Effect of different treatments and light quality on *Ulmus pumila* L. germination and seedling growth

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**ABSTRACT**

*Ulmus pumila* L. has potential benefits for erosion control, landscape ornamentals, and the bark contains substances that may have therapeutic value. To determine optimal growth conditions, we investigated the effect of water, sucrose, and exogenous plant growth regulator (6 benzylaminopurine: BA, indolebutyric acid: IBA and gibberellic acid: GA3) treatments as well as light quality (dark, fluorescence, and 2:1 or 4:1 Red:Blue (R:B) LED) on *U. pumila* seed germination and seedling growth. Seeds soaked in a 3% sucrose solution had the highest germination rate and germination energy in addition to the shortest germination time compared to seeds treated with water or PGRs, except the control. Light quality only affected germination energy, with seeds exposed to 2:1 R:B LED light having the highest germination energy and those exposed to fluorescent light having the lowest. Following transfer to a greenhouse with exposure to natural sunlight, the performance of seedlings varied depending on the light conditions under which they were germinated. In contrast, treatments during the germination stage such as incubation with PGRs had no effect on seedling growth in the greenhouse. Seedlings that germinated under 2:1 or 4:1 R:B LED lights grew taller and had thicker root collars compared to those grown in the dark or under fluorescent lights. The influence of light conditions persisted for at least seven months after the germination, even though the chlorophyll content was similar among seedlings exposed to different light quality; LED; seedlings

**KEYWORDS**

*Ulmus pumila*; Siberian elm; seed germination; light quality; LED; seedlings

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**ARTICLE**

**Introduction**

The Siberian elm (*Ulmus pumila* L.) belongs to Ulmaceae, the elm family that includes around 200 species ranging from shrubs to large trees that are either evergreen or deciduous and that are found across a range of climates from cool regions to tropical forests (Manchester 1990). *U. pumila* grows well in dry and cool temperatures found in mountainous areas of central or northern areas on the Korean peninsula. Although *U. pumila* is not useful for timber production by the forestry industry, its tolerance for sunlight, drought, cold and poor soil can be valuable in urban areas, particularly on southern slopes, to protect against soil erosion (Wesche et al. 2011). Ulmaceae species have undergone wide hybridization in native forests in North America (Zalapa et al. 2010) and *U. pumila* was introduced to improve resistance to Dutch elm disease (Solla et al. 2005). Despite these hardy characteristics, several conditions have led to the gradual and rapid disappearance of several Ulmaceae species from natural habitats. Wild populations of Ulmaceae species in China have been decreasing as seedlings are often buried in the sand (Shi et al. 2004). In addition, the bark of some species contains components that have beneficial pharmaceutical properties, such as antibacterial action (Lee et al. 1992), and harvesting to obtain these substances has decreased populations.

In Korea, species in the genus *Ulmus* have long been used as anti-inflammatory agents. Extracts of *U. pumila* were recently shown to inhibit the proliferation of lung cancer cells (In and Kim 2016). However, the production of these beneficial extracts requires the harvest of the tree bark or cortex that results in the death of trees. To maintain adequate supplies of these extracts and to protect natural habitats, understanding optimal methods for propagating these trees is essential. Although *U. pumila* produces large quantities of seeds, the period that these seeds are viable is short (Hirsch et al. 2012). Several studies on the germination of *U. pumila* have been conducted and showed that various factors influence seed germination, such as light and hormones. Environmental factors could also affect germination. These factors may affect the quality of seedlings during germination in terms of growth and biochemical properties (Jankausiene and Survilienne 2009; Flores et al. 2016; Simlat et al. 2016). In this study, we investigated the effects of different growth conditions during the germination stage such as incubation with PGRs as well as light quality (dark, fluorescence, and 2:1 or 4:1 Red:Blue (R:B) LED) on *U. pumila* seed germination and seedling growth. Seeds soaked in a 3% sucrose solution had the highest germination rate and germination energy in addition to the shortest germination time compared to seeds treated with water or PGRs, except the control. Light quality only affected germination energy, with seeds exposed to 2:1 R:B LED light having the highest germination energy and those exposed to fluorescent light having the lowest. Following transfer to a greenhouse with exposure to natural sunlight, the performance of seedlings varied depending on the light conditions under which they were germinated. In contrast, treatments during the germination stage such as incubation with PGRs had no effect on seedling growth in the greenhouse. Seedlings that germinated under 2:1 or 4:1 R:B LED lights grew taller and had thicker root collars compared to those grown in the dark or under fluorescent lights. The influence of light conditions persisted for at least seven months after the germination, even though the chlorophyll content was similar among seedlings exposed to different light quality; LED; seedlings

