

## SOYBEAN MOTHER PLANT EXPOSURE TO TEMPERATURE STRESS AND ITS EFFECT ON GERMINATION UNDER OSMOTIC STRESS

SHAD K. KHALIL<sup>1,2\*</sup>, JOHN G. MEXAL<sup>2</sup>, ABDUR REHMAN<sup>1</sup>, AMIR ZAMAN KHAN<sup>1</sup>, SAID WAHAB<sup>3</sup>, MUHAMMAD ZUBAIR<sup>4</sup>, I.H. KHALIL<sup>5</sup> AND FIDA MOHAMMAD<sup>5</sup>

<sup>1</sup>Department of Agronomy, <sup>2</sup>Department of Plant and Environmental Sciences, New Mexico State University, Box 3003, Las Cruces, NM 88003, USA, <sup>3</sup>Department of Food Science & Technology, <sup>4</sup>Department of Horticulture, <sup>5</sup>Department of Plant Breeding and Genetics, NWFP Agricultural University, Peshawar, Pakistan

### Abstract

High temperature reduces quality of soybean seed developed at different positions on the plant. The objective of this research was to study the quality of seed produced under different temperature regimes located at different position in the canopy. Soybean plants grown in pots were transferred at first pod stage to three growth chambers fixed at 18/10, 25/15 and 32/20°C day/night temperature having 13/11 hrs day/night length. The plants remained in growth chambers until physiological maturity. Seeds harvested from each growth chamber were exposed to osmotic stress having osmotic potential of -0.5 MPa and unstressed control. Both stressed and control treatments were germinated in three growth chambers fixed at 18, 25 and 35°C. Seed developed at lowest temperature (18/10°C day/night) had maximum germination. Germination decreased linearly with increased day/night temperature and lowest germination was recorded at highest temperature of 32/20°C (day/night). Seed developed at bottom position was heaviest and had better germination compared with seed developed at middle and top position. Seed germination was highest at 25°C and took fewer days to 50% germination than 18 and 25°C. Osmotic stress decreased germination and delayed days to 50% germination than control. It can be concluded that optimum temperature for seed development was 18/10°C (day/night) whereas best germination temperature was 25°C.

### Introduction

The pattern of seed germination varies according to seed source, parental nutrition, seed maturity and environmental condition during seed development (Chaisurisri *et al.*, 1992). Tropical conditions of high temperature and relative humidity during the final stages of seed development and maturation are serious seed production problems that result in rapid loss of viability in the standing crop (Delouche, 1980). Green *et al.*, (1965) reported that late maturing cultivars produced the best quality seed because they tend to fill seed after the hot, dry weather conditions had ended. They suggested that planting should be adjusted for cultivars with different maturity dates to avoid hot weather during seed fill. Egli *et al.*, (2005) and TeKrony *et al.*, (1980) concluded that high temperature during seed filling in the field reduced soybean seed germination.

Seed weight and location in the canopy are important parameters influenced by temperature. Modi & Asanzi (2008) and Gibson & Mullen (1996) reported that low production temperatures (20/10°C day/ night) gave heavier seeds of soybean and maize, while high temperature (30/20°C day/night) produced lighter seeds. Gbikpi & Crookston (1981) measured greater rates of dry matter accumulation in seeds from pods located in the upper canopy of indeterminate soybean compared with pods located in the lower portion of the canopy. However, Keigley & Mullen (1986) found that seeds from the middle stem region of indeterminate soybean cultivars produced heavier seedling compared with seeds from other regions of the stem.

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E-mail: shadkh2005@yahoo.com

There is little information available regarding the exposure of intact soybean plant to low and high temperature for longer durations and its effect on seed weight and germination, particularly during the seed filling stage. The effects of temperature and osmotic stress given to the seed by osmoticum such as polyethylene glycol (PEG 8000) on germination of soybean seed produced under different temperatures and germination temperature stress has yet to be elucidated. The seed developed at different temperature may also respond differently to the germination temperature. The present study was undertaken to examine the weight of soybean seed that developed and matured under different temperatures and evaluation of these seeds for germination at different temperatures after being exposed to mild PEG 8000 stress.

### Materials and Methods

**Plant growth in field:** Seed of indeterminate soybean cultivar William 82 was obtained from a commercial supplier (USA) and was stored at 4°C before use. Soybeans were planted on June 10, 1993 in 15 cm pots at the Fabian Garcia Science Center, New Mexico State University, Las Cruces, USA. One kg pots were filled with a mixture of 2:1 soil and vermiculite. Triple super phosphate was applied @ 10 g pot<sup>-1</sup> before sowing. Five seeds pot<sup>-1</sup> were planted and thinned to three plants, one month after planting. Urea was applied @ 2 g pot<sup>-1</sup> at two weeks interval after germination. Plants were watered twice a day in the first month and thereafter once a day.

