research article | International Journal of Plant Sciences, (July, 2010) Vol. 5 Issue 2 : 547-552

Studies on thermo-tolerance, germination behaviour and activity of guaiacol peroxidase enzyme in pea (*Pisum sativum* L.) and soybean [*Glycine max* (L.)Merr.] seeds during early stages of germination

K. BEENA ANTO, K.M. JAYARAM AND NABEESA SALIM

Accepted : April, 2010

SUMMARY

The effect of high temperature treatment on seeds of pea and soybean was investigated, taking the germination percentage, moisture content, seed vigour index and changes in the guaiacol peroxidase enzyme activity in the temperature- treated and control seeds of pea and soybean during the early stages of germination, as parameters of thermo-tolerance. Seeds could withstand the effect of high temperature treatment up to 70^0C for ten days continuously, retaining viability but high temperature treatment reduced the rate of germination percentage, moisture content, seed vigour index as well as increased the production of guaiacol peroxidase enzyme activity in order to protect the oxidative injury and heat-stress in the tissue of germinating seeds/seedlings. The pea and soybean showed metabolic adaptations to tolerate heat-stress and this is very relevant to agronomists in the changing climatic conditions.

Key words : Agronomists, Germination percentage, *Glycine max*, Guaiacol peroxidase enzyme activity, Heat-Stress, Metabolic adaptations, Moisture content, Oxidative injury, *Pisum sativum*, Seed vigour index, Thermo-tolerance

The experimental plants selected for the present study are green pea (*Pisum sativum* L.) and soybean [*Glycine max* (L.) Merr.], belong to family Fabaceae. *Pisum sativum* and *Glycine max* are the domesticated plants with the widest range of uses in both agricultural and horticultural field. Soybean is the third largest oil seed crop in India (Bhatnagar and Tiwari, 1991).

Viability is tested in terms of percentage of germination. Seed viability denotes the degree to which a seed is alive, metabolically active and possesses enzymes necessary for catalyzing metabolic reactions needed for germination and seedling growth (Basara *et al*., 2002). Cabrera and Boyd (1988) evaluated the effect of temperature in the range of 50 to 70^0C and moisture content of 7.5 to 14.5% on germination of gin-run cottonseed. They found that heat stress reduced the viability and vigour at 70^0C or higher. According to Gelmond *et al*. (1978) the seed vigour means a high rate of the overall biological activities of the seed, resulting in a high yield performance. They measured and predicted seed vigour according to the rate of root emergence of germination or field emergence. Vigour represents the potential ability of the seed to yield the maximum plant product at the earliest time under variable environmental field conditions.

When plants are subjected to environmental stress, the balance between the production of reactive oxygen species and the quenching activity of antioxidants is upset resulting in oxidative damage. Plants with high levels of antioxidants have been reported a greater resistance to oxidative damage (Hernandez *et al.*, 2001). The abiotic stress such as high level of temperature and radiation, salinity, drought etc. may cause the generation of oxidative stress (Foyer and Noctor, 2000). The oxidative stress is characterized by the over production of highly active oxygen species (AOS), represented predominantly by superoxide anion (O_2)-, hydrogen peroxide (H_2O_2), hydroxyl radical (·OH) and singlet oxygen ($^1O_2$). Plants have defensive mechanisms and utilize several biochemical strategies to avoid damage caused by AOS. Plant enzymatic defenses include antioxidative enzymes such as peroxidases, superoxide dismutase and catalase that promote the scavenging of AOS (Hernandez *et al*., 2001). Peroxidase is widely distributed in all higher plants and protects cells against the destructive influence of H_2O_2 by catalyzing its decomposition through oxidation into O_2 and H_2O (Dionisio-Sese and Tobita, 1998; Sudhakar *et al.*, 2001). Active oxygen species and the degree of damage depend on the balance between the

Correspondence to:
K. BEENA ANTO, Department of Botany, St. Joseph's College, Irinjalakuda, THRISSUR (KERALA) INDIA
Authors' affiliations:
K.M. JAYARAM AND NABEESA SALIM, Division of Plant Physiology and Biochemistry, Department of Botany, University of Calicut, MALAPPURAM (KERALA) INDIA

formation of an AOS and its removal by the antioxidative scavenging systems (Hernandez and Almansa, 2002). The enzyme peroxidases are hem proteins that catalyze the oxidation of a substrate and the reduction of H_2O_2. Plant peroxidases are typically glycoprotein of 30 to 60 KDa (van Huystee, 1987). These enzymes may participate in many processes of plant growth and defense (Gaspar *et al*., 1982; Greppin *et al*., 1986).

