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# Effects of brassinosteroids and gibberellin on water uptake and performance of soya bean seeds under different temperatures

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## Abstract

Low temperature has negative effects on seed performance and seedling growth in soya bean, reducing stand establishment. It was hypothesised, that seed treatment with plant hormones could improve seed imbibition and performance at low temperature. Therefore, the effects of 24-epibrassinolide (EBL) and gibberellic acid ($GA_3$) on soya bean seed germination were studied. Seeds of 'CM60' and 'CM0701-24' were imbibed with 0.25 or 0.50 ppm EBL, 50 or 100 ppm $GA_3$, or distilled water at 10, 15, 20 and 25°C. The seed imbibition patterns of the two soya bean varieties followed a similar trend. Low temperature delayed seed imbibition resulting in longer time to reach the end of phases 1 and 2 of seed germination as compared with high temperature. Soya bean seeds treated with 50 or 100 ppm $GA_3$ showed increased germination index, shoot and root length and reduced mean germination time. Thus, 50-100 ppm $GA_3$ could be used as a pre-soaking seed treatment to improve soya bean seed and seedling performance under low temperature.

**Keywords:** 24-epibrassinolide, gibberellic acid, primary root emergence, seed imbibition, seedling growth, soya bean, temperature

## Introduction

The optimum temperature for soya bean seed germination and seedling growth is 25-30°C (Uthayopas, 2006). Soya bean seed production fields in the north and northeast parts of Thailand can reach soil temperature less than 20°C in December and January during the dry season (Na Lampang, 1984; Polpanit *et al.*, 2015). Temperature <20°C decreases the first hours of seed imbibition (Bramlage *et al.*, 1978), reduces seedling emergence and retards seedling growth (Uthayopas, 2006; Cheng *et al*., 2010). At low temperature, soya bean seed imbibition is delayed resulting in slow primary root emergence (Jeschke *et al*., 2017). No seed germination occurs below 10°C (Jomol *et al*., 2000).

Brassinosteroids (BRs) and gibberellins (GAs) are two groups of phytohormones that have been shown to improve seed germination under low temperatures in various crops (Fujii and Saka, 2001; Li *et al*., 2013). BRs were reported to improve emergence percentage and seedling growth under low temperature in soya bean (Sathiyamoorthy and Nakamura, 1990; Thongsri *et al*., 2019), rice (Wang and Zeng, 1993), mungbean (Huang *et al*., 2006) and maize (Sun *et al*., 2020). While $GA_3$ could increase seedling emergence and establishment in soya bean and maize under temperature stress (Rood *et al*., 1990; Wang *et al*., 1996). Leubner-Metzger (2001) reported that 'Havana 425' tobacco seeds imbibed with 0.01 µM BL and 0.01 µM BL plus 10 µM $GA_4$ showed decreased imbibition time and greater seedling elongation and germination. Payal *et al*. (2013) reported that *Allium stracheyi* Baker seeds pre-soaked with 50 and 100 mg $L^{-1}$ $GA_3$ at 25°C showed slightly greater water imbibition and higher mean germination than those treated with hot water (80°C) or cold water (10°C). Dotto and Silva (2017) reported that priming beet seeds with 75 mg $L^{-1}$ $GA_3$ at 20°C for 30 hours reduced phases 1 and 2 of the imbibition process; beet seeds primed with 1 mM $GA_3$ for 48 hours had increased first count seed germination and shoot and root lengths. Xiong *et al*. (2021) found that japonica rice cultivar Nipponbare seeds imbibed with 2 µmol $L^{-1}$ brassinazole and 10 µmol $L^{-1}$ $GA_3$ had increased germination rate and shoot length at 26°C for 96 hours after imbibition.

Soya bean seeds are sensitive to low temperature especially during germination. It was hypothesised that BRs and GAs could improve physiological performance under low temperature. Therefore, the objective of this study was to evaluate the effects of EBL and $GA_3$ on water uptake and physiological performance of soya bean seeds under different temperatures.

## Materials and methods

### *Seed material*

Soya bean seeds (*Glycine max* (L.) Merr.) cv. 'Chiang Mai 60' (hereafter, 'CM60') and 'CM0701-24' were produced at Phitsanulok Seed Research and Development Center, Phitsanulok Province, Thailand. Initial seed moisture content, standard germination and vigour by accelerated ageing (AA) were measured followed ISTA (2020). 'CM60' and 'CM0701-24' soya bean seeds had initial moisture content of 8.6% (both varieties), and standard germination of 93 and 91% and seed vigour by AA of 73 and 70%, respectively. This means that the seed lots met the official seed standard of the Department of Agriculture, Bangkok, Thailand. The soya bean seeds were kept in aluminium foil bags and stored at 15 ± 2°C and 40 ± 5% relative humidity (RH) through the experiments.

### *Effects of EBL and $GA_3$ on seed imbibition of soya bean under different temperatures*

This experiment was carried out using a 4 × 5 factorial arrangement in a completely randomised design (CRD) with four replications. Factor A was the constant temperature during seed imbibition, 10, 15, 20 and 25°C; factor B was the phytohormone treatment, 0.25 or 0.50 ppm EBL or 50 or 100 ppm $GA_3$ with distilled water as the control. Four replications of 5 g of each soya bean seed sample were sown between paper towels (107 × 107 mm) moistened with phytohormone solution or distilled water. Each soaked-seed sample was put in a plastic box (114 × 114 × 65 mm) to prevent water evaporation and placed in a growth chamber at 10, 15, 20 and 25°C for 120 hours with a diurnal period of eight hours-light and 16 hours-darkness. Each soya bean sample was weighed using an electronic balance (Mettler toledo, ML 503, Switzerland) with three decimal places and the volume of water uptake measured at 6-hour intervals until 120 hours. To replace the solution loss, the phytohormone solution and distilled water were filled in moisten paper to reach original weight. The percentage of seed imbibition was calculated using the formula:

$$\text{Seed imbibition (\%)} = \left[\frac{(\text{M2} - \text{M1}) \times 100}{\text{M1}}\right]$$

Where M1 and M2 are the seed weight (g) before and after imbibition, respectively.

