LETTUCE ACHENE INVIGORATION THROUGH OSMOPRIMING AT SUPRAOPTIMAL TEMPERATURE

Muhammad Muzammil Jahangir[1], Muhammad Amjad[1]*, Irfan Afzal[2], Qumer Iqbal[1] and Aamir Nawaz[1]

[1]Institute of Horticultural Sciences, University of Agriculture, Faisalabad

[2]Department of Crop Physiology, University of Agriculture, Faisalabad

*Corresponding author's e-mail: amjaduaf@gmail.com

The effect of osmopriming on lettuce achene invigoration at supra optimal temperature (35ºC) was investigated in the present study. Osmopriming of lettuce achene with KNO_3 (0.25%, 0.5% and 1%), $CaCl_2$ (15 mM, 25 mM, 50 mM) and PEG 8000 (0.1 g/ml H_2O, 0.2 g/ml H_2O, 0.3g/ml H_2O) alleviated thermodormancy and improved lettuce achene's vigor. Moreover, priming significantly improved final germination % age, energy of germination, germination index, shoot length, root length, vigor index and reduced mean germination time and time taken to 50% germination, as compared to control, when seeds were subjected to supra-optimal germination environments. It can be concluded that osmopriming can act as effective tool to invigorate lettuce seeds at supra optimal temperature.

Keywords: Osmopriming, thermodormancy, lettuce, seed vigor

INTRODUCTION

Seed priming is a technique to control the hydration level within seeds so that the metabolic activities necessary for germination can take place at relatively slow rate but radicle emergence is prevented. The useful effects of priming have been related to different cellular, biochemical, and molecular processes including synthesis of RNA and proteins (Dell'Aquila and Bewley, 1989). Primed seeds germinate in a wider range of temperatures and are less sensitive to oxygen deprivation (Corbineau and Come, 1990) than unprimed ones. Priming increases respiratory activity of seeds (Halpin-Ingham and Sundstrom, 1992) and, when applied to aged seeds, restores activities of enzymes involved in the cell detoxifying mechanisms such as superoxide dismutase, catalase and glutathione reductase (Bailly *et al*., 1997).

Osmopriming is special seed hydration technique in which seeds are soaked in aerated low water potential solutions for a specific period of time and are re-dried to original weight. Osmotica are added to control the hydration process. Examples of such osmotica are $CaCl_2$, NaCl, KCl, PEG and KNO_3 etc (Afzal *et al.*, 2008).

Germination of lettuce seeds is strongly under temperature control; optimum being around 20°C for most of lettuce genotypes (AOSA, 1993), although the actual permissive temperature range varies among cultivars. In general, germination is adversely retarded at temperature above 30°C, declining with increase in temperature, approaching to zero at approximately 30°C to 35°C depending on cultivar (Cantliffe *et al.,* 2000). Seeds held at such supra-optimal temperatures in an un-germinated state, eventually lose their ability to germinate even when returned to a permissive temperature, thus entering a secondary dormancy termed as thermodormancy. Thermodormancy in lettuce seeds can be alleviated by priming the seeds in different salts (Cantliffe *et al.,* 1984), growth regulators (Saini *et al.,* 1986 and Gonai *et al.,* 2004) and distilled water (Warley, 2003).

Lettuce planting can be done by direct seeding or by transplant of seedlings. Recently, the specialized production of seedlings in trays has gained popularity. For a successful production using this technology, a high percentage of emergences in a fast and uniform way are required. Therefore seed vigor is crucial. A wide range in seedling emergence at supra optimal temperature (35ºC) due to thermodormancy is a strong hindrance in lettuce cultivation. Worst scenario appears when low vigor seeds are used for cultivation, resulting in poor germination; non-uniform stand establishment and subsequent variability in maturity at harvest.

Although very useful information is available regarding alleviation of thermodormancy in lettuce seeds however, the effect of hydro and osmopriming for achene invigoration at supra optimal temperatures, was not fully investigated and is still at preliminary phase. Present study was conducted to evaluate the efficacy of osmo and hydro priming techniques to alleviate thermodormancy and achene invigoration in lettuce cv. Grand Rapids at supra-optimal temperature.