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treatments and environmental factors on germination and other growth parameters of *U. pumila* seedlings.

**Materials and methods**

**Plant materials**

Seeds were collected from a *U. pumila* tree in the natural habitat of Youngwol, Gangwon-do, Korea in May 2018. Seeds were dried at 4°C for one week and the wings were removed before sowing. A 3 cm diameter peat pellet was used for the planting medium. Pellets were placed in 36 hole-tray and soaked with water before sowing the seeds, which were allowed to grow in a room with controlled temperature (23 ± 1°C) and light (16 h photoperiod).

**Germination test under different treatments and light quality**

Seeds were treated with 1 μM plant growth regulators BA, GA3, or IBA or 3% (w/v) sucrose before sowing by soaking in the indicated solution overnight (16 h). Untreated dried seeds were sown as a control. Different light sources, including fluorescent light and no light (dark), were used to illuminate the sowing plates to test the effect of light quality. Light wavelengths were controlled through the use of a LED light having different ratios of red and blue light (2:1 or 4:1). These ratios were selected based on that needed for vegetative growth of crops by general cultivation as the proper proportions of LED for tree species are unknown. The temperature was adjusted to 25 ± 2°C with 16 h photoperiods. Six seeds were sown for each treatment and the experiment was replicated three times. Seed germination was measured every day beginning four days after sowing until there was no emergence of seedlings, typically at 14 days. Germination performance parameters included germination rate, germination energy, and average days of germination. Each parameter was calculated using the following equations.

- Germination percentage \(= \frac{\text{(number of seeds germinated/total number of seeds)}}{100}\)
- Germination energy (%) \(= \frac{\text{(sum of the germinated seeds until the peak/total number of seeds)}}{100}\)
- MGT (mean germination time, days) \(= \frac{\text{(day of germination \times number of germinated seeds)}}{\text{total number of seeds}}\)

**Seedling growth in greenhouses**

Germinated seedlings were maintained under four different light conditions for one month before transfer to a greenhouse for acclimatization. Seedlings were planted in soil in an 8 × 8 cm square plastic pot and grown in a greenhouse for several months. One month after transfer to the greenhouse, ten seedlings for each light treatment and growth condition were evaluated by measuring seedling height and chlorophyll content. Seedlings entered a dormant stage after growth in winter. When seedlings began to grow in the spring, the growth was measured as described above. The diameter of the root collar and chlorophyll fluorescence were measured in mid-April. The chlorophyll content in leaves on the third node from the top of the plant was measured using a SPAD 502 plus instrument (Minolta Nikon, Osaka, Japan). Measurements were repeated three times for each leaf and the average value was calculated. Chlorophyll fluorescence comprising ground fluorescence (F0) and maximum fluorescence (Fm) was recorded with a FluorPen FP110 instrument (Photon Systems Instruments, Drasov, Czech Republic) using the same leaves that were used for chlorophyll measurement after a 30 min dark adaptation. The maximum photochemical efficiency of PSII (Fv/Fm) was calculated as (Fm–F0)/Fm (Genty et al. 1989).

**Data analysis**

Statistical analyses were carried out on the collected data using one-way or two-way ANOVA with Duncan’s post-hoc test and Pearson’s correlation with SPSS ver. 24 (IBM).

