Journal of Applied Horticulture, 16(3): 241-244, 2014

Physiology and biochemical changes in accelerated aged tomato (*Solanum lycopersicum* Mill.) seeds

V. Vijayalakshmi*, S. Poonguzhali, K. Ramamoorthy and N. Natarajan

Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore.
**E-mail: viji.seedscience@gmail.com*

Abstract

The effect of physiological and biochemical changes were studied in seeds of TNAU tomato hybrid (CO-3) exposed to accelerated ageing for a period of 10 days and investigated for speed of germination, per cent germination, shoot, root length, dry matter production and biochemical attributes *viz.*, free amino acids (FAA), electrical conductivity (EC), volatile aldehydes production - seedling length bio assay (BA), dehydrogenase (DH) and peroxidase (POD) activity against untreated control (fresh) seeds. E.C., FAA and BA were negatively correlated with speed and percentage germination, root/shoot lengths, dry matter production, DH and POD activity. Speed of germination was highly and positively correlated with per cent germination (0.923), root length (0.971), dry matter production (0.940), dehydrogenase (0.776) and peroxidase activity (0.676) and it was negatively correlated with free amino acid content (-0.990) and electrical conductivity (-0.936).

Key words: Tomato hybrid CO-3, accelerated ageing, physiological parameters, correlation, germination

Introduction

Storing of seed is a serious problem in tropical and subtropical countries like India where high temperature and relative humidity may greatly accelerate seed ageing resulting in loss of vigour and viability (Layek *et al.,* 2007; Pati and Bhattacharjee, 2011). Invariably all crops seeds require storage for one or more planting season, during such period the deterioration is inevitable (Coolbear, 1995). Seed ageing is an irreversible process and the physiology of seed ageing is not well understood (McDonald, 1999). Among the several factors responsible for seed ageing *viz.*, genetical, mechanical damage, relative humidity and temperature of the storage environment, seed water content, presence of microflora and seed maturity are prominent. The rate of loss of seed viability is mainly a function of temperature and seed moisture content (McDonald, 1999). Many hypotheses have been proposed regarding causes of seed ageing such as free radicals mediated lipid peroxidation, inactivation of enzymes or decrease in proteins, disintegration of cell membranes and genetic damage (Priestley, 1986; Smith and Berjak, 1995; Walters, 1998; McDonald, 1999; Murthy *et al.*, 2003). Degradation and inactivation of enzymes due to changes in their macromolecular structures is one of the most important hypotheses proposed regarding causes of ageing in seeds (Basavarajappa *et al.*, 1991; Kalpana and Rao, 1993; Salama and Pearce, 1993; Basra and Malik, 1994; Goel *et al.*, 2003; Bailly, 2004; McDonald, 2004 and Lehner *et al.*, 2008).

Accelerated ageing is the most widely used stress test to predict the storability of various crop seeds in short period (Younis *et al.*, 1990). The deterioration process of accelerated ageing condition is same as that in natural ageing but only the rate of deterioration is rapid. It increases catabolic changes at the cellular level beyond the threshold of tolerance leading to lethality (Balraj *et al.,* 2001 and Ramanadane *et al.,* 2004) and in several instances reduced the emergence of the plumule and radicle in soybean and barley (Chauhan *et al.,* 1984) and in tomato (Argerich and Bradfold, 1989). Deleterious effects of accelerated ageing on the germination process are associated with the damage occurring at the membrane, nucleic acid and protein levels (Fujikura and Karssen, 1995) in cauliflower. Artificially aged seeds showed an increase in lipid peroxidation and reduced the activity of free radical scavenging enzyme (Kalpana and Rao, 1994 in pigeon pea; Jeng and Sung, 1994 in peanut; Chiu *et al.,* 1995 in watermelon; Hsu and Sung, 1997 in watermelon and Bailly *et al.,* 1998 in sunflower). The knowledge on physiological and biochemical basis of seed deterioration is important for its potential use in judging seed vigor. Hence, studies were undertaken using accelerated ageing technique and its effects on physiological, biochemical changes in tomato seeds.

Materials and methods

Seed material: TNAU tomato hybrid CO-3 seeds with 96% germination and 8% moisture were used as base material for this study carried out in the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore.