MATERIALS AND METHODS

Seed materials

Seeds of lettuce (*Lactuca sativa* L. cv. Grand Rapids) used in this study were purchased from local vendor. Study was carried out in Vegetable Seed Lab, Institute of Horticultural Sciences, University of Agriculture,

Faisalabad Pakistan. The initial seed moisture content was 3.6% on a dry weight basis.

Seed hydration treatments

The following seed hydration treatments were integrated in the present study.

Hydropriming

Lettuce cv. Grand Rapids seeds were soaked in aerated distilled water at 15°C for 15 h in dark.

Osmopriming

Seeds were soaked in aerated solutions of salts of different concentrations i.e. KNO_3 (0.25%, 0.5% and 1%), $CaCl_2$ (15 mM, 25 mM, 50 mM) and PEG 8000 (0.1 g/ml H_2O, 0.2 g/ml H_2O, 0.3g/ml H_2O) at 15°C for 15 hours in dark, followed by re-drying near to initial moisture contents under shade with forced air. Salt solutions were prepared in distilled water. In all hydration treatments, the ratio of seeds and working solutions was kept 1:20 (g mL^{-1}) (Cantliffe *et al*., 1984). Aeration pumps provided aeration. While aluminum foils were used for dark provision.

Post priming operations

After soaking period seeds were washed with distilled water and redried near to original weight with forced air under shade at room temperature. Seeds were then sealed in polythene bags and stored in a refrigerator at ±5°C till further use.

Germination test

Seeds selected against deformities and discoloration, and for uniformity in size were germinated in 9 cm Petri dishes lined with two layers of Whatman No.1 filter papers at 35°C in an incubator and moistened with 4 ml of distilled water and rewetted again as and when necessary. Triplicates of 25 seeds were used. Seed germination was recorded at 8 h interval for the first 24 h and later on recorded after 24 h interval till the termination of experiment (14 days). Seeds were considered germinated when radicle protruded 2 mm from beneath the seed coat and pericarp (AOSA, 1983). Final germination percentage was taken at the end of experiment (after 14 days of sowing).

The time to achieve 50% germination [T_{50}] was calculated according to the following formula Farooq *et al*. (2004):

$$T_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right)(t_j - t_i)}{n_j - n_i}$$

Where N is the final number of germinating seeds and n_i, n_j are the cumulative number of seeds germinated by adjacent seed count at times t_i and t_j, respectively, when $n_i < N/2 < n_j$.

Mean germination time [MGT] was calculated according to the equation of Ellis and Roberts (1981):

$MGT = \sum (Dn) / \sum n$

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

The energy of germination was recorded on the 4th day after sowing. It represents the percentage of germinating seeds at 4th day after sowing relative to the total number of seeds tested (Ruan *et al*., 2002).

The germination index [GI] was calculated as described by the Association of Official Seed Analysts (1983).

At final count, five normal seedlings in each replication were taken at random and length of shoot and root was measured. Fresh weight of seedlings was obtained with the help of weighing balance and dry weight was obtained after drying 14 days older seedlings at 60^0C for 48 h in microwave oven.

The vigor index of seedlings was calculated using the formula suggested by Abdul-Baki and Anderson (1973):

Vigor Index=Germination (%)×Total Seedling Length (cm)

Where, total seedling length (cm) = Shoot Length +Root Length.

Statistical Analysis

The experiment was conducted in a completely randomized design and replicated thrice. The recorded data were analyzed statistically using Fisher's Analysis of Variance Techniques and Duncan's Multiple Range (DMR) test was applied at 5% probability level to compare the differences among treatment means (Steel and Torrie, 1984).

RESULTS

Compared with control, all osmopriming treatments had highly significant effect ($P< 0.05$) on time taken to 50 % germination (T_{50}). All osmopriming strategies decreased the T_{50} value except hydro priming (17.57 h) which behaved similar to control (19.11 h). Lowest value (12.47) for T_{50} was observed in seeds subjected to priming with 0.1 g/ml H_2O PEG 8000 that was statistically at par with 50 mM $CaCl_2$ (12.56 h), 0.5% KNO_3 (13.09 h), 0.25% KNO_3 (13.17 h), 1% KNO_3 (13.31 h), 0.2 g/ml H_2O PEG 8000 (13.89 h), 25 mM $CaCl_2$ (14.48 h), 15 mM $CaCl_2$ (14.93 h) and 0.3g/ml H_2O PEG 8000 (15.67 h).