**Results and discussion**

**Effects of treatment and light quality on germination**

The germination of *U. pumila* seeds varied significantly among the different treatments as evidenced by the parameters of germination (Table 1). Germination of dried, untreated seeds was superior to that for seeds treated with hormones, sucrose, or water. Among the different treatments, seeds soaked in 3% sucrose germinated at a similar rate (79%) to that seen for untreated dried seeds. The plant growth regulator IBA had the most adverse effect on germination, with only 29% of seeds germinating, which was less than half that seen for the control. In terms of germination energy, control seeds and those soaked in 3% sucrose solution were similar and had the highest percentage of the treatments examined. The average number of days until germination also varied among the treatments, with untreated dried seeds having the shortest germination time. None of the treatments resulted in shorter germination periods. Seeds soaked in IBA had the lowest germination rate and longest germination period.

| Factors          | Parameter          | F value | P value |
|------------------|--------------------|---------|---------|
| Light            | Germination rate   | 2.863   | 0.058   |
|                  | Germination energy | 4.539   | 0.012   |
|                  | Germination days   | 2.667   | 0.071   |
| Treatment        | Germination rate   | 7.445   | 0.000   |
|                  | Germination energy | 5.655   | 0.001   |
|                  | Germination days   | 3.072   | 0.028   |
| Light * Treatment| Germination rate   | 0.656   | 0.800   |
|                  | Germination energy | 0.888   | 0.585   |
|                  | Germination days   | 1.016   | 0.472   |
among the treatments tested. Although each of the treatments significantly influenced germination rate, germination energy, and germination (Tables 1 and 2), the only growth parameter that was significantly affected by light conditions was germination energy (Tables 1 and 3). The germination rate and days before germination were similar among the light treatments, but germination energy was highest for LED light having a 2:1 Red:Blue ratio. The second highest germination energy was seen by light followed by the dark condition, LED (R:B = 4:1), and the lowest energy was seen for growth under fluorescent light (Table 3). The mean germination time was 4.8–6.7 days, and seeds germinated within five days without light. Seeds exposed to light, regardless of the conditions, germinated later than those exposed to dark conditions (Table 3).

Ulmaceae species grow in a wide range of areas around the world and can endure harsh conditions such as dry and arid environments (Wesche et al. 2011) while also being more tolerant of Dutch elm disease relative to other species in the genus Ulmus (Collin et al. 2004). *U. pumila* produces many seeds and germinates under stressed conditions, although seed purity and survival after germination is low (Wesche et al. 2011). The germination rate in our study was highest for the control seeds that were left untreated. This result may reflect an eco-physiological trait of *U. pumila* that allows this tree to survive in dry conditions. Thus, soaking seeds in water or providing more plant growth regulators may in fact inhibit germination. On the other hand, 3% of sucrose solutions have increased osmotic pressure and allow less water to permeate the seed while also providing carbohydrate sources for germination. According to a study of *U. pumila* ecological characteristics, its seed germination rate was greater than that for any other competing species in riparian forests (Cabra-Rivas and Castro-Diez 2016) and the steppe slope of taiga (Dulamsuren et al. 2009). Hirsch et al. (2012) reported that precipitation significantly influenced the germination time, which decreases with increasing annual precipitation for both native and non-native *U. pumila*. They also concluded that this apparent tolerance for dry conditions is a driver of its invasive character. Cicek and Tilki (2007) reported that seeds of three different elm species except for *U. pumila*, likely need no treatment for germination, which is consistent with these earlier studies and for our current studies showing that excessive water uptake or exposure to plant growth regulators negatively affected seed germination.

### Table 2. Germination characteristics by pretreatment on seeds.

|        | Control | H₂O    | Sucrose | BA     | GA₃    | IBA    |
|--------|---------|--------|---------|--------|--------|--------|
| GP (%) | 82.3 a  | 45.9 ab| 79.2 a  | 37.5 b | 58.3 ab| 29.2 c |
| GE (%) | 68.8 a  | 39.6 b | 62.5 a  | 29.2 b | 50.0 ab| 20.9 c |
| MGT    | 4.5 a   | 5.8 ab | 5.6 ab  | 6.5 ab | 5.5 ab | 7.6 b  |

Concentration of BA, GA₃, IBA of the solution is 1 µM each. Same letters are not different in each parameter at a level of p = 0.05 by Duncan’s test.