Seed imbibition percentage was plotted and a curve fitting technique was used for the best equation with the highest polynomial regression ($R^2$) value. The end of phase 1 of seed imbibition was considered to be the intersection of the slope lines of phases 1 and 2 and the end of phase 2 was considered to be when there was 50% primary root emergence (root protrusion of 2 mm). The correlation between seed imbibition times and percentage of seed imbibition was expressed according to Cheng *et al.* (2010).

*Effects of EBL and $GA_3$ on soya bean seed performance under different temperatures*

This experiment was carried out using a 4 × 6 factorial arrangement in CRD with four replications. Factor A was the temperature during seed imbibition, 10, 15, 20 and 25°C, and factor B was the phytohormone treatment, 0.25 or 0.50 ppm EBL or 50 or 100 ppm $GA_3$ with distilled water and untreated seeds as the control. Fifty treated-seeds per replication were sown using between paper method for four replications and paper rolls were put in polypropylene plastic bags (229 × 305 mm) and incubated in a growth chamber at 10, 15, 20 and 25°C for eight days with a diurnal period of eight hours-light and 16 hours-darkness. Physiological performance was evaluated as follows:

1) Primary root emergence (RE) was counted 2 mm-long radicle at 36 hours after sowing according to ISTA (2020).
2) Germination percentage (GP) was at five (first) and eight days (final count) after sowing (ISTA, 2020).
3) Germination index (GI) was calculated according to Copeland and McDonald (1995):

$$\text{GI} = \Sigma \left(\frac{\text{Gt}}{\text{Dt}}\right)$$

Where Gt is the number of germinated seeds on day t, Dt is the number of days counted from the beginning of the 8-day germination test.

4) Mean germination time (MGT) was calculated according to Ellis and Roberts (1981):

$$MGT = \frac{\Sigma\ (Dn)}{\Sigma n}$$

Where D is the number of days counted from the beginning of germination, n is the number of germinated seeds on day D.

5) Shoot and root length were conducted by sowing 50 seeds per sample as the germination test. 20 seedlings per replication were randomly measured shoot and root length at eight days after sowing and expressed in mm. Results represented the mean.

*Statistical analysis*
Analysis of variance was carried out using R version 4.0.3 (R Core Team, 2020) followed by calculation of the least significant difference (LSD, $P < 0.05$). Data expressed in percentages were arcsine-transformed prior to analysis.

## Results

*Effects of EBL and $GA_3$ on seed imbibition of soya bean under different temperatures*
Seed imbibition patterns of 'CM60' and 'CM0701-24' in EBL, $GA_3$ and distilled water under different temperatures were similar (figure 1). The imbibition time of each phase was affected by temperature. The low temperature delayed the imbibition time of phase 1 and phase 2 compared to high temperature. The seed imbibition percentage decreased as temperature increased. At the end of phase 1 and phase 2, soya bean seeds with EBL and $GA_3$ had higher seed imbibition percentage than those of distilled water (figure 1, table 1).

As the temperature increased, primary root protrusion time decreased. Root protrusion from 'CM60' seeds occurred at 96, 72, 36 and 30 hours at 10, 15, 20 and 25°C, respectively, while root protrusion from 'CM0701-24' seeds occurred at 84, 48, 30 and 24 hours at 10, 15, 20 and 25°C, respectively (figure 2). Primary root protrusion was faster for 'CM0701-24' than for 'CM60' (figure 3).

*Effects of EBL and $GA_3$ on seed performance of soya bean under different temperatures*
*Primary root emergence*
At 25°C, 'CM60' seeds treated with 50 ppm $GA_3$ and 'CM0701-24' seeds treated with 0.50 ppm EBL showed the highest primary root emergence, significantly higher than that of control (table 2). However, primary root emergence of seeds treated with all concentrations of EBL and $GA_3$ at 20 and 25°C and 50 and 100 ppm $GA_3$ at 15°C were significantly higher than those of control.

*Germination percentage*
In both soya bean varieties, high temperature (20 and 25°C) resulted in higher germination than achieved at low temperature (10 and 15°C) (table 3). All EBL and $GA_3$-treated seeds