Similarly, all the salt seed hydration treatments had highly significant effect for mean germination time (MGT). Maximum mean germination time was recorded in control which was statistically at par with that of hydro priming and osmopriming with PEG 8000 (Table 1). All other osmopriming treatments exhibited lower mean germination time compared with control. Minimum value (52.08) for MGT was recorded in seeds subjected to priming with 0.1 g/ml H_2O PEG 8000 which was statistically similar to all the priming strategies used except hydropriming and control. Seeds subjected to KNO_3 exhibited maximum value (17.67%) of energy of germination (GE), which was statistically similar to other osmotic priming treatments.

Halopriming treatments had highly significant effect on final germination percentage (FGP). Seeds subjected to priming with 0.25% KNO_3 had maximum (76.64%) final germination percentage (FGP) that was statistically at par with that of priming with other osmotica. Maximum germination index was noted in seeds primed with 0.25% KNO_3 followed by 25 and 50 mM $CaCl_2$, 0.2 g/ml H_2O PEG 8000 (34.05), 50 mM $CaCl_2$ (33.52) 1% KNO_3 (33.36) and 0.1 g/ml H_2O PEG 8000 (32.99). Lowest value (8.38) was recorded for control, which was statistically similar to 0.3g/ml H_2O PEG 8000 (12.86).

All the osmopriming treatments significantly improved root and shoot lengths as compared to control. Maximum shoot length (17.80 mm) was recorded in case of seeds subjected to priming with 1% KNO_3 which was statistically at par with 0.5% KNO_3 (Fig. 1a). Maximum root length (21.33 mm) was recorded in seeds subjected to priming with 0.5% KNO3 which was statistically similar to 0.1 g/ml H_2O PEG 8000, 0.2 g/ml H_2O PEG 8000, 1% KNO_3, 25 mM $CaCl_2$, 0.25% KNO_3, 50 mM $CaCl_2$ and hydropriming (Fig. 1b). In the present study, maximum value of vigor index (268.2) was recorded in seeds subjected to priming with 0.5% KNO_3 which was statistically at par with 1% KNO_3, $CaCl_2$ and 0.1 g/ml H_2O PEG 8000 (Fig. 1c).

Table 1. Effect of various seed hydration treatments on germination of lettuce

Treatments	T50 (h)	MGT (h)	GE (%)	FGP (%)	GI
Control	19.11 a	59.99 a	4.66 c	18.67 c	8.38 c
Hydropriming	17.57ab	58.09ab	10.33 b	41.33 b	19.53 b
0.25% KNO_3	13.17 c	53.38 c	17.67 a	70.67 a	35.30 a
0.5% KNO_3	13.09 c	52.88 c	17.33 a	69.33 a	35.77 a
1% KNO_3	13.31 c	53.28 c	16.33 a	65.33 a	33.36 a
15mM $CaCl_2$	14.93 bc	54.45 c	9.66 b	38.67 b	19.80 b
25mM $CaCl_2$	14.87 bc	54.40 c	16.67 a	66.67 a	34.72 a
50mM $CaCl_2$	12.56 c	52.22 c	16.33 a	65.33 a	33.52 a
PEG-8000 (0.1g/mlH_2O)	12.47 c	52.08 c	16.00 a	64.00 a	32.99 a
PEG-8000 (0.2g/mlH_2O)	13.89 c	53.83 c	16.67 a	66.67 a	34.05 a
PEG-8000 (0.3g/mlH_2O)	15.67bc	55.39 bc	6.33 bc	25.30 bc	12.86 bc
LSD at 0.05	3.23	3.40	3.93	15.73	8.82

Figures not sharing the same letters differ significantly at $P = 0.05$.
T_{50}, time taken to 50 % germination; FGP, final germination percentage; GE, energy of germination; MGT, mean germination time; VI, vigor index; G I, germination index.