In the present study, an attempt was made to study the germination percentage, moisture content, seed vigour index and changes in the guaiacol peroxidase enzyme activity in the high temperature- treated seeds of pea and soybean during the early stages of germination.

MATERIALS AND METHODS

Seeds of pea (*Pisum sativum* L.) cv. BONEVILLE and soybean [*Glycine max* (L.) Merr.] cv. SL. 525 were used to study the thermo-tolerance as the germination percentage, moisture content, and seed vigour index and guaiacol peroxidase enzyme activity. From the seed lots, seeds having uniform size, colour and shape with intact seed coats were selected and were subjected to temperature treatments such as 50, 60 and 70^{0}C for 10 days each.

The temperature treated and fresh, healthy, untreated seeds, used as control were used for the present investigation. Germination studies were carried out in the Petridishes in etiolated condition and germination count was taken daily up to seven days. Parameters like germination percentage, moisture content and seed vigour index (SVI) were calculated (ISTA, 1985; Copeland and McDonald, 1995).

Germination studies were conducted by keeping the imbibed seeds in the Petri dishes lined with moistened filter paper and kept in darkroom and daily count of germinated seeds was taken up to seven days. Radicle emergence of 1 mm was scored as germinated and germination percentage was calculated (ISTA, 1985).

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seed taken for germination studies}} \times 100$$

Seed Vigour Index (SVI)

Thirty seeds each in triplicate from the control and temperature treated seed lot were sampled and sown in garden pots filled with garden soil, sand and dry powdered cow dung mixed in 2:1:1 ratio. Daily count of germinated seeds was taken and percentage of germination was calculated. The seed vigour index was calculated according to Copeland and McDonald (1995).

$$\text{SVI} = \frac{\text{Number of seeds germinated on first count}}{\text{days of the count}} + \ldots + \ldots + \ldots + \frac{\text{Number of seeds germinated on last count}}{\text{days of the last count}}$$

Determination of peroxidase activity:

The peroxidase enzyme was extracted from the seed samples of pea and soybean, control and temperature treated dry seeds as well as from the cotyledons of germinated seeds, sampled daily up to seventh day of germination.

The chopped cotyledons of seedlings as well as the powder of dry seeds were homogenized in 10 ml Tris buffer (50 mM with pH 7.5), using a clean mortar and pestle. The homogenate was centrifuged at 16,000 x g at 0^{o}C for 15 minutes in a Kubota KR 20000 T refrigerated centrifuge. The supernatant was collected and 2.0 ml of 10% trichloroacetic acid was added to 2.0 ml of supernatant and kept for flocculation in an ice-bath for one hour, for the estimation of soluble protein, in order to determine the specific activity of the enzyme. The unit activity of peroxidase enzyme was expressed as the change in absorbance per minute per gram fresh weight tissue. The rate of enzyme activity was measured by oxidation of guaiacol in the presence of H_2O_2 according to the procedure given by Abeles and Biles (1991).The method of Lowry *et al.* (1951) was followed to estimate the soluble protein.

RESULTS AND DISCUSSION

Seeds of *P. sativum* and *Glycine max* are orthodox and the seed lots exhibited cent per cent germination. When the seeds were subjected to temperature treatments at 50, 60 and 70^{0}C for 10 days continuously, the germination percentage was decreased (Table 1). The high temperature up to 70^{0}C given to the seeds reduced the germination percentage to 10% in pea and 17% in soybean.

Viability of the two legume seeds, *P. sativum* and *G. max* was inversely proportional to temperature and above 50^{0}C, the germination percentage was below 50%. The germination percentage declined to 10% from 33% when the temperature was raised from 60^{0}C to 70^{0}C in pea seeds. Soybean seeds exhibited higher resistance to temperature stress with a higher germination percentage and seed vigour index values than the pea seeds.