**Plant growth in growth chamber:** On September 30, 1993, 35 pots each were moved to 3 growth chambers fixed at day and night temperatures of 18/10, 25/15 and 32/20°C. Photosynthetic photon flux of 90 μ mol m<sup>-2</sup> s<sup>-1</sup> was provided by cool white light fluorescent tubes (F 20 T12/cw, Sylvania, USA). All plants were in pod formation/development stage. The day/ night length was 13/11 hr, respectively. Red color tags were used for pods formed at the bottom 1/3 of main stem, while green and yellow color tags were used for pods formed in the middle and upper 1/3 of the main stem respectively. Pods were formed in the first and second week after moving the plants in growth chambers. Fewer pods were formed after the second week especially in the growth chamber fixed at 25/15°C, while pods without seeds were formed in the growth chamber fixed at 32/20°C. Pods formed after the second week in the growth chamber were not tagged. The plants remained in the growth chambers for 8-10 weeks until the tagged seeds were fully developed and attained physiological maturity. Pods from all plants in the same growth chamber with the same color tags were picked together, dried, threshed, cleaned, and stored at 4°C before use.

### Experiment 1

**Hundred seed weight:** Seed harvested from the same position on the mother plant from each growth chamber were evaluated for hundred seed weight. The data was analyzed according to randomized complete block design with sub samples using SAS Institute (1990). Block was mother plant temperature and treatment was position. Because no seed produced at P3 in growth chamber fixed at 32/20°C, there was one missing combination. Least significant difference (LSD) test was applied upon obtaining significant F-value.

**Germination:** Seeds obtained from mother plants exposed to 18/10 and 25/15°C day and night temperature in growth chambers were split into two halves for each position on the mother plant. One half of the seeds were stressed with PEG 8000 having osmotic potential of -0.5 MPa according to Michel (1983), while one half of the seeds were unstressed (control). Seeds were placed in clear plastic germination boxes measuring 13 x 13.5 cm on Kim wipes paper wetted with PEG 8000, while the control was wetted with distilled water. Each box was properly covered to prevent evaporation and maintain the relative humidity close to 100%. Seeds were germinated in three growth chambers fixed at 18, 25 and 35°C. Each treatment was repeated three times. Germinated seeds were counted and removed daily until the 38th day; thereafter no germination occurred. Germination was defined as seeds with visible radicle about 5 mm long (ISTA, 1993). Days to 50% germination were calculated from the daily counts. The data was analyzed according to randomized complete block design using SAS Institute (1990). Chamber temperature was the blocking factor and treatments were in a 2 (PEG concentration) x 2 (mother plant temperature) x 3 (seed position). LSD test was used for mean separation when F-values were significant.

## Experiment 2

**Germination:** There was insufficient seed from each position on mother plants exposed to 32/20°C day and night temperature for experiment 1. Therefore, this experiment was designed to study the effect of exposure of mother plants to various temperatures ignoring seed position on the mother plant. Seeds obtained from mother plants exposed to 18/10, 25/15 and 32/20°C day/night temperature were evaluated for seed germination and days to 50% germination in three growth chambers fixed at 18, 25, and 35°C as in experiment 1. Seeds were placed in clear plastic germination boxes measuring 13 x 13.5 cm on Kim wipes paper wetted with distilled water as described in experiment 1. Each treatment was repeated three times. Seeds were sown according to randomized complete block design. Germination counts were made daily until 26th day, thereafter no germination occurred. Days to 50% germination were calculated in similar way as described in experiment 1. The data was analyzed according to randomized complete block design of SAS Institute (1990) and LSD test was applied upon obtaining significant F-values.

## Results

### Experiment 1

**Hundred seed weight:** Seed exposed to different temperature on mother plant (MPT) and seed position (POS) significantly affected seed weight (Table 1). Seeds harvested from mother plant exposed to 18/10°C day and night temperature resulted in heavier seeds (Fig. 1). Few seeds were formed at bottom and middle position; while severely shriveled few seeds were formed at top position on mother plant grown at 32/20 °C. Seeds weight decreased linearly with each increment of temperature. Lightest seeds were obtained from plants grown at 32/20°C day and night temperature. Heaviest seeds were obtained from bottom position (Fig. 2). Seed weight decreased as the seed position progressed from bottom to top. Lightest seeds were recorded from both middle and top position (Fig. 2).