DISCUSSION

Osmopriming exhibited highly significant effect ($P<0.05$) on alleviation of thermodormancy and demonstrated improvement of seedling vigor at supra optimal temperature (35°C). Osmopriming resulted in earlier and synchronized germination as depicted by lowered T_{50}, MGT and increased GI, GE, FGP, root length, shoot length and vigor index (Table 1 & Fig. 1). T_{50} is useful in determining the uniformity of germination and vigor of seeds, because the seeds, which take less time to complete 50% germination, are considered more vigorous which in turn result in better crop stand establishment. These results are in line with the findings of Ozbingol *et al*. (1999) who claimed that priming tomato seeds with PEG 8000 (-1.0 MPa) significantly reduced T_{50}. Significant reduction in T_{50} may be attributed to early reserve breakdown and early reserve mobilization. It might also be due to possible early activation or *de novo* synthesis of cell wall degrading enzymes (Hisashi and Francisco, 2005).

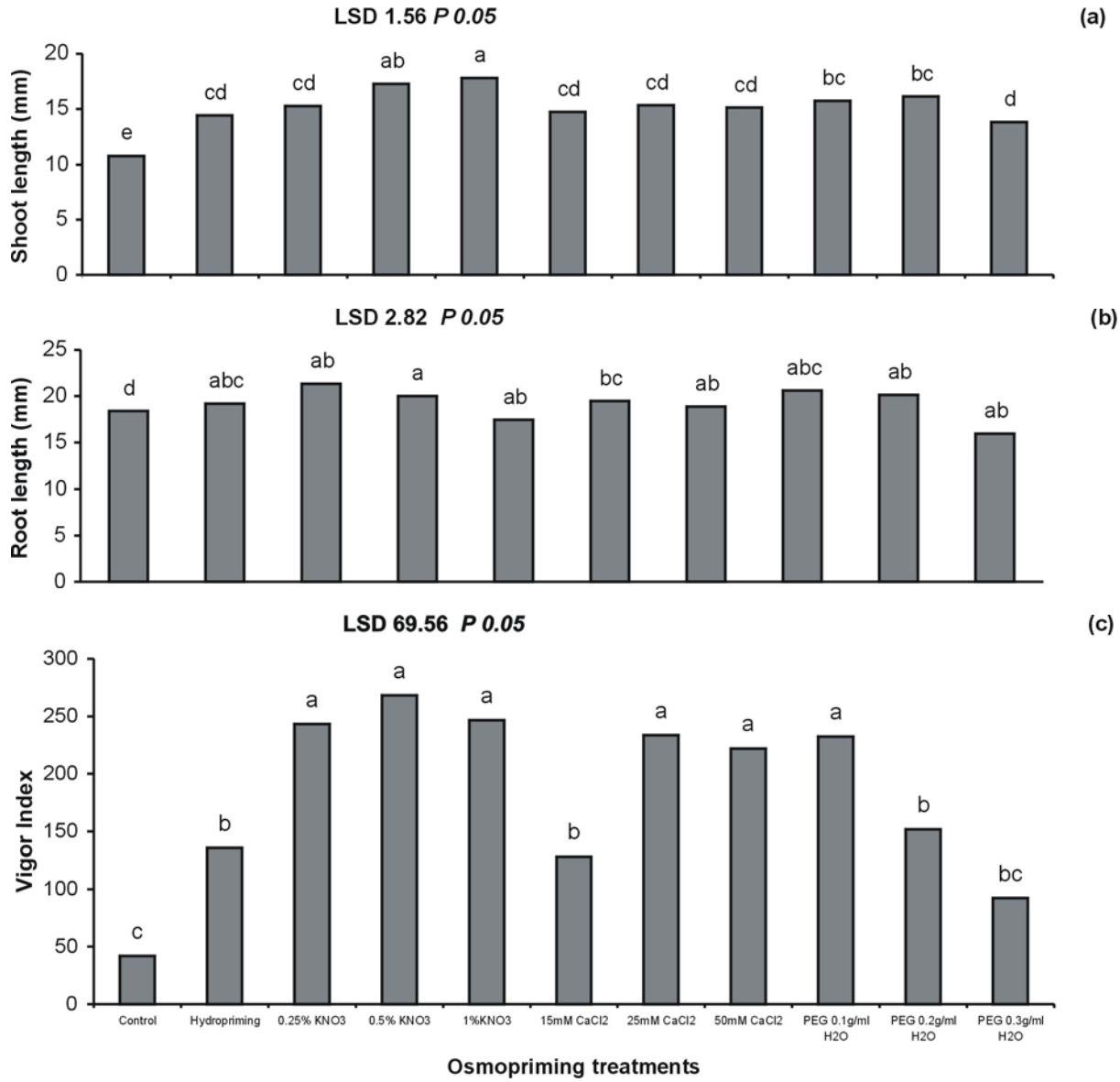

Fig. 1. Effect of different seed hydration treatments on seedling vigor of lettuce cv. Grand Rapids. The vertical bars with different alphabets are statistically different.

