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*Full Length Research Paper*

# Effects of seed coat colour on seed characteristics of honeylocust (*Gleditsia triacanthos*)

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**Honeylocust (*Gleditsia triacanthos*) seeds have hard seed coats of different colours that affect duration of dormancy. This study investigated the effects of seed coat colour on water uptake, germination, and quantity of seed parts. The results show that seed coat colour had a significant effect on seed moisture content, seed coat weight, endosperm weight, water uptake, and germination. However, the quantity of embryos having seed coats of various colours did not differ significantly. Seeds with a yellow seed coat contained the most moisture and had the highest water uptake, even though yellow seed coats were thicker than other coat colours. Yellow-coated seeds showed 100% imbibition after 48 h and also the highest germination (95%). As honeylocust seed coats darken, it slows down seed germination. This research suggests that yellow-coated honeylocust seeds should be recommended for nursery cultivation.**

**Key words:** Dormancy, germination, *Gleditsia triacanthos*, seed coat color, water uptake.

## INTRODUCTION

Honeylocust (*Gleditsia triacanthos* L.) is a leguminous tree that grows up to 20 m high and is native to North America. It has been widely planted throughout the world for various purposes, including windbreaks, shelterbelts, erosion control, wildlife food, and local wood products (Blair, 1990; Singh et al., 1991; Ürgenc, 1998). Honeylocust is also used for buffer strips along highways or in urban forests, where it can be successfully grown in areas with air pollution, poor drainage, salty soils, and drought (Gilman and Watson, 1993). Ornamental varieties of honeylocust include: "Rubylace", "Sunburst", "Moraine", "Shademaster", "Inermis", and "Skyline" (Pamay, 1992; Güçlü, 1993). Honeylocust is also used aesthetically to soften a landscape, a role in which evergreen species have traditionally been dominant. "Skyline" has a narrow crown and is preferable along highways, while "Inermis" has no spines and could be utilised in park and garden landscape design.

Honeylocust seeds mature in autumn (September or October). They have the typical hard seed coats of legumes, which are thin but not impermeable to water. Impermeability of the seed coat to water is frequently a cause of dormancy. Thus, dormant and non-dormant seeds behave differently with regard to their patterns of water uptake (Saha and Takahashi, 1981). If the seed coats are not pre-treated, germination can be erratic and prolonged (Fordham, 1965). Soaking seeds in either concentrated sulphuric acid or hot water has been used (Heit, 1942; Saatçioğlu, 1971; Bonner et al., 1974) to beak dormancy.

Colour is a seed coat characteristic associated with water absorption. Seed coat pigmentation is correlated with reduced rates of imbibition in several legume species. Seed coat development is related to the maternal genotype, but seed coat colour is influenced by environmental conditions (De Souza and Marcos-Filho, 2001) such as photoperiod. For example, in *Salsola volkensii* green seeds have virtually no dormancy, but non-green seeds show dormancy (Bewley and Black, 1994). In honeylocust, seed coat colour ranges from yellow to brown.

The cause of seed dormancy in honeylocust is related to an impermeable seed coat, as well as the status of both the embryo and endosperm. Moreover, seed coat colour may affect dormancy in this species. Thus, this study investigated the effect of seed coat colour on germination and other seed characteristics.

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## MATERIALS AND METHODS

Honeylocust seeds were collected from Izmir, Turkey, in autumn 2008. To determine coat colour, seeds were separated visually. A total of 100 seeds were randomly sampled from each of three replicates and sorted by hand into categories of colour (yellow, light brown, and dark brown). Seeds were stored at approximately 4°C in a cool room and tested for germination ability in 2009.

### Seed weight and moisture content

To determine the weight of the honeylocust seeds in relation to seed coat colour, average weights were measured for each of the three colour groups. Honeylocust seeds were also halved with a knife, and wet and dry weights were measured. Dry weights were obtained from samples placed in an aluminium can, heated at 103±2°C for 17 h (Saatçioğlu, 1971; ISTA, 1999), and then cooled in a glass desiccator for 60 min. Each measurement was replicated five times. The moisture content ($W_A$) was calculated as follows:

$$\text{Moisture content (\%)} = (A2 - A3)x\frac{100}{A2 - A1} \qquad (1)$$

where A1 is the weight of the aluminium can, A2 is the total initial seed and can weight, and A3 is the total dry weight of the seeds and can.