Accelerated ageing test : Seeds (100 nos) were packed in perforated butter paper bags with equal number of holes and placed in a desiccator maintained at 100% RH and 40 ± 1°C. During the period of accelerated ageing, seed packets were shuffled daily to ensure uniform exposure and the samples were drawn daily upto 10 days and tested for seed quality characters.

Seed quality analysis: Aged seeds were subjected to germination test outlined by ISTA (1999) with four replicates of 100 seeds each in roll towel medium and evaluated for seed quality parameters such as speed of germination, germination (%), seedling length and dry matter production along with non aged seeds serving as control. Number of seeds germinated with normal seedlings were

counted at 14th day after sowing and expressed in percentage (%). Seedling length was measured from the tip of primary leaf to the end of primary tap root and expressed in cm. Dry matter production was estimated by drying the normal seedlings at 80 °C and the values were expressed as mg/10 seedlings.

Free amino acid content (µg 50 seeds^{-1} 50 mL^{-1}): Fifty seeds were soaked in 50 mL of distilled water in four replicates for 9 h to obtain the seed leachate. One mL of 0.2 per cent Ninhydrin was added to 1 mL of seed leachate and boiled for 15 min in a water bath followed by cooling in running water and diluted to 10 mL and the intensity of colour developed was measured in an Optima UV-VIS spectrophotometer (Model SP-3000) at 620 nm against a leucine standard curve and expressed in µg.50 seeds^{-1}50 mL^{-1} (Ching and Ching, 1964).

Electrical conductivity (dSm^{-1}): Fifty seeds from each treatment were soaked in 50 mL of distilled water for 9 h in four replicates. The seed steep water was referred as leachate. The electrical conductivity of the seed leachate was measured in an electrical conductivity meter and expressed as dSm^{-1} (Presley, 1958).

Seedling growth in bio assay test (cm): Bioassay of seed vigour was done following the method of Sur and Basu (1990a) with minor modifications. Desiccators were added with water to create 100 per cent RH and two petriplates of different size were kept in such a way that one fit inside the other. In the inner petridish high vigour jute seeds in 25 numbers were placed equidistantly and in the outside petridish the tomato seeds of each treatment were placed equidistantly in four replicates and the dessicator was closed air tight. This set up was kept as such for 48 h and the seedling growth as influenced by the treatment was measured and the mean reported as seedling length in centimeter.

Dehydrogenase activity (OD): Twenty five seeds from each treatment were soaked in water for 18 h in four replicates. Ten embryos were separated and incubated in darkness with 5 mL of 0.2% TZ for 4 h. After incubation, the excess solution was decanted and the embryos were thoroughly washed with distilled water and surface dried with blotters. The formazon was eluted by soaking stained embryos in 5 mL methyl cellosolve (2 methoxy ethanol) overnight and the OD measured using UV-VIS spectrophotometer (Model SP-3000)at 470nm (Kittock and Law,1968).

Peroxidase activity (OD 10 min^{-1}): Two replicates of 500 mg pregerminated seed samples were homogenised in 5 mL of 0.25 M Tris buffer (pH 6.0) and centrifuged at 10,000 rpm for 10 min at 5 °C to extract enzymes. Pyrogallol solution (0.3 mL) was added to 0.1 mL of enzyme. Spectrophotometer was adjusted to read zero at 430 nm followed by addition of 0.5 mL of H_2O_2 and mixing. The change in absorbance was recorded every 30 seconds up to 3 minutes in a spectrophotometer. One unit of peroxidase is defined as the change in absorbance/minute at 430 nm (Malik and Singh, 1980).

Results and discussion

Highest germination speed was observed for untreated control (7.75). However with increase in ageing period there was gradual reduction in speed of germiantion, the value recorded on 10th day was (1.8) (Fig.1a). Accelerated ageing significantly decreased germination ability of non-aged seeds (98 to 45%) (Fig.1b). Similar trend was observed for shoot, root length and dry matter production (Fig.1c).