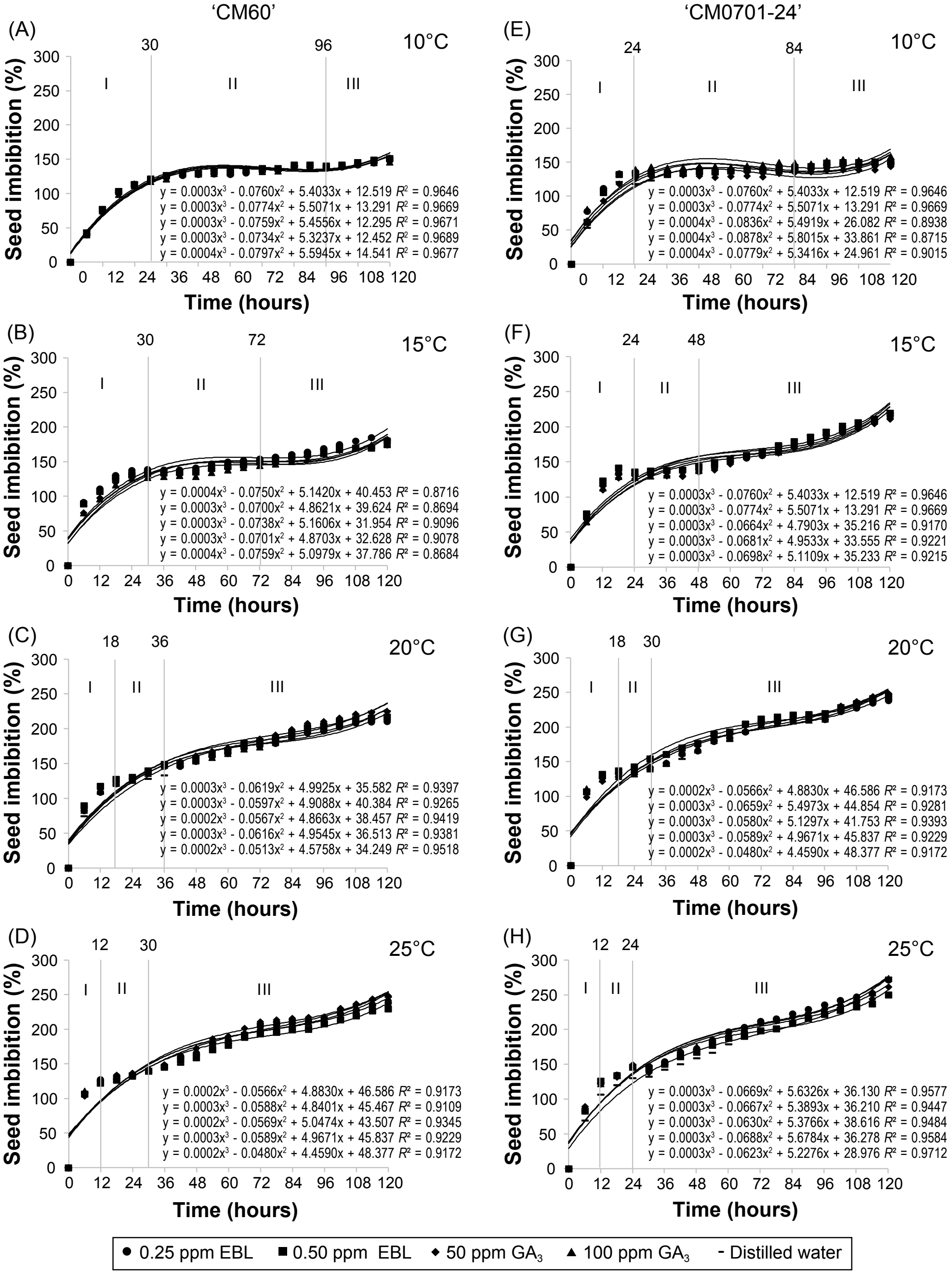


Figure 1. Seed imbibition (%) of 'CM60' (**A**-**D**) and 'CM071-24' (**E**-**H**) soya bean seeds under different concentrations of EBL and $GA_3$ at 10, 15, 20 and 25°C. The end of phase 1 was marked by the intersection of the slope line of each phase and the end of phase 2 was marked by 50% primary radicle emergence (visible radicle protrusion).

Table 1. Imbibition time and seed imbibition of 'CM60' and 'CM0701-24' soya bean seeds at the end of imbibition phase 1
EBL, $GA_3$ and distilled water at 10, 15, 20 and 25°C.

| Temperature (°C) | Seed imbibition (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | End of phase 1 | | | | | End of phase 2 | | | | |
| | Time (hours) | EBL | $GA_3$ | Distilled water | Mean | Time (hours) | EBL | $GA_3$ | Distilled water | Me |
| 'CM60' | | | | | | | | | | |
| 10 | 30 | 121.9 | 120.4 | 122.4 | 121.5$^{b}$ | 96 | 146.3 | 144.8 | 141.2 | 144 |
| 15 | 30 | 127.0 | 126.3 | 125.0 | 126.1$^{a}$ | 72 | 151.2 | 148.9 | 146.0 | 148 |
| 20 | 18 | 124.3 | 122.2 | 118.7 | 121.7$^{b}$ | 36 | 142.3 | 143.4 | 132.9 | 139 |
| 25 | 12 | 124.7 | 123.4 | 125.9 | 125.3$^{a}$ | 30 | 140.4 | 140.7 | 138.3 | 139 |
| Mean | | 124.4 | 123.0 | 123.5 | | | 145.0$^{A}$ | 144.5$^{A}$ | 139.6$^{B}$ | |
| $LSD_{0.05}$ (Temperature) | | | 1.89 | | | | | 2.06 | | |
| $LSD_{0.05}$ (Treatment) | | | 1.63 | | | | | 1.79 | | |
| $LSD_{0.05}$ (Temperature × Treatment) | | | 3.27 | | | | | 3.58 | | |
| 'CM0701-24' | | | | | | | | | | |
| 10 | 24 | 135.6 | 133.0 | 128.0 | 128.9$^{a}$ | 84 | 148.3 | 144.5 | 138.3 | 14 |
| 15 | 24 | 136.6 | 131.1 | 130.4 | 132.7$^{a}$ | 48 | 147.7 | 141.2 | 144.7 | 14 |
| 20 | 18 | 130.4 | 130.4 | 128.1 | 129.6$^{a}$ | 30 | 141.2 | 140.7 | 138.3 | 14 |
| 25 | 12 | 125.0 | 122.9 | 120.6 | 123.1$^{b}$ | 24 | 134.3 | 132.6 | 130.6 | 13 |
| Mean | | 132.1$^{A}$ | 129.3$^{A}$ | 124.3$^{B}$ | | | 142.9$^{A}$ | 139.8$^{AB}$ | 138.0$^{B}$ | |
| $LSD_{0.05}$ (Temperature) | | | 2.43 | | | | | 2.31 | | |
| $LSD_{0.05}$ (Treatment) | | | 2.11 | | | | | 2.00 | | |
| $LSD_{0.05}$ (Temperature × Treatment) | | | 4.21 | | | | | 4.01 | | |

Means within each column followed by the same lowercase letters are not significantly different at $P < 0.05$ by LSD. Mea
uppercase letters are not significantly different at $P < 0.05$ by LSD.

