

## HEAT STRESS INFLUENCES DORMANCY IN PEANUT SEEDS (*Arachis hypogea* L) cv. Khon Kaen 84-8

Peter OPIO and Songsin PHOTCHANACHAI\*

School of Bioresources and Technology, King Mongkut's University of Technology Thonburi,  
Bangkok, 10150 Thailand.

\*Corresponding author, S. Photchanachai, E-mail: songsin.pho@kmutt.ac.th

**ABSTRACT.** Peanut is one of the annual crops grown in both arid and semi arid areas whose germination can be affected by high temperatures. The effect of heat stress on seed germination and seedling vigor was investigated. Seeds were kept in sealed laminated aluminium foil bags and exposed to heat stress for 48 h at four temperatures;  $27\pm 2^{\circ}\text{C}$ ,  $70\pm 5\%$  RH (control: room temperature),  $40^{\circ}\text{C}$ ,  $50^{\circ}\text{C}$ , and  $60^{\circ}\text{C}$ . Seed germination and seed vigor decreased significantly as the heat stress temperature increased. The seed moisture content and total abnormal seedlings did not vary among treatments whereas occurrence of deformed seedlings was significant at temperatures greater than  $40\pm 1^{\circ}\text{C}$ . However,  $40^{\circ}\text{C}$  significantly induced dormancy by 31% compared to the control seeds, while heat stress beyond  $50^{\circ}\text{C}$  had detrimental effect on peanut seeds in the form of embryonic death.

**KEY WORDS:** Abnormal seedlings, Temperature; Seed germination, Seed vigor.

### INTRODUCTION

Temperature is one of the most important environmental factors that affect germination and dormancy in seeds (Bewley 2013). Moreover, the response of seed germination to temperature in crop production influences planting dates and growing seasons (Probert 2000, Bewley and Black 1994) thus influencing yields directly. Peanut seed survival in the field is also affected by high temperatures as is common in semi-arid and arid ar-

areas where it is grown (ICRISAT 1992). As an adaptation mechanism, some plant seed species enter a state of dormancy. It is therefore important to identify a temperature regime that provides for thermotolerance in peanut seeds to overcome the effects of high temperatures encountered prior to seed germination. Crop heat tolerance can be enhanced by preconditioning of plants under different environmental stresses or by exogenous application of osmoprotectants such as glycinebetaine and proline (Wahid 2007). An environmental stress in the form of high temperature has been implicated in bringing about regulation of changes in seed dormancy (Baskin and Baskin 1998; Benech-Arnold et al. 2000). Dormancy may be influenced through exposure of dry or imbibed seeds to certain temperature regimes before germination. This is one way of coping up with subsequent high temperatures. In *Arabidopsis* seeds, Silva-Correia (2014) reported that application of heat stress to the seeds had an effect on their dormancy and germination. Conditioning prior to high temperature treatment resulted in limited germination (Cadman et al. 2006). Baskin and Baskin (1998) reported that summer annual weed species seeds became dormant as a result of higher temperatures. In addition they noted that this response was species-dependent. There is a dearth of studies on the effect of heat stress on peanut seed germination, thus this study aimed to determine the temperature that maintained peanut seed viability.

## **MATERIALS AND METHODS**

### Plant material

Peanut seed (*Arachis hypogea* L.) cv. Khon Kaen 84-8 was obtained from a local market in Bangkok, Thailand. Sorting was done and broken seeds discarded. Samples weighing 375 g were packed in laminated aluminium foil bags. The bags were heat-sealed and subjected to various heat treatments ( $40\pm 1$ ,  $50\pm 1$ , and  $60\pm 1^\circ\text{C}$  for 48 h) in a hot air oven (Universal Oven, Model UNE 550, Memmert, Schwabach, Germany). The control was exposed to ambient room temperature ( $27\pm 2^\circ\text{C}$ ,  $70\pm 5\%$  RH).

### Analytical methods

Moisture content. This was carried out using the low constant temperature oven

method (ISTA 2007). Samples were blended and 4–5 g weighed in metallic moisture cans then placed in the hot air oven at  $103\pm 2^{\circ}\text{C}$  for 17 h. Each experimental unit was duplicated and the results were expressed as mean percentages.