**Table 1. Analysis of variance of hundred seed weight (g) of soybean as affected by exposure of mother plant to different temperature and seed position on mother plant. Values in next column are P values for mean squares values.**

Source	D.F.	Mean square	P-value
Mother plant temp. (MPT)	2	132.55	(<0.01)
Position (POS)	2	40.04	(0.01)
MPT x POS	3*	1.43	(0.23)
Error	32**	0.39	
<b>C. Total</b>	<b>39***</b>		

\* = Error term for MPT and POS for seed weight , \*\* = Sub-sample = Rep (MPT\*POS)

\*\*\* = Corrected total

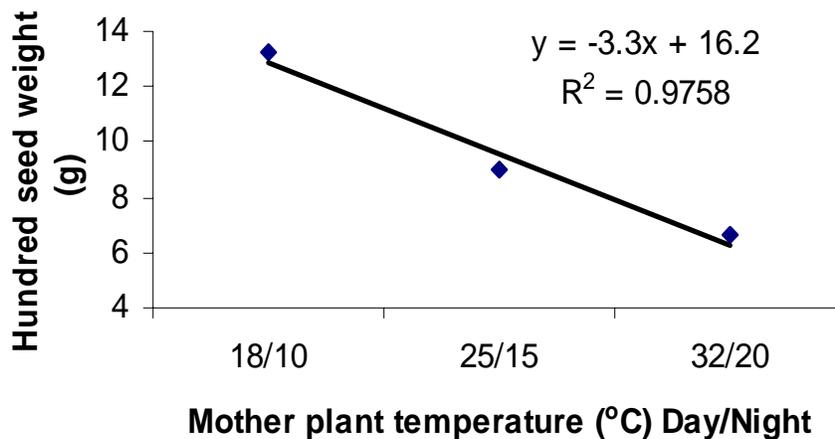


Fig. 1. Linear relationship between hundred seed weight (g) and mother plant temperature of soybean.

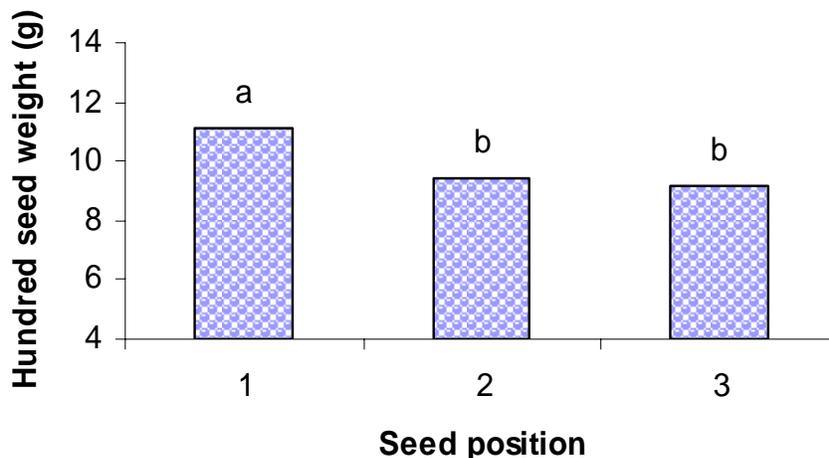


Fig. 2. Hundred seed weight (g) of soybean as affected by seed position on mother plant. Bars with similar letters are non significant at ( $p \leq 0.05$ ) using LSD. 1, 2, 3 are bottom, middle and top seed position on mother plant, respectively.

**Table 2. Analysis of variance of germination of soybean as affected by mother plant temperature, seed position, germination temperature and PEG 8000 stress. Values in parenthesis are P values for mean square values above it.**

Source	D.F.	Mean squares	
		Germination (%)	Day to 50% germination
Mother plant temp. (MPT)	1	3362 (<0.01)	11.20 (0.153)
Chamber temp. (CHT)	2	497.56 (<0.072)	2223.89 <0.01)
Position (POS)	2	2912.71 (0.012)	19.67 (0.037)
MPT x POS	2	1062.02 (<0.01)	3.43 (0.521)
Concentration (C)	1	968.00 (0.026)	1519.84 (<0.01)
MP x C	1	242.00 (0.247)	6.722 (0.263)
POS x C	2	825.53 (0.018)	5.07 (0.386)
MPT x POS x C	2	628.26 (0.042)	9.93 (0.167)
Error	69	375.42	7.66

