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Studies on influence of modified atmospheric storage conditions on biochemical parameters in pigeonpea seeds

B. Manjunatha[1*], S. N. Vasudevan[1], Umesha[1] and Chintalapati Sravani[2]

[1]Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, Raichur -584102 (Karnataka), INDIA.
[2]Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, GKVK, Bangalore-560065 (Karnataka), INDIA
[*]Corresponding author. E-mail: manjuraj522@gmail.com

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Abstract : An experiment was conducted to study the influence of modified atmospheric storage conditions on biochemical parameters of pigeonpea seeds. The experiment was conducted at the Department of Seed Science and Technology, College of Agriculture, Raichur, Karnataka. The seeds are exposed to various gaseous combinations and stored in 700 gauge polyethylene bag for ten months during July-2012 to April- 2013. The results revealed that, the seeds exposed to gaseous combination of 40% N_2+00% O_2+ 60% CO_2 showed less reduction in dehydrogenase enzyme activity and protein content (0.276 OD (*optical density)* value, 19.33 % respectively) as compared to the control (0.211 OD value and 18.13 % respectively) after ten months of storage. In addition less seed leachate (2.029 dSm^{-1}) was recorded in gaseous combination of 40% N_2+00% O_2+ 60% CO_2 as compared to control (2.207dSm^{-1}). It indicates the potential use of modified atmospheric storage technology for maintenance of seed viability and vigour during storage in pulses.

Keywords: Carbon dioxide, MAP, Nitrogen, Oxygen, Pigeonpea

INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.)] is an important pulse crop in India. It is also known as red gram, arhar and tur. In India, it is one of the very important grain legumes and occupies second position in area and production next to chickpea. It is mainly grown in the states of Maharashtra, Karnataka, Uttar Pradesh, Madhya Pradesh and Gujarat. It is grown on an area of 4.04 m ha with an annual production of 2.65 million tonnes and a productivity of 656 kg per ha. Its area, production and productivity trends in India in last five decades showed that there was about 2 per cent increase in area per year but the yield levels were stagnated around 600-700 kg per ha. In Karnataka, pigeonpea occupies second place in area (0.77mha) and ranks second in production (0.36 mt) with a productivity of 466 kg per ha (Anonymous, 2012).

Proper storage of seed in seed production programme is an important activity in order to maintain its quality parameters like viability, vigour and seed health until it is sown. Now a days seed consumer, tiller of the land demand for high quality seeds. Supplying high quality seeds can be achieved by an appropriate postharvest storage technology (Shivappa, 2011). A major cause of stored seed degradation and deterioration are insect pests and microorganisms. Many studies have shown that modified atmosphere of elevated carbon dioxide and depleted oxygen is effective method against insect and microorganisms during storage. Modified atmospheric storage is one of the seeds and food preservation methods that maintain the natural quality of food products in addition to extending the storage life. Modified atmosphere (MA) reduces the respiration rate of seeds and activity of insects or microorganisms in seeds (Jayas and Jeyamkondan, 2002).

The only technology that retains the special capacity of fumigation for in-situ treatment of stored commodities, as well as offering a similar diversity of application technologies, is the modified atmosphere (MA) method. Modified atmosphere offer an alternative that is safe and environmentally benign to the use of conventional residue-producing chemical fumigants for controlling insect pests attacking stored grains, oilseeds, pulses, processed commodities and packaged foods. Although the economics involved in the application of MA prevent their full replacement of conventional fumigants, novel approaches to the use of MA indicate their suitability for niche applications (Shlomo *et al*., 2004).

Disinfestations of stored seeds using modified atmospheric storage (MA) involves the alteration of the natural storage gases such as carbon dioxide (CO_2), oxygen (O_2) and nitrogen (N_2), to render the atmosphere in the stores lethal to pests. Modified atmosphere storage of seeds is a suitable alternative to the use of chemical fumigants and contact insecticides that are known to

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leave carcinogenic residues in the treated product (Bailey and Banks, 1980).The loss in viability is accompanied by certain biochemical changes in seed. Therefore, the present investigation was carried out to Study the influence of modified atmospheric storage conditions on biochemical parameters in pigeonpea seeds.