**Standard germination test.** Germination test was conducted using between paper (BP) method (ISTA 2007). One hundred seeds were placed in between three layers of paper towel moistened with deionized water. The paper towels were rolled and placed in a basket then covered with a plastic bag, and incubated in a germinator at  $27\pm 2^{\circ}\text{C}$ . Four replications for each treatment were examined. Seedlings were classified following ISTA (2007) with slight modifications. Normal seedlings were seedlings with all (100%) of their essential structures well-developed, complete and healthy with seed coats that were more than 75% removed from the cotyledons. Weak seedlings were those with normally emerged radicles with primary roots but with fused cotyledons. Abnormal seedlings were classified as: (A) deformed seedlings had less roots and stunted hypocotyl but with incomplete emergence of the embryo from the opened cotyledons; (B) imbalanced seedlings had only the radicle or seedlings with the radicle but only short hypocotyls with fused cotyledon; and (C) seedlings without hypocotyls but with fully opened cotyledons and a slight emergence of embryo.

Ungerminated seeds referred to decay and fresh ungerminated seeds. After the germination test, the fresh ungerminated seeds were classified by a tetrazolium chloride (TZ) test for their viability.

**Tetrazolium Chloride (TZ) test.** Fresh ungerminated seeds from the germination tests were washed, seed coats removed and soaked in 0.5% solution of 2,3,5-triphenyltetrazolium chloride and incubated at  $27^{\circ}\text{C}$  for 24 h in the dark (Universal Oven, Model UNE 550, Memmert, Schwabach, Germany) (ISTA 2007). Soaked seeds were washed twice with deionized water and then longitudinally opened to observe the staining of the embryo by TZ solution.

**Accelerated ageing test (AA- test).** Seeds were subjected to accelerated ageing at  $42\pm 1^{\circ}\text{C}$  and 100% relative humidity for 48 h (Universal Oven, Model UNE 550, Memmert, Schwabach, Germany) (ISTA, 2007). Thereafter, the seeds were subjected to a standard germination test.

**Germination index.** This was performed according to ISTA (2007) but with a slight modification. The first count was carried out after 5 days of germination with normal seedlings classified and recorded. The germination index (GI) was calculated from the equation below:

$$\text{GI} = \frac{\text{Number of normal seedlings after 5 days}}{5}$$

### Statistic

The experimental design used was a completely randomized design (CRD). Data were subjected to analysis of variance (ANOVA) using the SAS software (SAS Institute Inc., 2002. Cary, NC, USA. Version 9.00 (TS MO)). Means were calculated and separated by least significant difference procedure using Duncan's Multiple Range Test (DMRT).

## **RESULTS**

Moisture content: Heat stress did not affect the moisture content of the peanut seeds which ranged between 6.3–6.5%.

Germination and TZ test: The germination percentage decreased with the increase in heat stress temperature regimes (Table 1). Heat stress at 50 and 60°C had a markedly detrimental effect on peanut seed germination at 12.8 and 0.25% respectively. Of ungerminated seeds, there were more viable seeds at 40°C compared to other treatments. The highest percentage of dead seeds was observed at 50°C while at 40°C the lowest along with the control after TZ test. No viable as well as dead seeds were recorded in 60°C since a majority (99.8%) had decayed before these were subjected to the TZ test (Table 1).

Abnormal seedlings: Generally, the total abnormal seedling percentage increased with heat stress temperatures up to 50°C (Table 2). On further classification of abnormal seedlings, the percentage of deformed seedlings significantly increased likewise up to 50°C. For imbalanced seedlings, the stress effect was similar in ambient, 40 and 50°C. The percentage of abnormal seedlings classified as having open cotyledons without hypocotyls did not vary among treatments.

Germination index: The germination index significantly declined with increase in heat stress temperature (Table 2).

Accelerated ageing test (AA-test): After accelerated ageing, the germination percentage significantly decreased with increase in heat stress temperature (ambient > 40 > 50 > 60°C) (Table 3). Generally, the percentage of dormant seeds observed in AA-test had a similar trend as that in the standard germination test (Table 1). At 40°C, there was a two-fold increase of dormant

seeds after AA (Table 3) compared to that observed in the previous standard germination test (Table 1).

Table 1. Effect of heat stress for 48 h on germination of peanut seeds\*.