**Germination:** Exposure of mother plants to different temperatures (MPT), seed position on the mother plant (POS), growth chamber temperature (CHT), PEG concentration (C), and interactions between MPTxPOS, POSxC, and MPTxPOSxC significantly affected germination (Table 2). Seeds harvested from mother plants that were exposed to 18/10°C day and night temperature resulted in higher germination than the seeds harvested from mother plant grown at 25/15°C day and night temperature from each position (Fig. 3). Seed that were produced either in the middle or at the top resulted in better germination than seed produced at the bottom exposed to 18/10°C. However, seed produced in the middle resulted in higher germination when mother plants were exposed to 25/15°C day and night temperature. Seeds exposed to 18/10°C day and night temperature resulted in better germination for all three seed positions. However, germination drastically decreased for seed obtained from the top position of the mother plant grown at 25/15°C day and night temperature. Maximum germination was observed from seed germinated in the growth chamber fixed at 25°C (Fig. 4). Germination decreased when seed germinated at 18°C or 35°C.

Seed exposed to PEG 8000 (200 g PEG kg<sup>-1</sup> water) stress decreased the germination compared with control (no seed treatment) (Fig. 5). The reduction in germination of PEG stressed seed was more for the bottom and top position than middle position.

**Days to 50% germination:** Seed position (POS), germination chamber temperature (CHT), and concentration (C) significantly affected days to 50% germination (Table 2). Seed obtained from bottom position took fewer days to 50% germination than seed obtained from middle and top position (Fig. 6). Seeds germinated at 25°C and 35°C took fewer days to 50% germination, compared with germination at 18°C (Fig. 7). Seed germination showed inverse relationship with germination temperature (Fig. 7). Seeds stressed with PEG 8000 delayed days to 50% germination compared with control and showed linear relationship with PEG stress (Fig. 8).

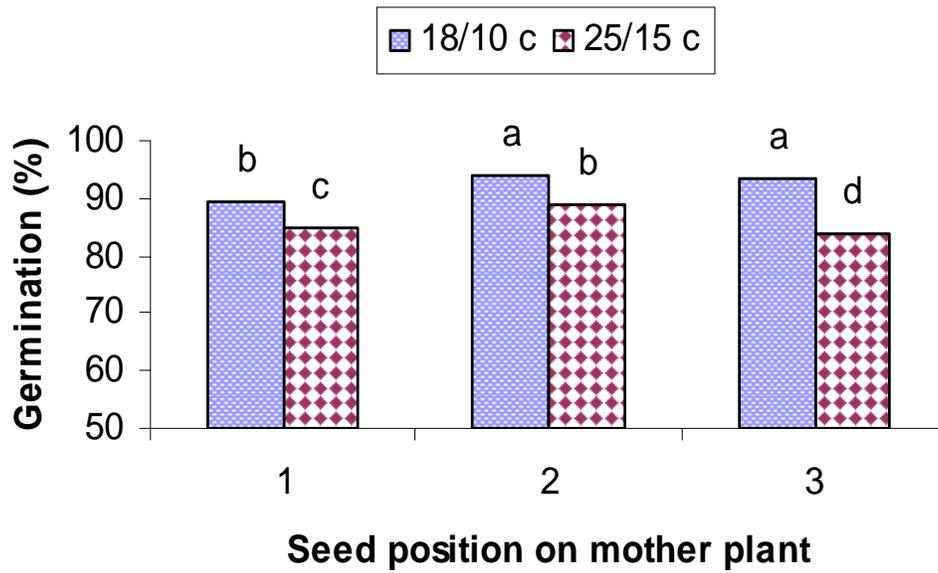


Fig. 3. Germination of soybean as affected by the interaction of seed position and mother plant temperature. Bars with similar letters are non significant at ( $p \leq 0.05$ ) using LSD. 1, 2, 3 are bottom, middle and top seed position on mother plant respectively.