Several researchers have reported cellular membrane degradation through free radicals mediated lipid peroxidation and peroxidative changes are the major cause of seed deterioration (Basra *et al.*, 2000; Stewart and Bewly, 1980). As seed quality declined, there was a concurrent increase in the levels of FAA, EC, BA and

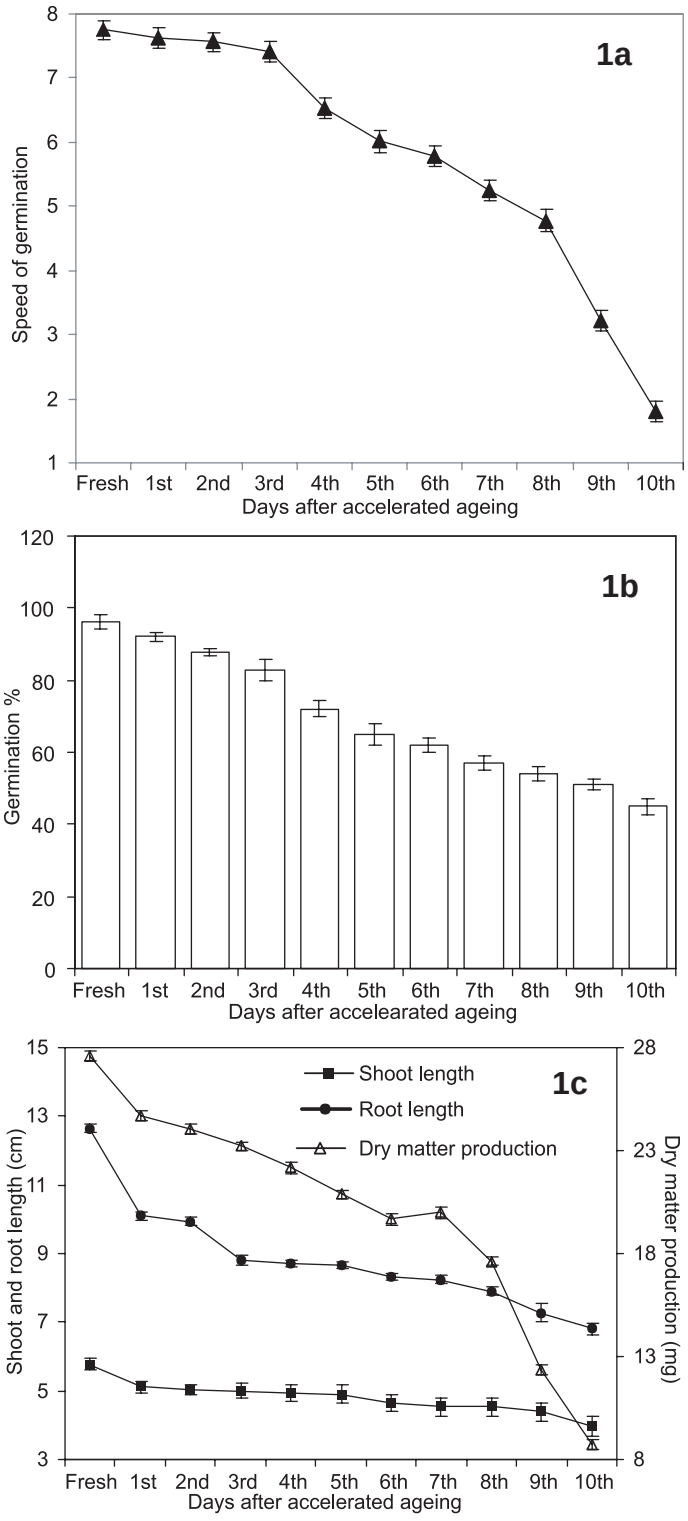

Fig.1. Effect of accelerated ageing on speed of germination (1a), germination percentage (1b), seedling length and dry matter production (1c) in TNAU tomato hybrid CO-3