For the maintenance of cent per cent germination, 12.8% and 13.9% of moisture content were found to be essential in pea and soybean seeds, respectively. When the moisture content was reduced to 7.3% in pea and 8.7% in soybean at 60^{0}C, the germination was reduced

to 33% and 40%, respectively and this reduction in germination is directly proportional to the temperature (Table 1).

Viability of the two legume seeds, *P. sativum* and *G. max* was inversely proportional to temperature and above 50^0C, the germination percentage was below 50%. The germination was found delayed in both the seed samples subjected to temperature treatment and soybean exhibited more seed vigour in samples treated at 70^0C than those of pea seeds.

Specific activity of peroxidase was measured minimum in dry control seeds of *P. sativum* and was found to increase significantly in the cotyledons of one day old seedlings (Fig. 1 A). No significant change in peroxidase activity in the cotyledons of seedlings was noticed up to 4 days. But in 5 days old seedlings, a significant increase ($p<0.01$) was recorded and thereafter no significant change was seen throughout the period of study.

Seeds treated at 50^0C exhibited a significant ($P < 0.05$) increase in peroxidase activity than that of control seeds. A significant increase in peroxidase activity was observed in the cotyledons of one day old seedlings and no significant changes were observed in the two succeeding days. Thereafter, the activity was found to increase in the cotyledons of 4, 5, 6 and 7 days old seedlings and the increase was significant ($P < 0.05$) on 5^{th} day. Similar pattern of peroxidase activity was observed in the cotyledons of 60 and 70^0C treated seeds. Among the different temperature treatments, cotyledons of 70^0C treated seeds exhibited the highest peroxidase activity.

In *G. max*, the specific activity of peroxidase of control seeds was very low on zero days and was found to increase significantly on the first day of germination (Fig. 1 B). No significant change in peroxidase activity was noticed in the cotyledons of two days old seedlings, but a continuous and significant increase was seen afterwards.

The temperature treated seeds showed an increase in peroxidase activity. The cotyledons of *G. max* seeds treated at 50^0C exhibited an identical trend as that of control seeds. But in 60^0C treated seeds the peroxidase activity was found to increase up to 3 days old seedlings and thereafter the changes were similar to that of 50^0C treated seeds. The peroxidase activity in the cotyledons of seedlings of 70^0C treated seeds showed a significant increase throughout the period of study. Of the various treatments, high peroxidase activity was observed in 70^0C treated seeds/seedlings of *G. max.*

These experiments were conducted to compare the thermo-tolerance in relation to germination percentage, moisture content, seed vigour index and guaiacol peroxidase enzyme activity of pea and soybean seeds. Seeds of *Pisum sativum* and *Glycine max* are orthodox and the seed lots used in the present study exhibited cent per cent germination. When the seeds were subjected to temperature treatments at 50, 60 and 70^oC for 10 days continuously, the germination percentage was decreased. The high temperature up to 70^0C applied to seeds reduced the germination percentage to 10% in pea and 17% in soybean. As a consequence of high temperature treatment, reduction in germination has been reported in *Solanum nigrum* seeds (Del Monte and Tarquis, 1997) and in *Raphanus sativum* (Meng *et al*., 2003). Viability of the two legume seeds, *P. sativum* and *G. max* is inversely proportional to temperature and above 50^0C; the germination percentage is below 50%. In the present study, soybean seeds showed higher resistance to temperature stress with a higher germination percentage and seed vigour than the pea seeds. The oil-rich nature of soybean seeds can be correlated to these qualities (Table 1).

In *P. sativum* and *G. max* seeds, loss of viability is inversely proportional to temperature treatments as has already been reported by various authors (Ellis *et al*., 1988; 1989; 1990; Sanhewe and Ellis, 1996). This is also confirmed by the present author that temperature treatment results in reduced germination with a concomitant reduction in moisture content (Table 1).