### Table 3. Germination characteristics by light conditions during germination.

| Light     | Control | H₂O    | Sucrose | BA     | GA₃    | IBA    |
|-----------|---------|--------|---------|--------|--------|--------|
| GP (%)    | 59.7 a  | 43.0 a | 68.0 a  | 50.0 a | 38.9 ab| 6.1 a  |
| GE (%)    | 52.8 ab | 29.2 a | 59.7 b  | 38.9 ab|        |
| MGT       | 5.8 a   | 6.7 a  | 6.0 a   | 6.1 a  |

Same letters are not different in each parameter at a level of p = 0.05 by Duncan’s test.

### Table 4. Statistical analysis of the effect of pretreatments and light conditions on shoot growth in the greenhouse.

| Factors   | F value | P value |
|-----------|---------|---------|
| Light     | 9.388   | 0.000   |
| treatment | 0.781   | 0.566   |
| Light × treatment | 1.110 | 0.365   |

Same letters are not different in each parameter at a level of p = 0.05 by Duncan’s test.

### Effect of light quality on growth of acclimatized seedlings in a greenhouse

In this study, germinated *U. pumila* seedlings were transferred to a greenhouse for acclimatization to natural sunlight for several months. We divided the seedlings into different treatment and light condition groups before measuring the seedling growth. Although treatments with growth regulators or sucrose significantly affected germination, growth after one and seven months was not affected (Table 4). The height of the one-month-old seedlings in the greenhouse ranged from 9.9 to 11.7 cm and no statistically significant differences were observed among the experimental groups (Table 5). However, the growth of the one-month-old seedlings was affected by different light conditions during germination and these differences persisted in 7-month-old plants (Table 6 and Figure 1). The root collar diameter and number of leaves per plant also significantly differed among the light conditions. Seedlings were grown under 2:1 R:B LED had the thickest root collar and the most leaves per plant, and those exposed to 4:1 R:B LED had the second-highest root collar thickness and number of leaves. Seedlings grown under fluorescent light or in the dark had a thin root collar and fewer leaves per plant relative to those grown under LED (Table 6). Although seedlings germinated in the dark were tallest at the time of transfer, they did not grow well in the greenhouse.

The chloroplast content was highest in leaves of 1-month-old seedlings grown under LED (R: B: 2:1) and lowest in the ones grown in the dark (Figure 2). After seven months of growth in the greenhouse, the chloroplast content was similar among the treatment group, although seedlings grown under fluorescent light continued to have the lowest chlorophyll content. There
was little difference in chlorophyll fluorescence (Fv/Fm) of one-month-old seedlings exposed to different light conditions, and these differences were also not significant after seven months (Figure 3).

Analysis of correlations between growth, physiological characteristics, and treatments indicated a strong correlation between the light conditions and growth characteristics such as shoot length, root collar diameter, number of leaves per plant, and chlorophyll content. This result suggests that *U. pumila* seedling growth is influenced by light conditions during the germination stage. Among the growth characteristics, those seedlings having more extended shoots also

Table 5. Growth of seedlings in greenhouse for 1 month after the transplanting by the pretreatment and light condition during the germination (length, unit: cm).

| Light           | Control | H2O  | Sucrose | BA*   | GA3* | IBA* | Mean  |
|-----------------|---------|------|---------|-------|------|------|-------|
| FL              | 10.8    | 13.3 | 10.0    | 13.2  | 11.4 | 10.0 | 11.2ab |
| LED (R:B, 2:1)  | 9.9     | 12.5 | 9.4     | 10.4  | 10.4 | 9.0  | 10.2a  |
| LED (R:B, 4:1)  | 11.9    | 11.0 | 15.0    | 14.5  | 12.9 | 13.4 | 13.1ab |
| Dark            | 8.2     | 8.3  | 9.3     | 9.0   | 7.2  | 8.0  | 8.2a  |
| Mean*           | 10.2    | 11.3 | 10.9    | 11.7  | 9.9  | 10.5 |

* Shoot length was not significantly different by pretreatment during the germination. Same letters are not different in each parameter at a level of $p = 0.05$ by Duncan’s test.