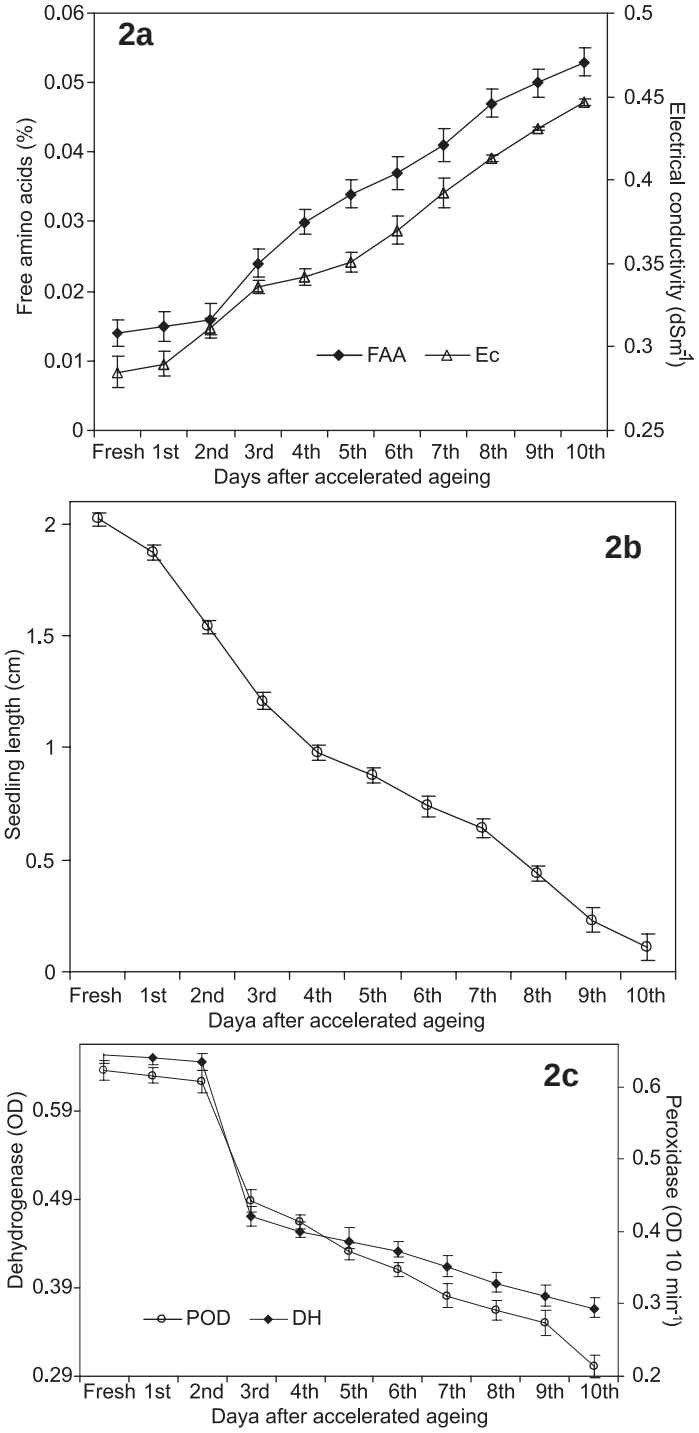

Fig. 2. Effect of accelerated ageing on (2a) free amino acids, electrical conductivity, (2b) volatile aldehyde production, dehydrogenase and (2c) peroxidase activity of TNAU tomato hybrid CO-3

decrease in enzymatic levels of DH and peroxidase. Peroxidase is an essential antioxidant enzyme that effectively scavenges free radicals (Sung, 1996), upto a certain period of ageing. In the present investigation, increase in free amino acid content was directly related with concurrent rise in seed leachate and volatile aldehyde production as well as decreased activity of peroxidase and dehydrogenase enzymes as a result of membrane integrity decline. The values recorded for untreated seeds were 0.014 %, 0.285 dSm^{-1}, 2.02 cm, 0.644 (OD) and 0.636 (OD 10 min^{-1}) respectively for FAA, EC, BA, DH and POD. The lowest values were observed for 10 days of accelerated aged seeds (Fig. 2a, 2b and 2c).

Correlation studies also revealed that EC, FAA and BA were negatively correlated with speed and percentage germination, root, shoot lengths and dry matter production, DH and POD activity. Speed of germination was highly and positively correlated with per cent germination % (0.923), root length (0.971), dry matter production (0.940), dehydrogenase (0.776) and peroxidase activity (0.676) and it was negatively correlated with free amino acid content (-0.990) and electrical conductivity (-0.936) (Table 1). The present results were in accordance with Basara *et al.* (2000) in cotton and Kapoor *et al.* (2011) in paddy.

It could be concluded that accelerated ageing test is a very suitable stress test and helps in predicting the seed vigour during seed storage with a specific temperature, relative humidity for a specific period of time. Further detailed studies may reveal the pattern and mechanism responsible for seed ageing.

References

Argerich, C.A., and K.J. Bradford, 1989. The effects of priming and ageing on seed vigour in tomato. *J. Expt. Bot.*, 40: 599-607.

Bailly, C. 2004. Active oxygen species and antioxidants in seed biology. *Seed Sci.*, 14: 93-107.