Mean germination time is an important indicator of seed vigor. The seeds with less mean germination time (MGT) are considered more vigorous and are able to complete their germination within less period of time. These results are in accordance with the observations of Jeong *et al*. (2000) who demonstrated that priming with PEG 8000 (-0.5 MPa) lowered the mean germination time of seeds of lettuce, carrot and onion. These results are also in close conformity with the findings of (Jin and Tylkowska, 2005) who reported that priming with PEG 8000(-1.25 MPa) significantly lowered the mean germination time of lettuce seeds at supra-optimal temperatures. Significant reduction in mean germination time could be the result of shortening the lag phase during priming process.

Energy of germination directly co-relates with seed vigor. Higher the value of energy of germination more will be the vigor of seeds. Significant improvement in energy of germination may be due to increased and synchronized seed germination as a result of additive effects of osmopriming. It might also be due to stimulation of internal regulatory metabolism of seeds. It might be due to early synthesis of nucleic acids e.g. DNA, RNA and proteins during salt hydration process,

which ultimately resulted in improved energy of germination of seeds. (Bray *et al.,* 1989; Dell'Aquila and Bewley, 1989).

Final germination percentage represents the total number of seeds germinated at the termination of experiment. An additive effect in the release of thermodormancy was observed when seeds were osmo primed. These results are supported by the findings of (Ozbingol *et al*., 1999) who reported that salt priming had additive effect on tomato seed germination. Results obtained are also in close association with the findings of (Jin and Tylkowska, 2005) who reported that priming with PEG 8000(-1.25 MPa) significantly improved lettuce seed germination at supra-optimal temperatures.

Germination index is a good indicator of seed vigor because higher the value of germination index more will be the vigor of seeds. Significant improvement in germination index might be the result of early and synchronized seed germination as a consequence of promotive effects of osmopriming. It might also be the result of better development of genetic repair mechanisms during the course of priming operations leading to improved germination index (Gallais *et al.,* 2000).

Those seeds, which can produce healthy and long shoots, are considered more vigorous compared with tender and weak shoots developed by untreated seeds. Similarly higher root length is also associated with high vigor of seeds. Significant improvement in root and shoot lengths may be attributed to earlier germination induced by osmopriming over control, which resulted in vigorous seedlings as indicated by longer shoots and roots. Significant improvement in root and shoot length might also be attributed to increased rate of cell division. The results are in accordance with the observations of (Jeong *et al*., 2000) who reported that priming with PEG 8000 (-0.5 MPa) increased the root length of seeds of lettuce, carrot and onion.

It may be concluded from present investigation that osmopriming can invigorate lettuce seeds at supra optimal temperature. Among various osmotica used in the present study, KNO^3 was found the most effective priming agent in triggering seedling growth of lettuce. However, hydropriming was proved to be less effective in enhancing germination and seedling vigor of lettuce achenes.

REFERENCES

Afzal I, S. Rauf, S.M.A. Basra and G. Murtaza. 2008. Halopriming improves vigor, metabolism of reserves and ionic contents in wheat seedlings under salt stress. Plant Soil Environ., 54: 382-388.

Abdul-Baki, A.A. and J.D. Anderson. 1973. Viability and leaching of sugars from germinating barley. Crop Sci., 1: 31-34.

Association of Official Seed Analysts (AOSA). 1983. Seed Vigor testing hand book. Contribution No. 32 to the handbook on seed testing. Association of Official Seed Analysis, Springfield, IL.

Association of Official Seed Analysts (AOSA). 1993. Rules for testing seeds. J. Seed Technol., 16: 1-113.