* Concentration of BA, GA3, IBA of a solution is 1 μM each.

* Shoot lengths were significantly different by light treatment; the same letters are not different at the level of $p = 0.05$ by Duncan’s post-hoc.

Table 6. Growth characteristics of the seedlings 7 months after the transplanting in the greenhouse by the light condition during the germination.

| Light           | Shoot length(cm) | Number of leaves | Diameter of root collar (cm) |
|-----------------|------------------|-----------------|-----------------------------|
| FL              | 11.3 ± 3.6b      | 16.8 ± 6.2ab    | 0.20 ± 0.05a                |
| LED (R:B, 2:1)  | 16.1 ± 2.1a      | 69.2 ± 27.7c    | 0.35 ± 0.08c                |
| LED (R:B, 4:1)  | 17.1 ± 5.6a      | 27.6 ± 10.9b    | 0.29 ± 0.06b                |
| Dark            | 9.71 ± 1.9b      | 11.6 ± 5.2a     | 0.17 ± 0.05a                |
| Mean*           | 10.2 ± 11.3      | 10.9 ± 11.7     | 0.99 ± 10.5                 |

* Same letters are not different in each parameter at a level of $p = 0.05$ by Duncan’s test.

![Figure 1](image1.png)  
*Figure 1.* Effect of light condition during the germination on shoot growth in the greenhouse. * Same letters are not different in each parameter at a level of $p = 0.05$ by Duncan’s test.

![Figure 2](image2.png)  
*Figure 2.* Effect of light condition during the germination on chlorophyll contents of leaves in a greenhouse. * Same letters are not different in each parameter at a level of $p = 0.05$ by Duncan’s test.
tended to have thicker root collars and more leaves. Although there was a negative correlation between growth and chlorophyll fluorescence (Fv/Fm), this relationship was not statistically significant (Table 7).

For pioneer tree species like *U. pumila* that have small seeds, light quality regimes can be a germination cue (Xia et al. 2016). When Cicek and Tilki (2006, 2007) tested the effect of light on the germination of three *Ulmus* species, they observed species-dependent light requirements for germination, although there were no significant differences among the light conditions in terms of germination rate. Their findings showed that the germination rate and average days for germination were not different among light conditions, although leaf thickness differed from the light intensity (Kwon and Woo 2015). As Song et al. (2011) reported, *U. pumila* seeds germinated within six days on average regardless of light conditions. However, in this study, we observed higher germination energy under LED (R:B, 2:1) and dark conditions, but significantly lower energy under white fluorescence and a higher Red:Blue ratio. High germination energy reflects seed quality and is an important factor in growth of nursery crops. Thus, our results suggest that light quality can indeed influence the germination phase and a certain light wavelength probably inhibited germination based on the results for seeds germinated in the dark. Germination is often influenced by light quality (Kettenring et al. 2006), as indicated by a report showing that blue LED improved stevia seed germination (Simlat et al. 2016). As such, the light quality is another eco-physiological characteristic of the ability of this species to adapt to varying conditions in habitats as Flores et al. (2016) observed for the germination of some species in Mexico. The germination of Ulmaceae species reflects the characteristics of the natural habitat, particularly when dry and arid conditions are present.

In contrast to germination, the light had a marked effect during the early seedling stage, but the differences were less pronounced upon transfer to the greenhouse. Following the development of LED light systems, researchers investigated their effects on various aspects of plants, such as the growth and content of chlorophyll and phytochemicals (Johkan et al. 2010; Chen et al. 2014; Bantis et al. 2016). The response to the quality of light or the ratio between red and blue varies from species to species or from cultivar to cultivar (Astolfi et al. 2012; Bantis et al. 2016). In two previous studies, a LED lighting system was used to grow tree seedlings and was demonstrated to increase the growth of some species (Astolfi et al. 2012; Apostol et al. 2015). In this study, plants were illuminated with LED lights only during the germination stage and then grew under natural light in the greenhouse. Growth parameters of plants germinated under LED lights (either 2:1 or 4:1 R:B) were improved relative to those plants exposed to fluorescent light or grown in the dark. This result is in agreement with that of Flores et al. (2016), who found that light conditions during germination were associated with the adult plant height. The growth of *U. pumila* seedlings in our study

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**Figure 3.** Effect of light condition during the germination on chloroplast fluorescence of leaves in a greenhouse. *Same letters are not different in each parameter at a level of p = 0.05 by Duncan’s test.