According to Ellis and Roberts (1982) and Ellis *et al*. (1990), the proportion of pea seeds germinated normally in standard germination tests declined progressively from 94 to 50% when the seeds were dried from 14.8 to 3.7% moisture content at ambient temperatures. Roberts (1973) opined that orthodox seeds can be dried to low moisture contents without damaging the embryo and their longevity increases with decrease in moisture content during storage over a wide range of conditions. Contradictory to this view, drying at high temperature leads to significant reduction of moisture content and concomitant loss of viability in pea and soybean seeds, plausibly due to the continuous treatment for 10 days at 50^0C to 70^0C (Table 1).

One of the important effects of high temperature on plant tissue is production of highly reactive oxygen species. Similarly peroxidase enzyme is widely distributed in all higher plants and protects cells against the destructive influence of H_2O_2 by catalyzing its decomposition through oxidation into O_2 and H_2O (Dionisio-Sese and Tobita, 1998; Sudhakar *et al.*, 2001). The seeds of pea and soybean subjected to temperature treatments showed an increased peroxidase activity throughout the period of study (Fig. 1

Table 1 : Effect of temperature on germination, moisture content and seed vigour index in *Pisum sativum* and *Glycine max* seeds

Seed Samples	Treatments	Germination percentage	Moisture content percentage	Seed vigour index
Pisum sativum	Control	100 ± 0	12.8 ± 1.68	21.7 ± 0.99
	50^0C	67 ± 1.27	8.9 ± 1.64	15.4 ± 0.88
	60^0C	33 ± 1.51	7.3 ± 1.48	4.90 ± 0.92
	70^0C	10.0 ± 0.91	6.4 ± 1.12	0.91 ± 0.61
Glycine max	Control	100 ± 0	13.9 ± 1.16	20.05 ± 1.52
	50^0C	70 ± 1.24	9.6 ± 1.68	12.87 ± 1.12
	60^0C	40 ± 1.16	8.7 ± 1.46	3.95 ± 0.94
	70^0C	17 ± 0.92	6.8 ± 0.95	1.50 ± 0.61

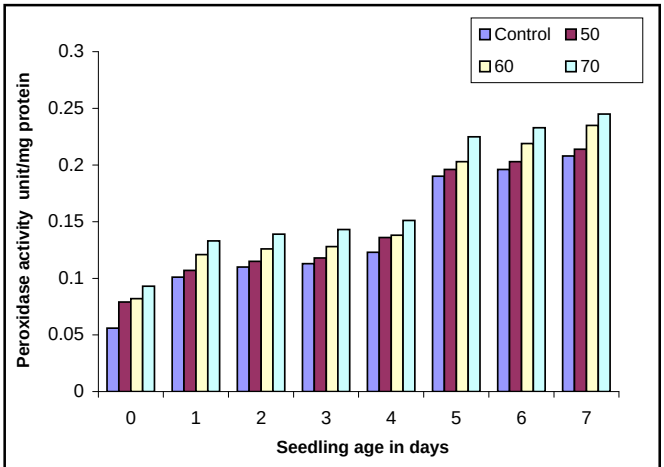

Fig. 1A : Effect of temperature on peroxidase activity in the seeds/ seedlings of *Pisum sativum*

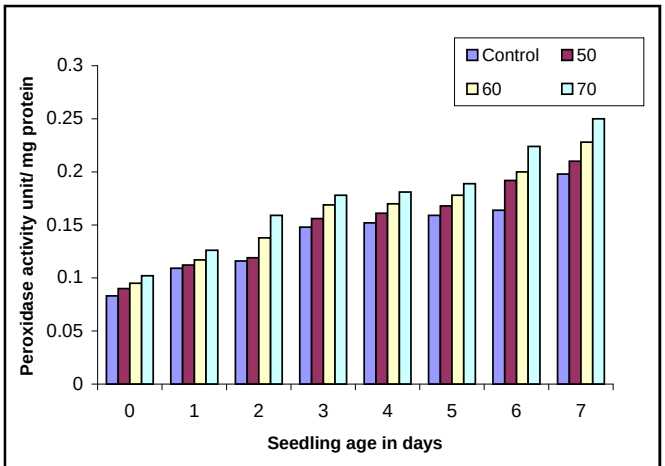

Fig. 1B : Effect of temperature on peroxidase activity in the seeds/ seedlings of *Glycine max*

A and 1B). The highest peroxidase activity was observed in 70^0C treated seeds and seedlings of both the plants. The increased peroxidase activity, proportional to the increase in temperature is indicative of the tolerance of both pea and soybean seeds towards temperature treatments up to 70^0C.