Bailly, C., A. Benamar, F. Corbineau and D. Come, 1998. Free radical scavenging as affected by accelerated ageing and subsequent priming in sunflower seeds. *Physiol. Plant.*, 104: 646-652.

Balraj, R., A.S. Channaveeraswamy, M. Shekhargowda and N.M. Shakuntala, 2001. Effect of ageing on seed quality in sunflower. (*Proc. National Symposium on Seed Science and Technology in the new Millenium – Vistas and Vision*, University of Mysore, Mysore, August 6 - 8, 2001. PP. 201-202.).

Basavarajappa, B.S., H.S. Shetty and H.S. Prakash, 1991. Membrane deterioration and other biochemical changes, associated with accelerated ageing of maize seeds. *Seed Sci. Technol.*, 19: 279-286.

Basra, A.S. and C.P. Malik, 1994. Amelioration of the effects of ageing in onion seeds. *Biol. Plantarum*, 36(3): 365-371.

Table1. Correlations between various vigor parameters of tomato seedling

Parameter	Speed	G %	RL	DMP	FAA	EC	DH	POD
Germination speed	1.000							
Germination (%)	0.923**	1.000						
Root length	0.971**	0.916**	1.000					
DMP	0.940**	0.951**	0.929**	1.000				
FAA	- 0.990	0.878*	-0.971	-0.901	1.000			
EC	-0.936	-0.991	-0.812	-0.661	0.788*	1.000		
DH	0.776*	-0.800	0.933**	0.741*	-0.921	-0.832	1.000	
POD	0.676*	0.914**	0.971**	0.837*	-0.974	-0.826*	0.979**	1.000

*Significant at 5% level; **Significant at 1% level

Speed – Germination speed, G % - Germination %, RL – Root Length (cm), DMP – Dry Matter Production (mg/ 10seedlings), FAA – Free Amino Acid (%), EC – Electrical Conductivity (dSm^{-1}), DH – Dehydrogenase activity(OD), POD – Peroxidase activity (OD 10 min^{-1}).

Basara, S.M.A., K.U. Rehman and S. Iqbal, 2000. Cotton seed deterioration: Assessment of some physiologicaland biochemical aspects. *Int. J. Agril. Boil.,* 3: 195-198.

Chauhan, K.P.S., J.K. Purkar and S.K. Banerjee, 1984. Ageing induced changes in seeds soybean and barley. *Seed Res.,* 12(1): 53-71.

Ching, T.M. and K.K. Ching, 1964. Freeze drying of pine pollen. *Plant Physiol.,* 39: 705-709.

Chiu, K.Y., C.S.Wang and J.M. Sung, 1995. Lipid peroxidation and peroxide-scavenging enzymes associated with accelerated ageing and hydration of watermelon seeds differing in Ploidy**.** *Physiol. Plant.,* 94: 441-446.

Coolbear, P. 1995. Mechanisms of seed deterioration. In: *Seed Quality: Basic Mechanisms and Agricultural Implications.* Basra A.S. (Eds.) Food Products Press, New York. pp. 223-277.

Fujikura, Y. and C.M. Karssen, 1995. Molecular studies on osmoprimed seeds of cauliflower: a partial amino acid sequence of a vigour-related protein and osmopriming-enhanced expression of putative aspartic protease. *Seed Sci. Res.,* 5: 177-178.

Goel, A., A. Goel and S. Inder Singh, 2003. Changes in oxidative stress enzymes during artificial ageing in cotton (*Gossypiumhirsutum* L.) seeds. *J. Plant Physiol.,* 160: 1093-1100.

Hsu, J.L. and J.M. Sung, 1997. Antioxidant role of glutathione associated with accelerated ageing and hydration of triploid watermelon seeds. *Physiol. Plant.,* 100: 967-974.

ISTA.1999. *International Rules for Seed Testing.* International Seed Testing Association, Switzerland.

Jeng, T.L. and J.M. Sung, 1994. Hydration effect on lipid peroxidation and peroxide scavenging enzymes activity of artificially aged peanut seed. *Seed Sci. & Technol.,* 22: 531-539.

Kalpana, R. and M.K.V. Rao, 1993. Lowered lipoxygenase activity in seeds of pigeonpea (*Cajanus cajan* L. Mill.) cultivars during accelerated ageing. *Seed Sci. Technol.,* 21: 269-272.