### Measurements of seed coat, endosperm and embryo

40 seeds, with three replicates of each seed colour, were randomly selected and weighed. Seeds were placed in a 100-ml beaker and imbibed with 75 ml distilled water at 40±2°C for 48 h. After this time, the imbibed seeds had more than doubled in size and the seed coats were peeled off using forceps. Each of the three components (seed coat, endosperm, and embryo) were separated, placed in pre-weighed aluminium cans, dried at 100°C for 24 h, and then weighed (Liu et al., 2007).

### Swelling test and water uptake

For the swelling test of seeds of each coat colour, three replicate samples of 40 seeds were randomly selected. Seeds were soaked in distilled water in a 100-ml beaker at ambient temperature (30 – 38°C) for 96 h, and the number of swollen seeds was recorded after 0, 48, 54, 60, 72, 84, and 90 h. The percentage swollen seeds (SP) were calculated as follows:

$$SP \quad \% = \frac{number \quad of \quad swelled \quad seeds}{number \quad of \quad soaked \quad seeds} \times 100 \qquad (2)$$

For the seed water uptake of yellow, light brown and dark brown seeds of honeylocust, three replicates of 40 seeds of each colour were randomly selected and weighed, added to a 100-ml beaker with 75 ml distilled water, and kept at 22°C in the dark for 24 h. Water uptake of each sample beaker was calculated by dividing the hydrated seed weight by the initial dry seed weight (Liu et al., 2007).

### Seed germination test

Germination testing was based on complete randomized design with three replicates, using 100 seeds in each replicate for each of the three seed coat colours. Seeds were germinated in 12-cm petri dishes on two layers of filter paper moistened with distilled water. Petri dishes were placed in a plant growth chamber (MMM Clima Cell) with a daily photoperiod of 24°C for 16 h at 175 µmol $m^{-2}$ $s^{-1}$ and 18°C for 8 h in the dark. Moisture of the dishes was maintained with distilled water. Germination was assessed every day. A seed with a radicle at least a 5-mm long was considered to have germinated and was removed from the petri dishes. Final germination was counted on day 13 after the start of imbibition because no germination was observed after 13 days. Germination percentage was calculated as the proportion of germinated to total number of seeds.

### Statistical analysis

Analysis of variance was conducted to test the effects of seed coat colour on germination, weights of seed coat, endosperm, embryo, and imbibition. Percentage data were arcsin $(p)^{1/2}$ transformed before analysis (Zar, 1996). To determine significant differences, transformed means were subjected to analysis of variance using the software package SPSS 9.0 (SPSS Inc., USA) and differences among means were analysed by Duncan's multiple range test.

## RESULTS

### Effect of seed coat colour on seed characteristics

The results of analyses of variance on the effect of seed coat colour on seed moisture, seed coat weight, endosperm weight, water uptake, and germination were significantly different at the 0.01 or 0.001 confidence level. In addition, there were no significant differences in weight of 100 seeds or in embryo weight among yellow, light brown and dark brown seeds of honeylocust (Table 1).

The seed coat colour was not affected by honeylocust seed weight; however, seed moisture and water uptake were affected by coat colour, with the yellow seed coat showing the highest moisture content and water uptake, even though the yellow seed coats were also the thickest. The weight of the seed coat and endosperm of the three coat colours varied significantly. However, embryo weight did not differ significantly among the three coat colours. Honeylocust seeds were composed of 27 – 30.2% endosperm, 68.1 – 71% seed coat, and 1.7 – 2.1% embryo.

### Effect of seed coat colour on imbibition

The imbibitions of honeylocust seeds of the three seed coat colours differed in terms of percentage and duration. After soaking for 96 h, the swelling percentages of yellow, light brown and dark brown seeds were 100, 46 and 34%, respectively (Figure 1).