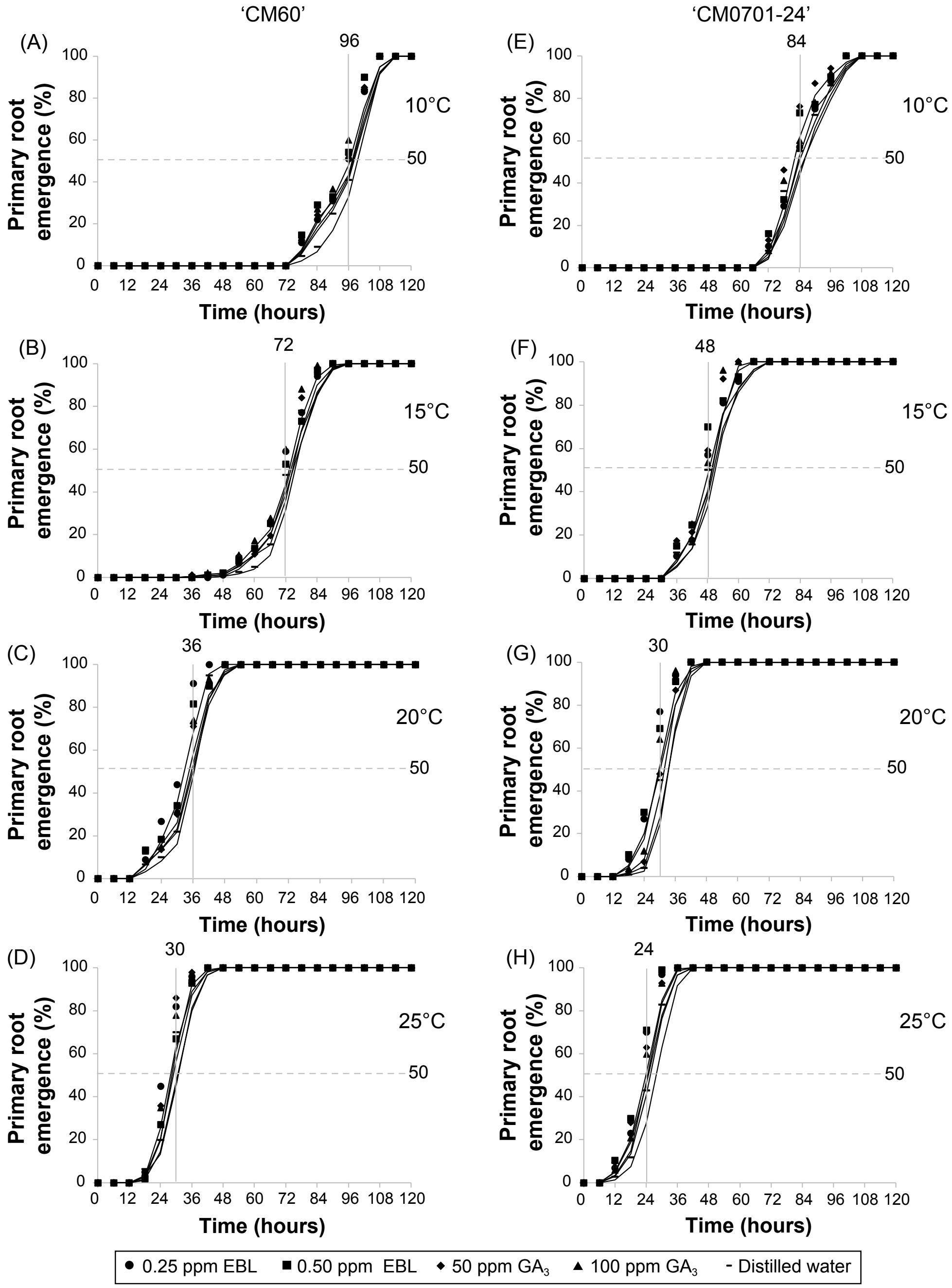


Figure 2. Primary root emergence (%) of 'CM60' (**A**-**D**) and 'CM0701-24' (**E**-**H**) soya bean seeds under different concentrations of EBL and $GA_3$ at 10, 15, 20 and 25°C.


Figure 3. Soya bean seed physical changes during seed imbibition of 'CM60' (**A**) and 'CM0701-24' (**B**) at 10, 15, 20 and 25°C. Scale bar, 5 mm.

Table 2. Effects of temperature and EBL and $GA_3$ treatment on primary root emergence at 36 hours for 'CM60' and 'CM0701-24' soya bean seeds at 10, 15, 20 and 25°C.

| Treatment | Primary root emergence (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 'CM60' | | | | | 'CM0701-24' | | | | |
| | 10°C | 15°C | 20°C | 25°C | Mean | 10°C | 15°C | 20°C | 25°C | Mean |
| Untreated | $0^{g}$ | $0^{g}$ | $72.75^{e}$ | $93.00^{c}$ | $41.44^{d}$ | $0^{k}$ | $10.25^{j}$ | $84.00^{g}$ | $84.50^{g}$ | $44.69^{c}$ |
| Distilled water | $0^{g}$ | $0^{g}$ | $72.50^{e}$ | $93.00^{c}$ | $41.38^{d}$ | $0^{k}$ | $12.00^{ij}$ | $85.00^{g}$ | $83.00^{g}$ | $45.00^{c}$ |
| 0.25 ppm EBL | $0^{g}$ | $0^{g}$ | $91.00^{c}$ | $96.00^{b}$ | $46.75^{a}$ | $0^{k}$ | $10.20^{j}$ | $94.00^{cd}$ | $97.00^{b}$ | $50.30^{b}$ |
| 0.50 ppm EBL | $0^{g}$ | $0^{g}$ | $81.50^{d}$ | $93.00^{c}$ | $43.63^{c}$ | $0^{k}$ | $14.91^{hi}$ | $91.00^{ef}$ | $99.00^{a}$ | $51.23^{a}$ |
| 50 ppm $GA_3$ | $0^{g}$ | $1.00^{f}$ | $71.00^{e}$ | $98.00^{a}$ | $42.50^{b}$ | $0^{k}$ | $17.30^{h}$ | $90.00^{f}$ | $93.00^{de}$ | $50.08^{b}$ |
| 100 ppm $GA_3$ | $0^{g}$ | $1.00^{f}$ | $74.00^{e}$ | $96.00^{b}$ | $42.75^{bc}$ | $0^{k}$ | $15.00^{h}$ | $96.00^{bc}$ | $93.00^{de}$ | $51.00^{ab}$ |
| Mean | $0^{D}$ | $0.33^{C}$ | $77.13^{B}$ | $94.83^{A}$ | | $0^{D}$ | $13.28^{C}$ | $90.00^{B}$ | $91.58^{A}$ | |
| $LSD_{0.05}$ (Temperature) | | | 0.83 | | | | | 1.22 | | |
| $LSD_{0.05}$ (Treatment) | | | 1.01 | | | | | 1.49 | | |
| $LSD_{0.05}$ (Temperature × Treatment) | | | 2.03 | | | | | 2.98 | | |

Means within each column followed by the same lowercase letters are not significantly different at $P < 0.05$ by LSD. Means within each row followed by the same uppercase letters are not significantly different at $P < 0.05$ by LSD.