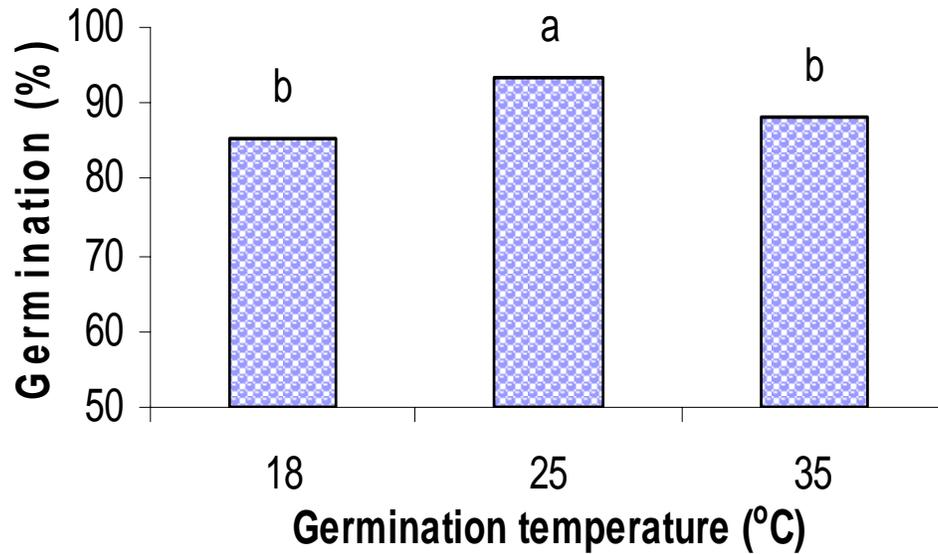


Fig. 4. Germination of soybean as affected by growth chamber temperature. Bars with similar letters are non significant at ( $p \leq 0.05$ ) using LSD.

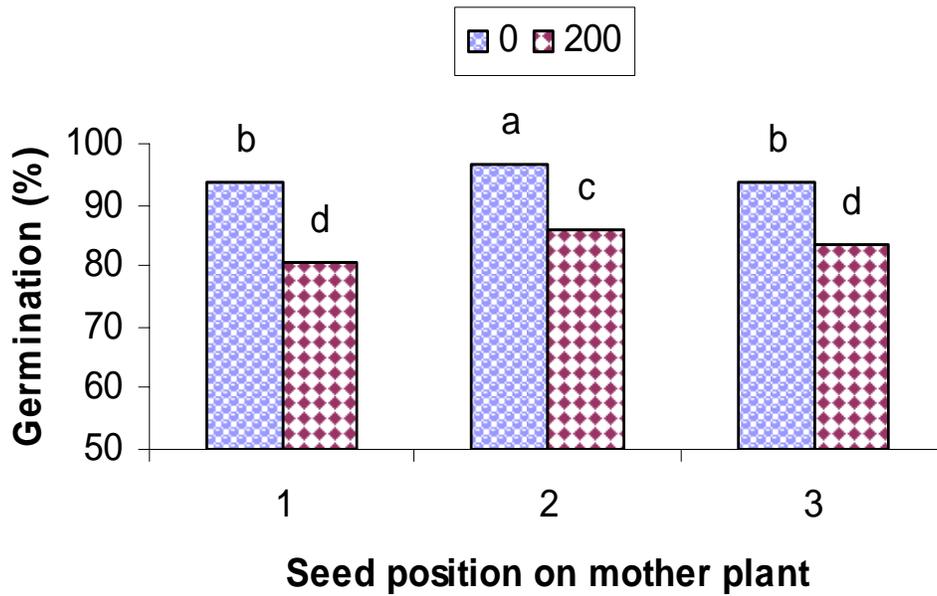


Fig. 5. Germination of soybean as affected by the interaction of seed position and PEG stress. Bars with similar letters are non significant at ( $p \leq 0.05$ ) using LSD. 1, 2, 3 are bottom, middle and top seed position on mother plant, respectively.