Bailly, C., A. Benamar, F. Corbineau and D. Come. 1997. Changes in superoxidedismutase, catalase and glutathione reductase activities in sunflower seeds during accelerated ageing and subsequent priming. pp. 665-672. In: R.H. Ellis, M. Black, A.J. Murdoch and T.D. Hong (eds.). Basic and applied aspects of seed biology. Dordrecht: Kluwer Academic Publishers.

Bray, C.M., P.A. Davison, M. Ashraf and R.M. Taylor. 1989. Biochemical changes during priming of leek seeds. Ann. Bot., 63: 185-193.

Cantliffe, D.J., J.M. Fischer and T.A. Nell. 1984. Mechanism of seed priming in circumventing thermodormancy in Lettuce. Plant Physiol., 75: 290-294.

Cantliffe, D.J., Y. Sung and W.M. Nascimento. 2000. Lettuce seed germination. Hort. Rev., 24: 229-275.

Corbineau, F. and D. Come. 1990. Effects of priming on the germination of *Valerianella olitoria* seeds in relation with temperature and oxygen. Acta Hort., 267: 191-197.

Dell'Aquila, A. and J.D. Bewley. 1989. Protein synthesis in the axes of polyethylene glycol treated pea seeds and during subsequent germination. J. Exp. Bot., 40: 1001-1007.

Ellis, R.A. and E.H. Roberts. 1981. The quantification of ageing and survival in orthodox seeds. Seed Sci Technol., 9: 373–409.

Farooq, M., S.M.A. Basra, K. Hafeez and E.A. Warriach. 2004. Influence of high and low temperature treatments on the seed germination and seedling vigor of coarse and fine rice. Int. Rice Res. Notes, 29: 69–71.

Gallais, S., D.E. Crescenzo, M.A.P. Laval and D.L. Martin. 2000. Changes in soluble and membrane bound isoforms of calcium calmodulin dependent and independent NAD^+ kinase, during the culture of after ripened and dormant seeds of *Avena sativa.* Aus. J. Plant. Physiol., 27: 649-658.

Gonai, T., S. Kawahara, M. Tougou, S. Sato, T. Hashiba, N. Hirai, H. Kawaide, Y. Kamiya and T. Yoshioka. 2004. Abscisic acid in the thermoinhibition of lettuce seed germination and enhancement of its catabolism by gibberellin. J. Exp. Bot., 55(394): 111-118.

Halpin-Ingham, B. and F.J. Sundstrom . 1992. Pepper seed water content, germination response and respiration following priming treatments. Seed Sci Technol., 20: 589- 596.

Hisashi, K.N and A.M. Francisco. 2005. Effects of 6-methoxy-2-benzoxazolinone on the germination and a-amylase activity in lettuce seeds. J. Plant Physiol., 162: 1304-1307.

Jeong, Y.O., S.M. Kang, L.J. Cho and Y. Jeong. 2000. Germination of carrot, lettuce, onion, and Welsh onion seeds as affected by priming chemicals at various concentrations. Korean J. Hort. Sci. Technol., 18(2): 93-97.

Jin, H.U. and K. Tylkowska. 2005. Effects of priming in combination with fungicides on germination and infestation of lettuce (*Lactuca sativa* L.). Agricultural Sciences in China, 4(6): 449-454.

Ozbingol, N., F. Corbineau, S.P.C. Groot, R.J. Bino and D. Come. 1999. Activation of the cell cycle in tomato (*Lycopersicon esculentum* Mill.) seeds during osmoconditioning as related to temperature and oxygen. Ann. Bot., 84(2): 245-251.

Ruan, S., Q, Xue and K. Tylkowska. 2002. The influence of priming on germination of rice (*Oryza sativa* L.) seeds and seedling emergence and performance in flooded soils. Seed Sci. Technol., 30: 61–67.

Saini, S.H., E.D. Consolacion, P.K. Bassi and S.M. Spencer. 1986. Requirement for ethylene synthesis and action during relief of thermoinhibition of lettuce seed germination by combinations of gibberellic acid, kinetin, and carbon dioxide. Plant Physiol., 81: 950-953.

Steel, R.G.D. and J.H. Torre. 1984. Principles and Procedures of Statistics. McGraw Hill Book Co., Inc., New York: 173-191.

Warley, M.N. 2003. Preventing thermoinhibition in a thermosensitive lettuce genotype by seed imbibition at low temperature. Sci. Agri., 60(3): 477-480.