Table 3. Effects of temperature and EBL and $GA_3$ treatment on germination for eight days of 'CM60' and 'CM0701-24' soya bean seeds at 10, 15, 20 and 25°C.

| Treatment | Germination (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 'CM60' | | | | | 'CM0701-24' | | | | |
| | 10°C | 15°C | 20°C | 25°C | Mean | 10°C | 15°C | 20°C | 25°C | Mean |
| Untreated | $67^{k}$ | $72^{ijk}$ | $94^{cd}$ | $96^{bc}$ | $82^{b}$ | $64^{k}$ | $82^{i}$ | $88^{gh}$ | $85^{hi}$ | $80^{c}$ |
| Distilled water | $70^{k}$ | $78^{hi}$ | $95^{c}$ | $93^{cd}$ | $84^{b}$ | $60^{k}$ | $83^{i}$ | $85^{hi}$ | $83^{i}$ | $78^{c}$ |
| 0.25 ppm EBL | $77^{hij}$ | $82^{gh}$ | $99^{a}$ | $96^{bc}$ | $88^{a}$ | $82^{i}$ | $83^{i}$ | $94^{cde}$ | $97^{b}$ | $89^{ab}$ |
| 0.50 ppm EBL | $72^{jk}$ | $92^{de}$ | $95^{c}$ | $93^{cd}$ | $88^{a}$ | $73^{j}$ | $82^{i}$ | $91^{efg}$ | $99^{a}$ | $86^{b}$ |
| 50 ppm $GA_3$ | $78^{hi}$ | $84^{fg}$ | $94^{cd}$ | $98^{ab}$ | $89^{a}$ | $76^{j}$ | $92^{ef}$ | $90^{fg}$ | $93^{def}$ | $88^{b}$ |
| 100 ppm $GA_3$ | $78^{hi}$ | $88^{ef}$ | $93^{cd}$ | $96^{bc}$ | $89^{a}$ | $75^{j}$ | $96^{bc}$ | $96^{bcd}$ | $93^{ef}$ | $90^{a}$ |
| Mean | $74^{C}$ | $83^{B}$ | $95^{A}$ | $95^{A}$ | | $72^{D}$ | $86^{C}$ | $91^{B}$ | $92^{A}$ | |
| $LSD_{0.05}$ (Temperature) | | | | 2.16 | | | | | 1.58 | |
| $LSD_{0.05}$ (Treatment) | | | | 2.64 | | | | | 1.94 | |
| $LSD_{0.05}$ (Temperature × Treatment) | | | | 5.29 | | | | | 3.87 | |

Means within each column followed by the same lowercase letters are not significantly different at $P < 0.05$ by LSD. Means within each row followed by the same uppercase letters are not significantly different at $P < 0.05$ by LSD.

had significantly higher germination than those of untreated seeds. At high temperature, the germination of 'CM60' and 'CM0701-24' seeds treated with EBL and $GA_3$ was not significantly different from that of untreated seeds and still had germination higher than 90 and 80%, respectively. 'CM60' seeds treated with EBL and $GA_3$ had significantly higher germination than those of untreated seeds at low temperature. For 'CM0701-24', treating seeds with $GA_3$ resulted in higher germination than untreated seeds at 15°C. While the germination of seeds treated with EBL and $GA_3$ were significantly higher than those of untreated seed at 10°C.

*Germination index*

The highest germination index was observed in soya bean seeds sown at 25°C, whereas the lowest germination index was found at 10°C (table 4). Seeds treated with EBL and $GA_3$ were significantly different in germination index compared with untreated seeds at high temperature. In 'CM60', seeds treated with EBL and $GA_3$ had higher germination index than those of untreated seed, whereas 'CM0701-24' seeds treated with $GA_3$ gave higher germination index than untreated seed at 15°C. It is indicated that 50 and 100 ppm $GA_3$ had a positive effect on germination index at 15°C.

*Mean germination time*

Soya bean seeds of 'CM60' and 'CM0701-24' had significantly lower mean germination time at high temperature than at low temperature (table 5). Seeds treated with $GA_3$ had significantly lower mean germination time than that of untreated seeds. At 25°C, seeds treated with $GA_3$ had significantly lower mean germination time than untreated seeds in both varieties. EBL and $GA_3$-treated seed had significantly higher mean germination time than those of untreated seeds at 10 and 15°C. It can be noted that soya bean seeds treated with EBL and $GA_3$ had lower mean germination time and higher speed of germination than those of untreated seeds at all temperatures.

*Shoot and root length*

The highest shoot and root lengths was found at 25°C (tables 6 and 7). 'CM60' and 'CM0701-24' seeds treated with $GA_3$ had significantly higher shoot lengths than those of untreated seeds. The shoot length of $GA_3$-treated seeds was significantly different from that of untreated seeds when germinated at both low and high temperatures (table 6). While $GA_3$-treated seeds had higher root lengths than those of untreated seeds at low temperature (table 7). The results indicated that 'CM60' and 'CM0701-24' seeds treated with $GA_3$ had the highest shoot length under all temperatures (table 6). Seeds treated with 100 ppm $GA_3$ had the highest root length in 'CM60' while the highest root length of 'CM0701-24' was found for seeds treated with 0.25 and 0.50 ppm EBL (table 7).

## Discussion

In this study, the patterns of seed imbibition and primary root protrusion were affected by temperature in both 'CM60' and 'CM0701-24' soya bean varieties (figures 1 and 2).

Table 4. Effects of temperature and EBL and $GA_3$ treatment on the germination index of 'CM60' and 'CM071-24' soya bean seeds at 10, 15, 20 and 25°C.