The percentage imbibition rose more rapidly in yellow-coated seeds with a higher moisture content than in light and dark brown seeds. Imbibition of yellow-coated seeds

**Table 1.** Analyses of variance, *F* values, and mean values of seed characteristics in honeylocust.

| | Mean squares | | | | | | |
|---|---|---|---|---|---|---|---|
| **Seed characteristics** | **100 seed weight (g)** | **Seed Moisture (halved) (%)** | **Seed coat weight (mg)** | **Endosperm weight (mg)** | **Embryo weight (ng)** | **Water uptake (%)** | **Germination (%)** |
| Between groups | 2.984 | 49.100 | 0.00123 | 0.000428 | 0.0542 | 2572.58 | 49.281 |
| Within groups | 2.388 | 0.113 | 0.00012 | 0.000033 | 0.0471 | 0.150 | 3.712 |
| *F* value | 1.249$^{NS}$ | 434.083*** | 10.856** | 12.965** | 1.152$^{NS}$ | 17121.8*** | 13.275** |
| | | | **Mean coat** | | | | |
| Yellow | 20.99 | 65a[1] | 0.171a[1] | 0.076a[1] | 440 | 100a[1] | 95a[1] |
| Light brown | 19.27 | 55b | 0.131b | 0.055b | 370 | 46b | 89b |
| Dark brown | 19.26 | 51c | 0.147b | 0.056b | 360 | 34c | 87b |

$^{NS}$ Non-significant, ** significant at p < 0.01, *** significant at p < 0.001, [1]) numbers not followed the same letter are significantly different at the 5% level (Duncan's criterion).

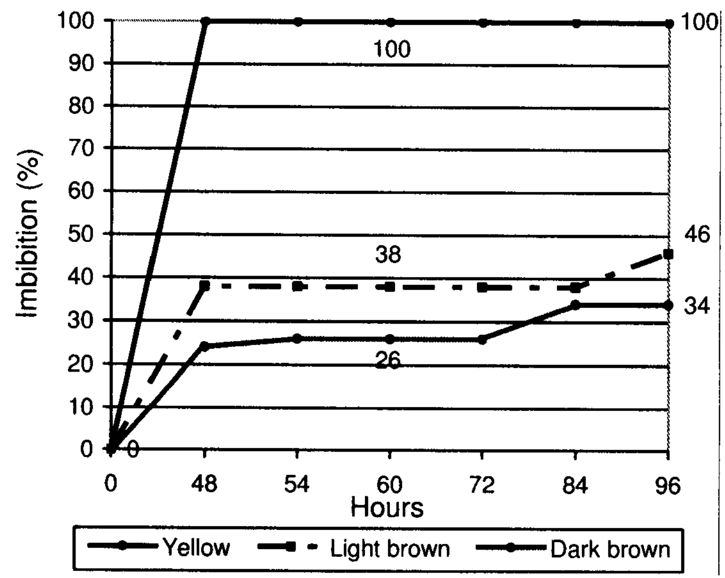


**Figure 1.** Imbibition of yellow, light brown and dark brown seeds.

reached 100% after 48 h in distilled water, while the percentage imbibition of light brown-coated seeds was 38% after 84 h and only reached 46% at 96 h. Dark brown-coated seeds showed a slow increase in imbibition but had the lowest swelling percentage (34%).

### Effect of seed coat colour on seed germination

Water uptake is the first stage of germination, leading to seed swelling and loss of seed coat-induced dormancy. In this study, germination differed significantly among honeylocust seeds with different seed coat colours (Figure 2).

The yellow, light brown and dark brown honeylocust seeds showed average germination of 95, 89 and 87%, respectively. The yellow-coated seeds had the highest germination because of maximum water uptake and highest percentage imbibition. Moreover, germination of yellow-coated seeds rose rapidly when seeds reached about 65% moisture content. In contrast, dark brown-coated seeds had a low moisture content (51%) and germinated more slowly. Therefore, as the colour of honeylocust seeds darkens, seed germination rate slows down (Figure 2).