Peroxidase, catalase and superoxide dismutase play major roles in protecting cells from oxidative damage (Scandalios *et al*., 1980). According to Bhattacharjee and Mukherjee (2006), abiotic stresses like heat and salinity during early germination of *Amaranthus* seeds may result in the induction of oxidative stress in germinating tissues, which increases the vulnerability of newly assembled membrane systems to oxidative damage. Resistance to environmentally induced oxidative stress has been shown to be associated with high levels of peroxidases (Zheng and van Huystee, 1992; Scandalios, 1993; Tsang *et al*., 1991). According to Wise and Naylor (1987), temperature stress is an environmental factor that may cause "oxidative injury". Peroxidases probably play a key defense role against peroxidative stress (Zheng and van Huystee, 1992). Plants possess mechanisms to sense stress and transduce these into a change in activity of different classes of enzymes (Perl-Treves and Galun, 1991). The coordinated induction of antioxidant enzymes in response to environmental stress has been reported in plants (Scandalios, 1990; Perl-Treves and Galun, 1991; Tsang *et al*., 1991). The defensive role of peroxidase activity against temperature stress is evidently shown by both pea and soybean because even the seeds treated at 70^0C showed high peroxidase activity and a germination of 10% and 17% in pea and soybean, respectively, indicating the role of peroxidases in ameliorating the temperature stress.

Enhanced activity of peroxidases is a characteristic feature of both pea and soybean seeds subjected to temperature treatment. This served as a mechanism to protect the tissue from the destructive effect of H_2O_2 generated as a consequence of high temperature treatment.

Acknowledgement:

The first author Beena K. Anto is thankful to the

Head of the Department of Botany, University of Calicut, Kerala, India for providing necessary facilities and the University Grants Commission, South Western Regional Office, Bangalore, India for granting financial assistance to carry out the work.

REFERENCES

Abeles, F.B. and Biles, C.L. (1991). Characterization of peroxidases in lignifying Peach fruit endocarp. *Plant Physiol*., **95**: 269-273.

Basara, S.M.A., Ahmad, N., Rehman, K. and Iqbal, N. (2002). Cotton seed invigoration by pre-sowing seed humidification. *Internat. J. agric. Biol*., **4**: 127-130.

Bhatnagar, P.S. and Tiwari, S.P. (1991). Soybean in edible oil economy of India. *Agri. Situ. India*, **46**: 295-301.

Bhattacharjee, S. and Mukherjee, A.K. (2006). Heat and salinity induced oxidative stress and changes in protein profile in *Amaranthus lividus* L. *Indian J. Plant Physiol*., **11**: 41-47.

Cabrera, E.R. and Boyd, A.H. (1988). Effect of moisture and temperature on germination of cotton seed. Proc. Beltwide Cotton Prod. Res. Conf. Nat. Council. Memphis. 88- 89.

Copeland, L.O. and McDonald, M.B. (1995). *Principles of Seed Science and Technology* 3rd Ed. Chapman and Hall, New York.

Del Monte, J.P. and Tarquis, A.M. (1997). The role of temperature in the seed germination of two species of the *Solanum nigrum* complex. *J. Exp. Bot*., **48**: 2087-2093.

Dionisio-Sese, M.L. and Tobita, S. (1998). Antioxidant responses of rice seedlings to salinity stress. *Plant Sci.*, **135**: 1-9.

Ellis, R.H. and Roberts, E.H. (1982). Desiccation, rehydration, germination, imbibition injury and longevity of pea seeds (*Pisum sativum*). *Seed Sci. Technol*., **10** : 501-508.

Ellis, R.H., Hong, T.D. and Roberts, E.H. (1988). A low moisture content limit to logarithmic relations between seed moisture content and longevity. *Ann. Bot*., **61**: 405-408.

Ellis, R.H., Hong, T.D. and Roberts, E.H. (1989). A comparison of the low moisture content limit to the logarithmic relation between seed moisture and longevity in twelve species. *Ann. Bot.*, **63** : 608-611.