| Treatment | Germination index | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 'CM60' | | | | | 'CM0701-24' | | | | |
| | 10°C | 15°C | 20°C | 25°C | Mean | 10°C | 15°C | 20°C | 25°C | Mean |
| Untreated | $8.39^{m}$ | $10.37^{k}$ | $18.93^{f}$ | $29.58^{cd}$ | $16.82^{c}$ | $8.04^{l}$ | $11.83^{hi}$ | $17.78^{f}$ | $27.59^{c}$ | $16.31^{c}$ |
| Distilled water | $8.77^{m}$ | $11.26^{j}$ | $19.25^{f}$ | $29.08^{d}$ | $17.09^{c}$ | $7.57^{l}$ | $12.25^{i}$ | $17.20^{f}$ | $27.38^{c}$ | $16.10^{c}$ |
| 0.25 ppm EBL | $9.64^{kl}$ | $11.94^{ij}$ | $20.37^{e}$ | $31.42^{b}$ | $18.34^{ab}$ | $10.36^{j}$ | $12.41^{hi}$ | $19.39^{e}$ | $31.71^{b}$ | $18.46^{b}$ |
| 0.50 ppm EBL | $9.04^{lm}$ | $13.40^{g}$ | $19.72^{ef}$ | $30.25^{c}$ | $18.10^{b}$ | $9.27^{k}$ | $12.46^{h}$ | $18.98^{e}$ | $32.81^{a}$ | $18.38^{b}$ |
| 50 ppm $GA_3$ | $9.77^{k}$ | $12.30^{hi}$ | $19.65^{ef}$ | $33.17^{a}$ | $18.72^{a}$ | $9.64^{k}$ | $13.95^{g}$ | $18.76^{e}$ | $31.45^{b}$ | $18.45^{b}$ |
| 100 ppm $GA_3$ | $9.82^{k}$ | $12.95^{gh}$ | $19.48^{ef}$ | $32.42^{ab}$ | $18.67^{a}$ | $9.52^{k}$ | $14.54^{g}$ | $20.14^{d}$ | $31.29^{b}$ | $18.87^{a}$ |
| Mean | $9.24^{D}$ | $12.03^{C}$ | $19.57^{B}$ | $30.99^{A}$ | | $9.07^{D}$ | $12.91^{C}$ | $18.71^{B}$ | $30.37^{A}$ | |
| $LSD_{0.05}$ (Temperature) | | | 0.32 | | | | | 0.28 | | |
| $LSD_{0.05}$ (Treatment) | | | 0.40 | | | | | 0.34 | | |
| $LSD_{0.05}$ (Temperature × Treatment) | | | 0.79 | | | | | 0.69 | | |
| C.V. (%) | | | 3.14 | | | | | 2.74 | | |

Means within each column followed by the same lowercase letters are not significantly different at $P < 0.05$ by LSD. Means within each row followed by the same uppercase letters are not significantly different at $P < 0.05$ by LSD.

Table 5. Effects of temperature and EBL and $GA_3$ treatment on mean germination time of 'CM60' and 'CM071-24' soya bean seeds at 10, 15, 20 and 25°C.

| Treatment | Mean germination time (days) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 'CM60' | | | | | 'CM0701-24' | | | | |
| | 10°C | 15°C | 20°C | 25°C | Mean | 10°C | 15°C | 20°C | 25°C | Mean |
| Untreated | $7.98^{l}$ | $6.96^{k}$ | $4.97^{h}$ | $3.30^{d}$ | $5.80^{d}$ | $7.97^{o}$ | $6.94^{l}$ | $4.97^{h}$ | $3.11^{d}$ | $5.75^{e}$ |
| Distilled water | $7.99^{l}$ | $6.94^{k}$ | $4.95^{h}$ | $3.25^{c}$ | $5.78^{c}$ | $7.93^{n}$ | $6.82^{k}$ | $4.96^{h}$ | $3.06^{c}$ | $5.69^{d}$ |
| 0.25 ppm EBL | $7.99^{l}$ | $6.89^{j}$ | $4.86^{g}$ | $3.11^{b}$ | $5.71^{b}$ | $7.93^{n}$ | $6.75^{j}$ | $4.88^{g}$ | $3.11^{d}$ | $5.67^{c}$ |
| 0.50 ppm EBL | $7.97^{l}$ | $6.86^{ij}$ | $4.85^{fg}$ | $3.14^{b}$ | $5.70^{b}$ | $7.89^{m}$ | $6.66^{i}$ | $4.84^{ef}$ | $3.08^{c}$ | $5.62^{b}$ |
| 50 ppm $GA_3$ | $7.99^{l}$ | $6.86^{ij}$ | $4.82^{ef}$ | $3.00^{a}$ | $5.67^{a}$ | $7.89^{m}$ | $6.67^{i}$ | $4.84^{ef}$ | $2.99^{a}$ | $5.60^{a}$ |
| 100 ppm $GA_3$ | $7.95^{l}$ | $6.83^{i}$ | $4.81^{e}$ | $3.01^{a}$ | $5.65^{a}$ | $7.89^{m}$ | $6.67^{i}$ | $4.82^{ef}$ | $3.02^{b}$ | $5.60^{a}$ |
| Mean | $7.98^{D}$ | $6.89^{C}$ | $4.88^{B}$ | $3.14^{A}$ | | $7.92^{D}$ | $6.75^{C}$ | $4.89^{B}$ | $3.06^{A}$ | |
| $LSD_{0.05}$ (Temperature) | | | 0.01 | | | | | 0.01 | | |
| $LSD_{0.05}$ (Treatment) | | | 0.01 | | | | | 0.01 | | |
| $LSD_{0.05}$ (Temperature × Treatment) | | | 0.03 | | | | | 0.02 | | |
| C.V. (%) | | | 0.33 | | | | | 0.29 | | |

Means within each column followed by the same lowercase letters are not significantly different at $P < 0.05$ by LSD. Means within each row followed by the same uppercase letters are not significantly different at $P < 0.05$ by LSD.

Table 6. Effects of temperature and EBL and $GA_3$ treatment on shoot length eight days after germination for ‘CM60’ and ‘CM071-24’ soya bean seeds at 10, 15, 20 and 25°C.

