 64,54% klíčivosti. V případě vyluštěných semen byla nejlepší variantou aplikace GA_3 o koncentraci 500 ppm, použitá po stratifikaci v délce 120 dnů, u níž bylo dosaženo klíčivosti 79,74 %, což představovalo ve srovnání s kontrolou zvýšení klíčivosti semen o 29 %.

Klíčová slova: dormance; klíčení; kyselina giberelová; dusičnan draselný; *Prunus avium* L.; semena; tiomočovina

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