Heat stress (°C)	Germination (%)	Abnormal seedlings (%)	Decayed (%)	Un germinated seeds (%)	
				Viable	Dead
ambient	75.3 a	4.7ab	2.0 c	7.5 b	10.5 b
40	58.0 b	9.5 a	4.8 c	20.0 a	7.5 b
50	12.8 c	6.2 a	52.5 b	5.0 b	23.5 a
60	0.2 c	0 b	99.8 a	0 c	0 c

\*Means (n=4) within a column with different lower case letters indicate that values are significantly different at  $P \leq 0.05$ , DMRT

Table 2. Germination index and classification of abnormal seedlings in peanut seeds on exposure to heat stress for 48 h\*

Heat stress (°C)	Germination index	Classification of abnormal seedlings (%)		
		Deformed	Imbalanced	Open cotyledon without hypocotyls
ambient	5.9 a	1.5 b	3.5 a	1.0
40	3.9 b	3.8 a	2.0 ab	1.5
50	1.1 c	4.8 a	4 a	1.5
60	0 c	0 b	0 b	0

\*Means (n=4) within a column with different lower case letters indicate that values are significantly different at  $P \leq 0.05$ , DMRT

Table 3. Germination percentage, dormant, dead and decayed seeds after accelerated ageing of peanut seeds heat stressed for 48 h\*

Heat stress (°C)	Germination (%)	Decayed (%)	Un germinated seeds (%)	
			Viable	Dead
ambient	40.3 a	4.5 b	25.5 b	12.8 a
40	33.0 b	5.3 b	39.8 a	10.8 a
50	0.3 c	94.5 a	0 c	0 b
60	0 c	0 c	0 c	0 b

\*Means (n=4) within a column with different lower case letters indicate that values are significantly different at  $P \leq 0.05$ , DMRT

## DISCUSSION

Ecologically, temperature plays an important role in the seeds' responsiveness to appropriate time detection for germination to be initiated. This study aimed to identify an appropriate temperature that could maintain peanut seed viability longer during periods of heat stress. This study validates that temperature played a significant role in the regulation of dormancy in peanut seeds. Temperature had a dual effect, on one hand, it regulated dormancy and on the other, germination, which could occur only when the actual temperature was within the germination range and otherwise detrimental if beyond the maximum temperature range. Furthermore, 40°C heat stress temperature showed a significantly lower germination percentage compared to the control. This could be due to the higher percentage of dormant seeds. In relation to this, Tamura (2006) reported that incubation of *Arabidopsis* seeds at 34°C suppressed germination by 90% whereas there was complete germination at 22°C. The peanut response in this study is similar to *Poa bulbosa*, a herbaceous plant species of Mediterranean origin whose germination was limited also by high temperatures as well as long days and water deficits during summer (Ofir and Kerem 1982). Roberts (1988) pointed out that some physiological processes such as seed deterioration which depends on seed moisture content and temperature had a significant effect on germination. Other authors argue that alterations in normal physiological processes in a seed can be brought about by exposure to harsh environmental conditions while still on the parent plant, harvesting and processing method and postharvest storage (Heydecker 1977; Maguire 1977). All these can have a profound effect on seed germination of which sub-cellular mechanisms are not yet properly well understood. XueJun et al. (2009) elucidated that pea seedlings improved in heat tolerance due to enhanced synthesis of heat shock proteins (HSPs) after exposure to high temperatures. This suggests that normal seedlings that emerge after heat stress may have a tendency to survive harsh environmental conditions such as high field temperatures.

Highest seed dormancy was shown by seeds exposed to 40°C. Several authors have explained how heat stress influences dormancy in seeds and

our findings seemed to be in agreement with their results. Toh et al. (2008) reported that high temperatures induce accumulation of reactive oxygen species (ROS) and abscisic acid (ABA) which are central ideas in dormancy and germination control in *Arabidopsis* seeds. On the other hand, Piskurewicz et al. (2009) stressed that the heat shock proteins (HSPs) produced as a result of heat stress on seeds is linked to dormancy such that endosperm rupture during germination is inhibited. Furthermore, HSPs produced after exposure to high temperatures maintain the proteins in the seed in a folding-competent manner and this limits germination until favorable conditions occur (Smykal et al. 2000). However, the effect of heat stress on germination may be dependent on plant species and duration of stress plus intensity (Wahid et al. 2007). Generally, early establishment of any plant species in high temperature area is related to germination response of seeds to temperature (Huang et al. 2003). Therefore, for seed germination and seedling growth to be successful a plant species has to depend on adaptive mechanisms like dormancy to overcome harsh environmental conditions (Mijani et al. 2013).