| Treatment | Shoot length (mm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ‘CM60’ | | | | | ‘CM0701-24’ | | | | |
| | 10°C | 15°C | 20°C | 25°C | Mean | 10°C | 15°C | 20°C | 25°C | Mean |
| Untreated | 64$^{m}$ | 428$^{j}$ | 1,184$^{ef}$ | 1,218$^{e}$ | 723$^{b}$ | 174$^{j}$ | 379$^{g}$ | 871$^{e}$ | 1,127$^{bc}$ | 638$^{b}$ |
| Distilled water | 64$^{m}$ | 401$^{j}$ | 1,338$^{cd}$ | 1,144$^{fg}$ | 737$^{b}$ | 184$^{j}$ | 363$^{gh}$ | 947$^{d}$ | 1,190$^{b}$ | 671$^{b}$ |
| 0.25 ppm EBL | 54$^{m}$ | 286$^{k}$ | 1,084$^{gh}$ | 1,061$^{h}$ | 621$^{d}$ | 156$^{jk}$ | 251$^{i}$ | 937$^{d}$ | 999$^{d}$ | 586$^{d}$ |
| 0.50 ppm EBL | 61$^{m}$ | 395$^{j}$ | 1,221$^{e}$ | 1,040$^{h}$ | 679$^{c}$ | 138$^{k}$ | 239$^{i}$ | 859$^{e}$ | 942$^{d}$ | 545$^{c}$ |
| 50 ppm $GA_3$ | 109$^{l}$ | 391$^{j}$ | 1,384$^{bc}$ | 1,500$^{a}$ | 846$^{a}$ | 336$^{h}$ | 544$^{f}$ | 1,069$^{c}$ | 1,452$^{a}$ | 850$^{a}$ |
| 100 ppm $GA_3$ | 116$^{l}$ | 566$^{i}$ | 1,296$^{d}$ | 1,428$^{ab}$ | 851$^{a}$ | 326$^{h}$ | 540$^{f}$ | 1,194$^{d}$ | 1,459$^{a}$ | 880$^{a}$ |
| Mean | 78$^{C}$ | 411$^{B}$ | 1,251$^{A}$ | 1,232$^{A}$ | | 219$^{D}$ | 386$^{C}$ | 979$^{B}$ | 1,195$^{A}$ | |
| $LSD_{0.05}$ (Temperature) | | | 24.33 | | | | | 25.17 | | |
| $LSD_{0.05}$ (Treatment) | | | 29.80 | | | | | 30.83 | | |
| $LSD_{0.05}$ (Temperature × Treatment) | | | 59.60 | | | | | 61.66 | | |
| C.V. (%) | | | 5.69 | | | | | 6.30 | | |

Means within each column followed by the same lowercase letters are not significantly different at $P < 0.05$ by LSD. Means within each row followed by the same uppercase letters are not significantly different at $P < 0.05$ by LSD.

Table 7. Effects of temperature and EBL and $GA_3$ treatment on root length eight days after germination for ‘CM60’ and ‘CM071-24’ soya bean seeds at 10, 15, 20 and 25°C.

| Treatment | Root length (mm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ‘CM60’ | | | | | ‘CM0701-24’ | | | | |
| | 10°C | 15°C | 20°C | 25°C | Mean | 10°C | 15°C | 20°C | 25°C | Mean |
| Untreated | 253$^{kl}$ | 383$^{h}$ | 876$^{e}$ | 1,274$^{bc}$ | 696$^{c}$ | 198$^{l}$ | 408$^{h}$ | 1,025$^{f}$ | 1,209$^{d}$ | 710$^{c}$ |
| Distilled water | 263$^{k}$ | 342$^{hij}$ | 879$^{e}$ | 1,331$^{ab}$ | 704$^{c}$ | 271$^{ij}$ | 396$^{h}$ | 1,060$^{ef}$ | 1,214$^{d}$ | 735$^{d}$ |
| 0.25 ppm EBL | 225$^{l}$ | 313$^{j}$ | 907$^{e}$ | 1,357$^{a}$ | 700$^{c}$ | 246$^{jk}$ | 467$^{g}$ | 1,265$^{cd}$ | 1,386$^{a}$ | 841$^{a}$ |
| 0.50 ppm EBL | 238$^{kl}$ | 355$^{hi}$ | 1,001$^{d}$ | 1,283$^{ab}$ | 719$^{c}$ | 234$^{k}$ | 469$^{g}$ | 1,309$^{bc}$ | 1,358$^{ab}$ | 843$^{a}$ |
| 50 ppm $GA_3$ | 458$^{g}$ | 338$^{ij}$ | 1,053$^{d}$ | 1,207$^{c}$ | 764$^{b}$ | 290$^{i}$ | 478$^{g}$ | 1,064$^{ef}$ | 1,223$^{d}$ | 764$^{b}$ |
| 100 ppm $GA_3$ | 520$^{f}$ | 511$^{f}$ | 1,014$^{d}$ | 1,348$^{ab}$ | 848$^{a}$ | 295$^{i}$ | 475$^{g}$ | 1,086$^{e}$ | 1,206$^{d}$ | 766$^{b}$ |
| Mean | 326$^{D}$ | 373$^{C}$ | 955$^{B}$ | 1,300$^{A}$ | | 256$^{D}$ | 449$^{C}$ | 1,135$^{B}$ | 1,266$^{A}$ | |
| $LSD_{0.05}$ (Temperature) | | | 23.59 | | | | | 18.93 | | |
| $LSD_{0.05}$ (Treatment) | | | 28.89 | | | | | 23.19 | | |
| $LSD_{0.05}$ (Temperature × Treatment) | | | 57.77 | | | | | 46.37 | | |
| C.V. (%) | | | 5.55 | | | | | 4.24 | | |

Means within each column followed by the same lowercase letters are not significantly different at $P < 0.05$ by LSD. Means within each row followed by the same uppercase letters are not significantly different at $P < 0.05$ by LSD.

The time of imbibition and to reach 50% primary root emergence were delayed when temperature decreased whereas high temperature reduced the time for seeds to complete phases 1 and 2 of the triphasic pattern of imbibition described by Bewley (1997). It is well known that higher temperatures increase the energy of water and diffusion pressure resulting in activation of the metabolic and physiological changes during seed imbibition that could accelerate germination (Castro *et al*., 2004). Conversely, lower temperatures during seed imbibition usually slow down seed water uptake and metabolic pathways as the phospholipids are unable to reorganise from the hexagonal phase to the lipid bilayer phase (Simon, 1974).

Comparing the two soya bean genotypes, ‘CM0701-24’ reached the end of phase 2 slightly earlier than ‘CM60’ whereas the patterns of water uptake in EBL- and $GA_3$-treated seeds were quite similar. A factor associated with this difference is the variation in the physical process of imbibition, the seed coat as a protection against imbibitional injury, membranes during imbibition relative to seed vigour that low vigour soya bean seeds has some broken seed coats which is resulted in rapid water uptake causing damage to the cotyledon cells and adherence of the seed coat to the embryo could play an important role in controlling water uptake in soya bean (Oliveira *et al*., 1984; Woodstock, 1988).