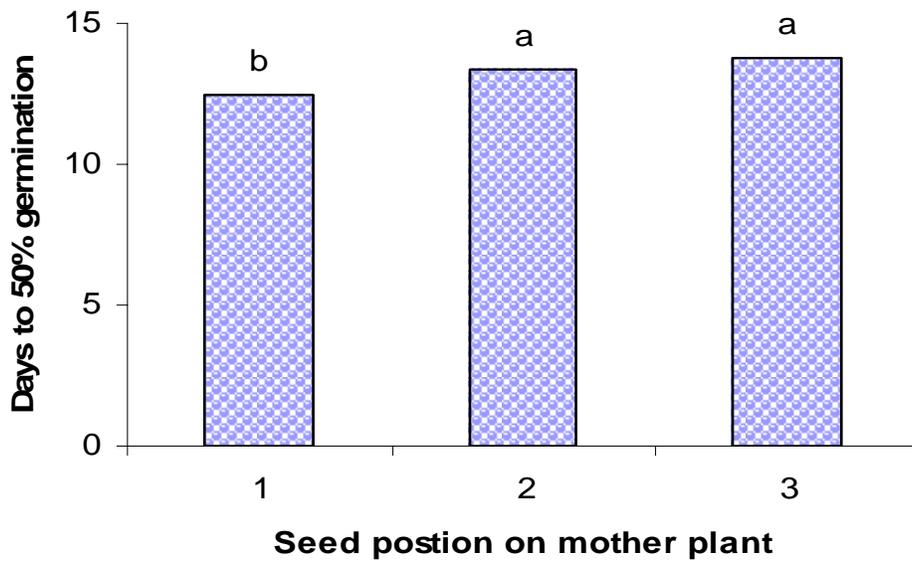


Fig. 6. Days to 50% germination of soybean as affected by seed position on mother plant. Bars with similar letters are non significant at ( $p \leq 0.05$ ) using LSD. 1, 2, 3 are bottom, middle and top seed position on mother plant, respectively.

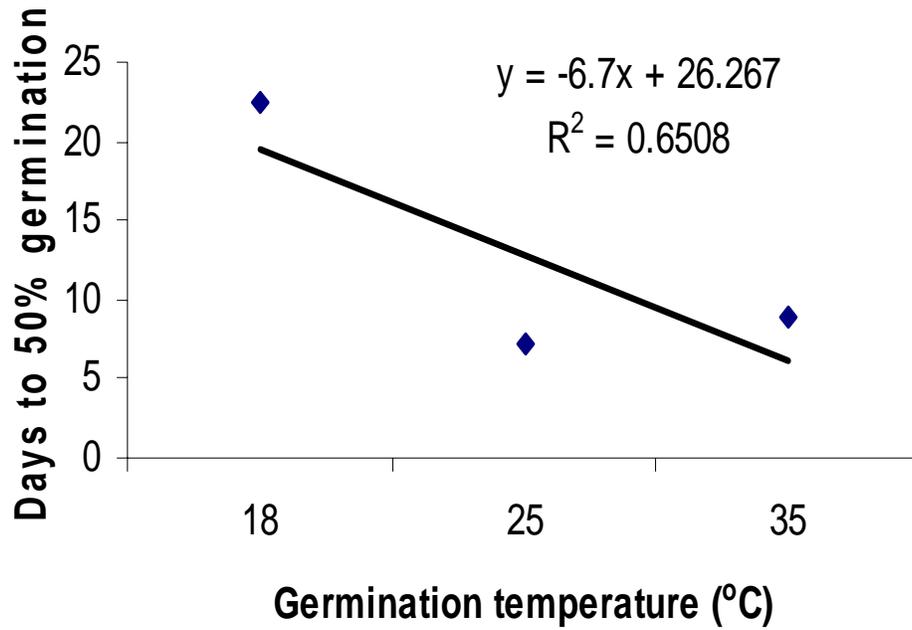


Fig. 7. Linear relationship between days to 50% germination and germination temperature of soybean.

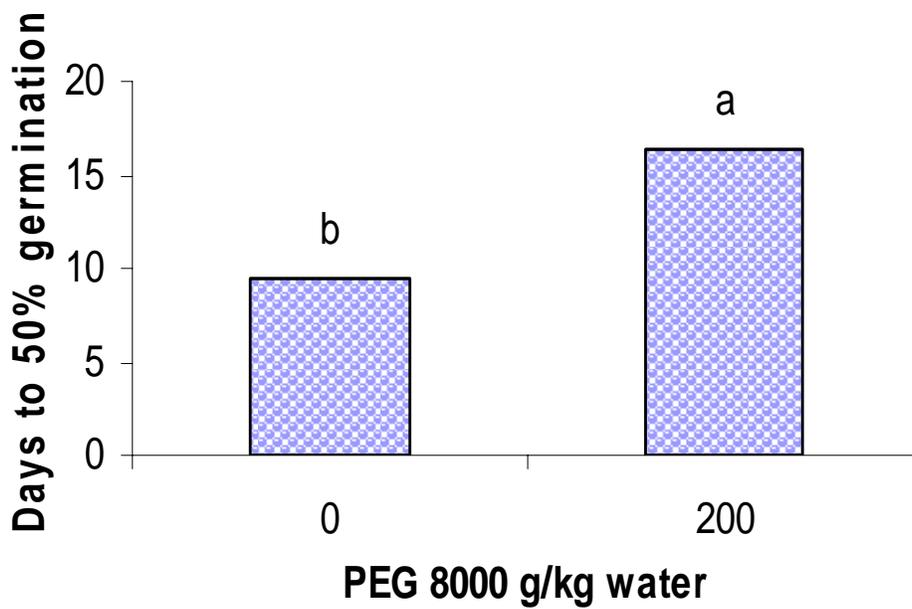


Fig. 8. Days to 50% germination of soybean as affected by PEG stress. Bars with similar letters are non significant at ( $p \leq 0.05$ ) using LSD.