Ellis, R.H., Hong, T.D. and Roberts, E.H. (1990). Effect of moisture content and method of rehydration on the susceptibility of pea seeds to imbibitional damage. *Seed Sci. Technol*., **18**: 131-137.

Foyer, C.H. and Noctor, G. (2000). Oxygen processing in photosynthesis: regulation and signalling. *New Phytol*., **146** : 359-388.

Gaspar, T., Penel, C., Thorpe, T. and Greppin, H. (1982). Peroxidases 1970-1980. *A survey of their biochemical and physiological roles in higher plants*. University of Geneva Press, Geneva, Switzerland.

Gelmond, H., Luria, I., Woodstock, L.W. and Perl. M. (1978). The effect of accelerated ageing of *Sorghum* seeds on seedling vigour. *J. Exp. Bot*., **29**: 489-495.

Greppin, H., Penel, C. and Gaspar, T. (1986). *Molecular and physiological aspects of plant peroxidases*. University of Geneva Press, Geneva, Switzerland.

Hernandez, J.A. and Almansa, M.S. (2002). Short-term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. *Plant Physiol.*, **115**: 251-257.

Hernandez, J.A., Ferrer, M.A., Jimenez A., Barcelo, A.R. and Sevilla, F. (2001). Antioxidant systems and O_2/ H_2O_2 production in the apoplast of pea leaves. Its relation with salt induced necrotic lesions in minor veins. *Plant Physiol*., **127** : 817-831.

ISTA. (1985). International rules for seed testing. (ISTA). *Seed Sci. Technol.*, **13**: 299-355.

Lowry, O.H., Rosenbrough, N.J., Farr, F.L. and Randall, R.J. (1951). Protein measurement with folin phenol reagent. *J. Biol. Chem*., **193** : 265-275.

Meng, S.C., Zhang, H.Y. and Kong, X.H. (2003). Effect of dry-heat treatment at 76^0C in different times and moisture content on seed vigour of Xin-Li- Mci radish. *Seed Sci. Technol*., **31** : 193-197.

Perl-Treves, R. and Galun. E. (1991). The tomato Cu, Zn Superoxide dismutase genes are developmentally regulated and respond to light and stress, *Plant Mol. Biol*., **17**: 745-760.

Roberts, E.H. (1973). Predicting the storage life of seeds. *Seed Sci. Technol.*, **1**: 499-514.

Sanhewe, A.J. and Ellis, R.H. (1996). Seed development and maturation in *Phaseolus vulgaris*. II. Post-harvest longevity in air-dry storage. *J. Exp. Bot*., **47**: 959-965.

Scandalios, J.G. (1990). Response of plant antioxidant defence genes to environmental stress. *Adv. Genet*., **28**: 1-41.

Scandalios, J.G. (1993). Oxygen stress and superoxide dismutases. *Plant Physiol*., **101**: 7-12.

Scandalios, J.G., Tong, W.F. and Roupakias, D.G. (1980). Cat 3, a third gene locus coding for a tissue-specific catalase in maize: Genetics, intracellular location, and some biochemical properties. *Mol. Gen. Genet.*, **179**: 33-41.

Sudhakar, C., Lakshmi, A. and Giridarakumar, S. (2001). Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. *Plant Sci.*, **161**: 613-619.

Tsang, E.W.T., Bowler, C., Herouart, D., Van Camp. W., Villarroel, R., Genetello, C., van Montagu, M. and Inze. D. (1991). Differential regulation of superoxide dismutase in plants exposed to environmental stress. *Plant Cell*, **3**: 783-792.

van Huystee, R.B. (1987). Some molecular aspects of plant peroxidase biosynthetic studies. *Annu. Rev. Plant Physiol.*, **38**: 205-219.

Wise, R.R. and Naylor, A.W. (1987). Chilling-enhanced photo-oxidation. Evidence for the role of singlet oxygen and superoxide in the breakdown of pigments and endogenous antioxidants. *Plant Physiol.*, **83**: 278-282.

Zheng, X. and van Huystee, R.B. (1992). Anionic peroxidase catalysed ascorbic acid and IAA oxidation in the presence of hydrogen peroxide: A defence system against peroxidative stress in peanut plant. *Phytochemistry*, **31**: 1895-1898.

****
*****