As compared with EBL- and $GA_3$-treated seeds, the patterns of water uptake of the two genotypes in distilled water were quite similar under different temperatures (figure 1). However, there was a tendency for seed imbibition and times to reach 50% primary root protrusion and the end of phase 2 to be slightly earlier for seeds treated with EBL and $GA_3$ than those treated with distilled water, depending on the temperature. Primary root emergence of the ‘CM60’ and ‘CM0701-24’ seeds treated with EBL and $GA_3$ could be increased under high temperatures. Whereas, the primary root emergence of both soya bean genotypes were 0% at 10°C and less than 20% at 15°C in the case of ‘CM0701-24’. These results demonstrated that the temperature below than 15°C were likely to be inapplicable for primary root emergence in the both ‘CM60’ and ‘CM0701-24’ soya bean seeds. No seed germination found when the temperature is below 10°C (Jomol *et al*., 2000).

There was higher germination at 20 and 25°C than at 10 and 15°C (table 3). It can be noted that germination increased when the temperature increased. Similarly, Tyagi and Tripathi (1983) reported that the optimal temperature for soya bean seed germination is 25°C. There are some reports that at 15°C, soya bean seeds required 10 days to reach 80% germination while, at 10°C seeds required nearly 15 days to complete the process, resulting in 60% reduction in final germination compared with 25°C (Khamassi *et al*., 2013).

Germination of seeds of both soya bean genotypes seeds treated with EBL and $GA_3$ did not increase compared with distilled water and untreated seeds under high temperature (table 3). Whereas, 0.25 and 0.50 ppm EBL or 50 and 100 ppm $GA_3$ could increase the germination of soya bean seeds at low temperatures (10 and 15°C). The results show that EBL and $GA_3$ play important roles in seed germination. Gibberellins promote germination by mobilising the resources necessary for embryo development and can stimulate the germination process of non-dormant seeds (Tuan *et al*., 2018). The expanding embryo begins to leave space during cotyledon development, GA-biosynthesis is activated and followed by the activation of proteolytic enzymes and α-amylases which is secreted to promote the degradation of starch to glucose (Sreenivasulu and Wobus, 2013). The exogenous application of $GA_3$ has been reported to stimulate seed germination in many plants (Bewley and Black, 1994; Yang *et al*., 2009), promoting germination and seedling development, cell division, hypocotyl growth as well as stand establishment (Karmoker, 1984). According to Wang *et al*. (1996), 0.1 mM $GA_3$ resulted in the highest seedling emergence of corn and soybean at 10°C. EBL has various roles in plant growth and development including cell growth, seedling elongation and endosperm rupture (Leubner-Metzger, 2001) and interacts with both GAs and ABA to coordinate seed germination (Li *et al.*, 2016). According to Li *et al*. (2002), 0.1 to 0.4 ppm EBL increased seed germination and reduced the time of germination of Chinese red pine and black locust by 32 and 24 hours. 0.1 ppm EBL effectively increased the germination rate, seedling growth and plant biomass of maize under chilling stress (Sun *et al*., 2020).

High temperature reduced vigour in terms of MGT or speed of germination (table 5). The speed of germination is directly dependent on temperature. Likewise, Srivastava *et al*. (2015) found that kidney bean seeds grown under low temperature (3°C) for 15 days had germination rate, seed vigour and germination index lower than normal room temperature. In this study, soya bean seeds treated with EBL and $GA_3$ had significantly increased GI and reduced MGT (tables 4 and 5). It is supported by Thongsri *et al*. (2019) reported that soya bean 'CM60' seeds were pre-soaked with 100 ppm $GA_3$ or 0.50 ppm EBL decreased MGT compared with untreated seed at 10°C. In *Triticum aestivum* L. cv. 'Koyuki', pre-soaked seeds with 0.1 mM $GA_3$ or 0.1 mM $GA_3$ plus 10 mM Proline improved speed of germination and reduced MGT at 4°C (Sultana *et al*., 2000).

High temperature (25°C) enhanced shoot and root length of soya bean seeds (tables 6 and 7). Temperature is also fundamental for the development of specific parts of seedlings and primary root, generally the first part to protrude during germination. Inadequate temperature directly affects root growth, a process in which cells are rapidly dividing, and any adverse environmental factor diminishes the capacity of the root for development (Larcher, 2003). Likewise, Janas *et al*. (2000) found that root growth was inhibited under low temperature. Compared with the relative growth rate at 25°C, 10°C resulted in a 10-fold decrease in root growth.

Seeds treated with 50 and 100 ppm $GA_3$ had greater shoot length than seeds treated with EBL, distilled water or untreated seed at all temperatures (table 6). $GA_3$ influences cell elongation of shoot and seedling vigour and affects shoot elongation but did not increase primary and secondary root (Gupta and Chakrabarty, 2013). Likewise, Wang *et al*. (1996) reported that $GA_3$ stimulated soya bean seedling emergence and improved soya bean seedling development under low temperature at 10°C due to cell expansion. Nevertheless, $GA_3$ produced in roots plays important roles in growth, promoting cell division, increasing nitrogen metabolism, stimulating shoot growth and root elongation, and reducing the effects of stress (Bai *et al*., 2016). According to Suo *et al*. (2017), germination and seedling growth of sweet corn seeds coated with 200 ppm $GA_3$ were improved. The results of shoot and root length are supported by those of Leite *et al*. (2002) who confirmed the positive effect of $GA_3$ on the vegetative growth of soya bean treated with 50 ppm $GA_3$. However, 'CM0701-24' seeds treated with 0.25 or 0.50 ppm

EBL had increased root length at 15, 20 and 25°C (table 7). EBL promoted cell elongation and cell division of seedlings (Wei and Li, 2016). Furthermore, EBL can induce chilling-tolerance of seedlings and increase seedling size, whereas low concentrations of BRs can promote root growth (Singh *et al*., 2012). In addition, Anwar *et al*. (2018) reported that EBL treatments significantly enhanced cucumber seedling growth and improved vigour. In this study, EBL increased root length of ‘CM0701-24’ compared with distilled water and untreated seeds at 15°C but not at 10°C.

In conclusion, this study recommends 50-100 ppm $GA_3$ as a pre-soaking and seed treatment for improved physiological quality of soya bean seeds sown at low temperature.

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