**Table 3. Analysis of variance of radical germination of soybean as affected by exposure of mother plant to different temperature and germination temperature. Values in parenthesis are P values for mean squares values above it.**

Source	D.F.	Mean squares	
		Germination (%)	Day to 50% germination
Mother plant temp. (MPT)	2	1492.6 (<0.01)	8.26 (0.673)
Chamber temp. (CHT)	2	212.03 (0.110)	259.15 (0.020)
Error	22	90.57	67.37
<b>C. Total</b>	<b>26</b>		

## Experiment 2

**Germination:** Exposure of mother plant to different temperatures significantly affected germination (Table 3). Seeds obtained from mother plant grown at 18/10°C day and night temperature resulted in maximum germination (Fig. 9). Exposure of mother plant to temperature higher than 18/10°C produced seeds with lower germination. Poor germination was observed when mother plants were exposed to 32/20°C day and night temperature. Germination showed inverse relationship with mother plant temperature (Fig. 9).

**Days to 50% germination:** Germination chamber temperature significantly affected days to 50% germination (Table 3). Fewer days to 50% radicle germination were observed for the seed grown at 25 or 35°C. However, germination was delayed when seed was grown at 18°C (Fig. 10).

## Discussion

Temperature directly influenced germination, days to 50% germination and seed weight. Germination of the seed lots consistently decreased with increased growth chamber temperature to which the mother plants were exposed. The linear response indicates that exposure of mother plant to different temperatures is important in determining the extent of high temperature injury to seed germination. Khalil *et al.*, (2001) correlated temperature during seed development with seed germination and found that plants that matured during hot dry conditions produced seeds of poor germination. Similar effects of high temperature on germination were reported by Keigley & Mullen (1986), Siddique & Goodwine (1980), Green *et al.*, (1965). However, the above studies were conducted in the field where the temperature can not be controlled and the mother plants can not be exposed to a specific temperature for a specific duration.

The results of the present study indicate that seed weight decreased with increased temperature of mother plant. These results agree with the findings of Gibson & Mullen (1996), Zanakis *et al.*, (1994), Keigley & Mullen (1986), Green *et al.*, (1965), Drew & Brocklehurst (1990) who reported lighter seeds from soybean and lettuce that developed and matured at high temperature compared with low temperature. However, it contradicts our field results (Khalil *et al.*, 2001) where heavier soybean seed developed at higher temperature compared with seed developed at lower temperature. The possible variation in seed weight may be due to differences in photoperiod length. The photoperiod showed linear relationship with seed weight (Khalil *et al.*, 2001).

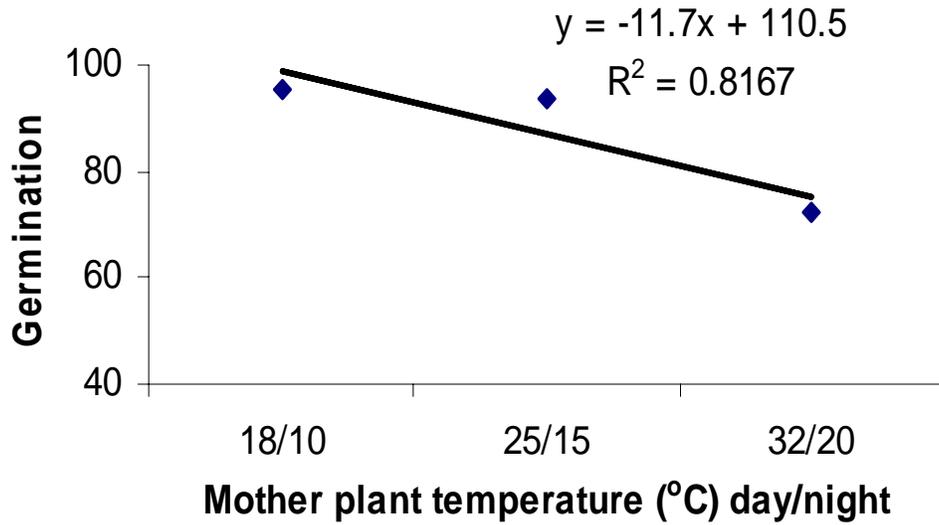


Fig. 9. Germination of soybean as affected by mother plant temperature.