## DISCUSSION

Dormancy of honeylocust seeds is caused by an

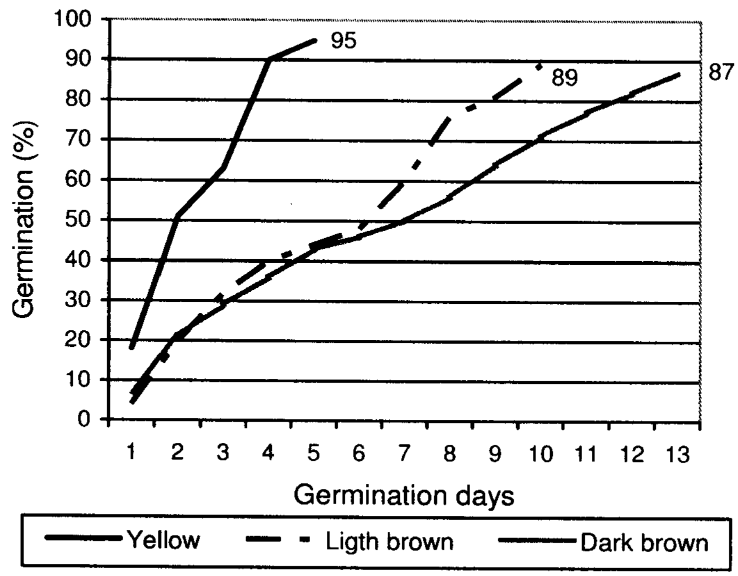


**Figure 2.** Germination of yellow, light brown and dark brown seeds.

impermeable seed coat. This impermeability to water is typical of many species and families of *Fabaceae* (Copeland and McDonald, 2001; De Souza and Marcos-Filho, 2001). If the seed coats are not pre-treated, germination can be erratic and prolonged (Fordham, 1965). Therefore, to overcome dormancy, seeds require pre-treatment that emulates natural conditions or satisfies certain physiological requirements. Stratification, scarify-cation and hot water are effective treatments to release dormancy (Saatçioglu, 1971; Bonner et al., 1974; Bewley and Black, 1994). Dormancy can be expressed as a result of interaction among the seed coat, embryo and endosperm; however, the relationship between seed dormancy and seed coat colour is unclear. Seed coat colour is an important factor that regulates seed dormancy (Torada and Amano, 2002). In *Chenopodium album*, Bewley and Black (1994) reported four types of brown or black seeds having reticulate or smooth coats; of these, smooth, black seeds had the deepest dormancy. Torada and Amano (2002) did not find an influence of seed coat colour on seed dormancy in wet environments. In the present study, seed coat colour affected seed moisture, coat weight, endosperm weight, imbibition, and germination. Seed coat weight of yellow seeds was higher than that of light or dark brown seeds. This finding agrees with that of Liu et al. (2007), who reported a lower seed coat weight in black-coated seeds than in dull white seeds. In our study, yellow seed coats were thickest but also had the highest percentage water uptake, and thus the highest germination of 95%. Saatçioglu (1971) and Bonner et al. (1974) also examined honeylocust seed germination, finding 75% germination after 40 days, while Fordham (1965) submerged seeds in water and recorded 20% germination in 13 days. In this study, all seeds completed germination in 13 days because no germination was observed after 13 days.

Seed coat colour affected seed swelling as well as germination. Seed swelling is one of the most apparent characteristics of water absorption and is thus a visible indication of dormancy breaking. In this study, swelling percentage rose more rapidly in yellow-coated seeds (100% in 48 h) than in light and dark brown seeds. Chachalis and Smith (2000), using soybean seeds, found similar results for seeds with black seed coats. In this study, the swelling percentages of yellow, light brown and dark brown seeds were 100, 46 and 34%, respectively, after soaking for 90 h (Figure 1). This result agrees with that of Yang et al. (2008), who also found that the swelling percentage was lower in dark-coated seeds of *Ammopiptanthus nanus* and *A. ongolicus*. However, seeds with unpigmented coats may deteriorate more rapidly and are more susceptible to swelling damage than are those with a pigmented seed coat (Abdullah et al., 1991; Asiedu and Powell, 1998).

In conclusion, yellow-coated honeylocust seeds had a greater water uptake rate and higher germination (95%) than light or dark brown-coated seeds. Dormancy from an impermeable seed coat in honeylocust can be quickly overcome in yellow-coated seeds. Thus, seeds with a yellow coat are recommended for use in honeylocust propagation.

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