Kalpana, R. and K.A.M. Rao, 1994. Absence of the role of lipid peroxidation during accelerated ageing of seeds of pigeon pea (*Cajanus cajan* Mill.) cultivators. *Seed Sci. & Technol.,* 22: 253-260.

Kapoor, N., A. Arya, M.A. Siddiqui, H. Kumar and A. Amir, 2011. Physiolgical and biochemical changes during seed deterioration in aged seeds of rice. *American J., Plant. Physiol.,* 6(1): 28-35.

Kittock, D.L. and A.G. Law, 1968. Relationship of seedling vigour, respiration and tetrazolium chloride reduction by germination of wheat seeds. *Agron. J.,* 60: 286-288.

Layek, N., B.K. De, S.K. Mishra and A.K. Mandal, 2007. Seed invigoration treatments for improved germinability and field performance of gram (*Cicer arietinum* L.). *Legume Res.,* 29(4): 257-261.

Lehner, A., N. Mamadou, P. Poels, D. Come, C. Bailly and F. Corbineau, 2008. Changes in soluble carbohydrates, lipid peroxidation and antioxidant enzyme activities in the embryo during ageing in wheat grains**.** *J. Cereal Sci.,* 47: 555-565

Malik, C.P. and M.B. Singh, 1980. *Plant Enzymology and Histo enzymology.* Kalyani Publishers, New Delhi. p. 286.

McDonald, M.B. 1999. Seed deterioration: Physiology, repair and assessment. *Seed Sci. Technol.,* 27: 177-237.

McDonough, C.M., C.D. Floyd, R.D. Waniska and L.W. Rooney, 2004. Effect of accelerated ageing on maize, sorghum, and sorghum meal. *J. Cereal Sci.,* 39: 351-361.

Murthy, U.M.N., P.P. Kumar and W.Q. Sun, 2003. Mechanisms of seed ageing under different storage conditions for *Vigna radiata* (L.) Wilczek: Lipid peroxidation, sugar hydrolysis, Maillard reactions and their relationship to glass state transition. *J. Exp. Bot.,* 54: 1057-1067.

Pati, C.K. and A. Bhattacharjee, 2011. Sunflower seed invigoration by chemical manipulation. *African J. Plant Sci.,* 5(15): 867-872.

Presley, J.T. 1958. Relation of protoplast permeability of cotton seed viability and predisposition of seedling diseases. *Plant Disease Report.,* 42(7): 582.

Priestley, D.A. 1986. *Seed Ageing: Implications for Seed Storage and Persistence in the Soil*. Cornell University Press, Ithaca, New York. pp. 304.

Ramanadane, T. and A.S. Ponnuswamy, 2004. Ageing and anatomical influence on seed storability in rice (*Oryza sativa* L.) hybrids and parental lines. *Trop. Agric. Res.,*16: 37-50.

Salama, A.M. and R.G. Pearce, 1993. Ageing of cucumber and onion seeds: Phospholipase d, lipoxygenase activity and changes in phospholipid content. *J. Exp. Bot.,* 44: 1253-1265.

Smith, M.T. and P. Berjak, 1995. Deteriorative changes associated with the loss of viability of stored desiccation tolerant and desiccation sensitive seeds. In: *Seed Development and Germination,* J. Kigel and G. Galili (Eds.). New York. pp. 701-746.

Stewart, R.R.C. and J.D. Bewly, 1980. Lipid peroxidationa associatedwith accelerated ageing of soybean axes. *Plant. Physiol.,* 65: 245-248.

Sung, J.M. 1996. Lipid Peroxidation and peroxidase scavenging in soybean seeds during ageing. *Physiol. Plant.,* 97: 85-89.

Sur, K. and R.N. Basu, 1990. Reduced volatile aldehyde production in wheat by seed invigoration treatments. *Curr. Sci.,* 59: 799-800.

Walters, C. 1998. Understanding the mechanisms and kinetics of seed ageing**.** *Seed Sci. Res.,* 8: 223-244.

Younis, S.A., F.I. Al-Ravi and E.G. Hagop, 1990. Physiological and biochemical parameters of seed quality in some rice varieties (*Oryza sativa* L.). *Seed Res.,* 18(2): 148-153.

Received: October, 2013; Revised: May, 2014; Accepted: May, 2014