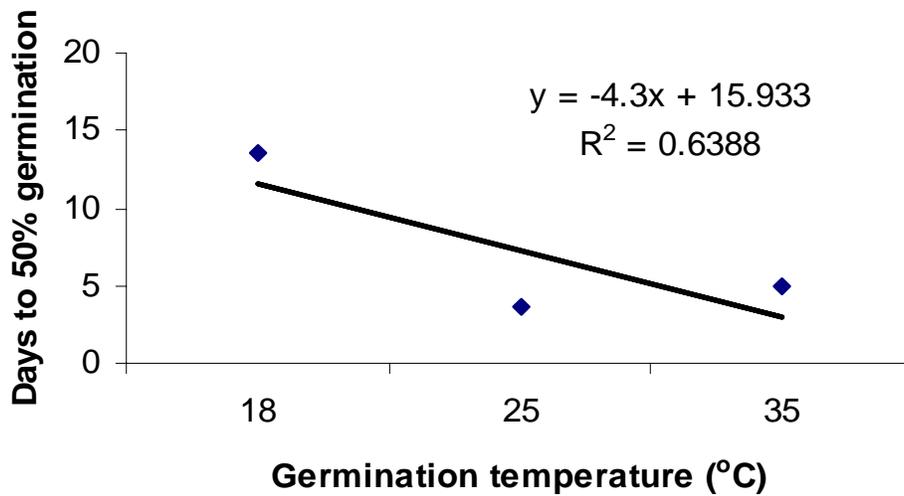


Fig. 10. Linear relationship between days to 50% germination and germination temperature of soybean.

Heavier seeds were obtained from bottom position, followed by middle position, while top position produced the lightest seeds. Similar results were reported by Keigley & Mullen (1986). However, Smiciklas *et al.*, (1992) reported heavier seeds from the top main stem of soybean compared with the bottom. Heavier seeds from top in Smiciklas *et al.*, (1992) study may be due to differences in temperature during the seed development stage being grown in the field. The temperature was lower at the time of seed formation and development at the top because they developed late in the season compared with pods that developed at the bottom.

Seed position plays a role in germination. Seed harvested from middle position gave the best germination, while top position resulted in poor germination. These results contradict the findings of McDonald *et al.*, (1983) who reported that seeds from top position exhibited higher germination compared with seeds from bottom position. The contradiction may be due to environmental differences in which soybeans were grown. McDonald *et al.*, (1983) grew soybeans in the field in which the bottom pods were likely to have more potential disease problems than the bottom seeds in the present growth chamber study, because the bottom pods developed at high temperature and humidity.

The best germination temperature was 25°C, while temperature above or below 25°C decreased germination. These findings agree with Weiss (1983) who reported maximum germination of soybean at 25°C. Lower temperature (18°C) took more days to 50% germination compared with 25 or 35°C which may be due slow rate of imbibitions and enzymatic activities and thus slowed down the metabolic process of seed.

The results showed that seed germinated faster at 25°C compared with 18 or 35°C. The temperature of 25°C was found to be an optimum germination temperature. The optimum temperature above and below which the rate of germination declines has been noted in several studies (Modi & Asanzi, 2008, Tiliki & Cicek, 2005; Humara *et al.*, 2000; Bewley & Black, 1994; Bradford, 1995).

PEG 8000 stress (osmotic stress) decreased germination and delayed days to 50% germination. Decreased germination due to PEG (osmotic stress) have also been reported by Sadeghian & Yavari (2004), Murray *et al.*, (1993), Murray *et al.*, (1992), Murray (1990), Carpenter (1990). The decrease in osmotically stressed seed may be due to slow rate of imbibition by primed seed compared with non primed (Kader & Jtzi, 2002). However, Khan *et al.*, (2008), Arif *et al.*, (2008), Afzal *et al.*, (2005), Khan *et al.*, (2005), Khalil *et al.*, (2003), Khalil *et al.*, (2001), Madakadze *et al.*, (1993), Odell *et al.*, (1992), Xiang *et al.*, (1996), Parera & Cantliffe (1992), Parera & Cantliffe (1993), Hardegree & Emmerich (1992), Carpenter, (1989), Simak *et al.*, (1984), Carpenter, (1989) observed increased total germination of primed seed compared with unprimed seed.

## Conclusions

High temperature during soybean seed filling and maturation in growth chambers decreased the germination and seed weight. Seed located at bottom and middle position were heavier and gave better germination than seed located at top position. Seeds germinated better at 25°C compared with 18 and 35°C. PEG stress decreased germination and also delayed germination. Avoidance of hot weather during seed development by optimal planting date or other management that decrease exposure of mother plant to high temperature during seed filling and maturation would result in better seed quality.

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