Seedlings classified as abnormal were those with missing or defective in one or more essential structures with no capacity to develop into a normal plant once grown in the field under favorable conditions. In this study, the percentage of deformed seedlings characterized by having less roots, stunted hypocotyls and incomplete emergence of embryo was significant while the imbalanced seedlings (with short hypocotyls and fused cotyledons) were similar in ambient, 40 and 50°C. As temperature increased, so did the occurrence of abnormal seedlings. Generally, the results in this study suggest that a heat stress temperature regime greater than 40°C but not at 60°C could have accentuated the occurrence of deformed seedlings. Specifically, heat stress at 40°C and 50°C greatly had an impact on the occurrence of deformed seedlings at 3.8 and 4.8% respectively. XueJun et al. (2009) reported that heat stress greater than 40°C restricted the emergence and elongation of hypocotyls in three pea (*Pisum sativum*) varieties. In addition, investigations on heat stress tolerance in soybean by Nemeskéri (2004), revealed that temperatures greater than 28°C significantly increased occurrence of abnormal seedlings which matches with our

findings. These results concur with Aswathaiah et al. (1993) who reported that occurrence of abnormal seedlings can be a result of embryo damage in seeds caused by mechanical handling, heat, drought or insect damage. In savanna grasses, Ernst (1991) suggested that the likely occurrence of deformed seedlings after exposure to dry heat could have been due to embryonic tissue damage especially the radicle or plumule. The extent of existence of abnormal seedlings in a given seed lot could be used as a test for seed vigor and a degree of tolerance to high temperatures during germination. This study showed that the germination index decreased with increase in temperature (ambient > 40 > 50 > 60°C). This result is in agreement with Sharma (1976) who reported that the germination rate of three semi-arid plant species increased positively up to a certain limit at 20–25°C then eventually declined with a further increase in temperature. However from this result, it is necessary to highlight that the germination index may not have been a reliable test given the fact that a 40°C heat stress temperature regime appeared to influence dormancy in the peanut seeds. Consequently, on subjecting peanut seeds to further stress, during an accelerated ageing test, there was a significant decline in germination percentage with increase in heat stress. Bailly et al. (1996) reported a deleterious effect of high accelerated ageing temperature on sunflower seeds' ability to germinate within the optimal temperature of 25°C. The very low germination observed reflected that a further heat stress detrimentally affected the peanut seed germination capacity. It is suggested that occurrence of low seedling vigor could be associated with seed physiological deterioration (Hampton and Coolbear 1990) and lipid peroxidation as a result of heat stress. In addition, lipid peroxidation causes cellular degeneration through free radical assault on important cellular molecules and structures (Stewart and Bewley 1980). This then results into cell damage in the form of mitochondrial dysfunction, enzyme inactivation, membrane degradation and genetic damage (McDonald 1999) eventually leading to low germination index as well as germination. Generally, seed vigor is considered a very significant test since the results obtained are usually correlated with those in field germination under unfavorable environmental conditions compared to standard germination test revelations (Dornbos 1995; Hampton and Coolbear 1990). This study

also pointed out that there was similarity in seed dormancy after accelerated ageing and standard germination test with the former registering about a two-fold increase in dormant seeds at a 40°C. Moist heat stress in the form of accelerated ageing appeared to induce more dormancy on peanut seeds compared to dry heat. Roberts et al. (1988) reported that high temperatures coupled with hydration in seeds may induce or generally reinforce dormancy. The process in which this occurs is not yet well explained.

## CONCLUSION

Heat stress has a wide range of effects in seed germination. It is concluded that a 40°C heat stress for 48 h induced dormancy in peanuts. Likewise abnormal seedlings tended to increase with temperature with 50°C showing the highest percentage of deformed seedlings. The heat stress of 60°C proved to be detrimental to the peanut seed survival. However, further studies are required to establish biochemical changes in peanut seeds that influence dormancy when subjected to 40°C for 48 h.

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