**Table 7.** Correlation analysis among the growth, physiological parameters of seedlings, and light condition during the germination.

| Light | Shoot length | Diameter of root collar | No. of Leaves | Chlorophyll content | Fv/Fm |
|-------|--------------|-------------------------|---------------|---------------------|-------|
| Light | 1            | 0.594**                 | 0.550**       | 0.458**             | 0.421** | -0.312 |
| Shoot length | 0.594** | 1                       | 0.556**       | 0.604**             | -0.032 | -0.389* |
| Diameter of root collar | 0.550** | 0.556** | 1               | 0.639**             | 0.005  | -0.121 |
| No. of Leaves | 0.458** | 0.604** | 0.639** | 1                   | 0.040  | -0.177 |
| Chlorophyll content | 0.421** | -0.032 | 0.005 | 0.040 | 1 | -0.106 |
| Fv/Fm | -0.312 | -0.389* | -0.121 | -0.177 | -0.106 | 1 |

* Statistically significant at p = 0.05 by Pearson’s correlation analysis.
** Statistically significant at p = 0.01 by Pearson’s correlation analysis.
showed a latent effect of light conditions on subsequent plant development. The most significant difference among the seedlings is that those germinated under LED lights had more leaves than those exposed to fluorescent light or grown in the dark. In particular, seedlings from the 2:1 R:B group had more leaves than the 4:1 R:B group. Light is a crucial factor in morphogenesis, and Macedo et al. (2011) found that light quality altered the morphological characteristics of the tropical plant *Alternanthera brasiliana*, which had more leaves following exposure to blue light that was previously shown by Hogewoning et al. (2010) to play a vital role in leaf morphogenesis. Although in this study we used mixed lights having different blue to red ratios and illuminated plants during the germination stage only, we did see that seedlings had more leaves following germination under light having a high blue light ratio.

In analyzing the correlation among the parameters, light conditions were strongly related to most of the parameters except chlorophyll fluorescence (Fv/Fm), an indirect measurement of photosynthesis. However, we saw little difference in chlorophyll content and maximum photochemical efficiency of PSII (Fv/Fm) among the various light conditions, and the values, in general, were lower (~0.8) than those seen by other researchers (Hogewoning et al. 2010). The lack of correlation between light and growth characteristics that we observed could be because the seedlings were young and the leaves had not fully matured at this stage. Moreover, determining whether there might be a positive or negative correlation between the Fv/Fm ratio and growth is difficult, particularly given that Zheng and Van Labeke (2017) found that some dicot and monocot plant leaves have low Fv/Fm under red light only, but that this type of light did not influence dry weight production. Nevertheless, the ratio of red to blue light in the LED used in our study did affect growth, consistent with an earlier finding that in some plant species blue light is associated with enhanced leaf thickness and palisade parenchyma, which is important for light absorption (Zheng and Van Labeke 2017).

In summary, here we measured the morphological and physiological characteristics of Siberian elm to investigate the effect of treatment with growth regulators, water or sucrose and light quality on both seed germination and growth during the early seedling stage. Siberian elm seeds showed eco-physiological characteristics consistent with pioneer species that can grow in dry and arid regions. The germination rate was high under dry conditions and was mainly influenced by water uptake rather than light quality. Meanwhile, light quality affected germination energy, and light having a higher ratio of Red:Blue inhibited the germination of this species. The effects of light quality during the germination stage appeared during the early seedling stage. Seedling growth was superior when the seeds were germinated under the LED light. Furthermore, seedling morphogenesis in addition to leaf number and leaf size were significantly influenced by blue light.

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