MATERIALS AND METHODS

The experiments were conducted in the laboratory of Department of Seed Science and Technology, College of Agriculture, University of Agricultural Sciences, Raichur, Karnataka for ten months during July-2012 to April- 2013. Polyethylene bags of 700 gauge measuring 40 cm length and 25 cm breadth were used for packing purpose. In these bags, one kg of pigeonpea seeds (variety BSMR-736) was packed along with the gases like carbon dioxide, nitrogen and oxygen in different concentrations according to the treatments *viz.*, (T_1-Control, T_2-(70% N_2+20% O_2+10% CO_2), T_3-(60 % N_2+20% O_2 +20% CO_2), T_4-(40% N_2+20% O_2+40% CO_2),T_5-(20% N_2+20% O_2+ 60% CO_2),T_6-(80% N_2 +10% O_2+10% CO_2), T_7-(70% N_2+10% O_2+20% CO_2),T_8-(50% N_2+10% O_2+40% CO_2),T_9-(30% N_2+10% O_2+60% CO_2),T_{10}-(90% N_2+00% O_2+10% CO_2),T_{11}-(80% N_2+00% O_2+20% CO_2),T_{12}-(60% N_2+00% O_2+40% CO_2), T_{13}-(40% N_2+00% O_2+ 60% CO_2), T_{14}-Vacuum). Firstly, the valves of the gas cylinders were opened and were released at a pressure of 7 kg/cm^2 and the different combinations of carbon dioxide, nitrogen and oxygen were mixed in the mixing chamber. According to the treatments given, gas flow rate was controlled in the buffer tank which was directly connected to the packaging unit. The pigeonpea seeds of 1 kg were packed using the packaging machine by evacuing the air, then flushing with the gases of required combinations followed by sealing automatically. Composition of the gas *i.e.*, O_2 and CO_2 gas concentrations inside the package was checked by Check mate head space gas analyzer with the help of septum which prevents leakage of gas from polyethylene bag. The experiment was carried out in completely randomized design in three replications and observations on various seed quality parameters were recorded (2, 4, 6, 8 and 10) bimonthly.

Representative seeds (25) from each treatment were taken and preconditioned by soaking in water overnight at room temperature. Seeds were taken at random and the embryos were excised. The embryos were steeped in 0.25 per cent solution of 2,3,5-triphenyl tetrazolium chloride solution and kept in dark for two hours at 40^{o}C for staining. The stained seeds were thoroughly washed with water and then soaked in 10 ml of 2 methoxy ethanol (methyl cellosolve) and kept overnight for extracting the red colour formazan. The intensity of red colour was measured using ELICO UV -VIS spectrophotometer (model SC-159) using blue filter (470 nm) and methyl cellosolve as the blank. The OD value obtained was reported as dehydrogenase activity (Kittock and Law, 1968).

Five grams of seeds in four replicates were soaked with acetone for half a minute and thoroughly washed in distilled water for five times. Then the seeds soaked in 25 ml distilled water and kept in an incubator maintained at 25 ± 1^{o}C for 24 hours. The seed leachate was collected and volume was made up to 25 ml by adding distilled water. The electrical conductivity of the seed leachate was measured in the digital conductivity bridge (ELICO) with a cell constant of 1.0 and the mean value expressed in deci Simons per meter.

Seed samples from each treatment were ground into fine powder. The seed samples were analyzed for protein content by Lowry's method (Lowry, *et al.*, 1951) and protein content was finally expressed in mg per 100 g seed sample by referring to standard graph.

RESULTS AND DISCUSSION

Tetrazolium is a rapid test to estimate seed viabil-ity and vigor based on color alterations of seed living tissues in contact with a solution of 2,3,5 triphenyl tetrazolium chloride, thus reflecting the degree of activity of the dehydrogenase enzyme system closely related to seed respiration and viability (Julio, 2015).The dehydrogenase enzyme activity is a good stable metabolic marker to estimate the degree of vigour in seeds (Saxena *et al*., 1987) and have positive association with vigour and viability of seeds (Rudrapal and Basu, 1970; Halder and Gupta, 1982 and Kharlukhi, 1983).

Dehydrogenase activity declined with the advancement of storage period. The mean dehydrogenase activity decreased from 0.802 OD value at the initial stage to 0.240 OD value after ten months of storage. The 5% significant difference due to modified atmospheric storage conditions on dehydrogenase activity was recorded throughout the storage period. At the end of ten months of storage period, highest dehydrogenase activity was recorded in T_{13} (40% N_{2+} 0% O_2+60% CO_2) 0.276 followed by T_{12}-(60% N_2 + 0% O_2+40% CO_2) 0.271, while the lowest dehydrogenase activity was recorded in T_1 (control) 0.211(Table1).This gradual decline in dehydrogenase activity may be due to reduction in vigour level of seed as the deterioration occurs rapidly over a period of time under uncontrolled condition. The similar results of dehydrogenase activity were also reported by Shivappa (2011) in onion and Shrishail (2011) in groundnut.

The damage caused to membrane through deterioration that provides lower selectivity and hence increase in the leakage of solutes to the environment has been one of the main causes of the decline in the physiological quality of seeds. As result, the electrical conductivity test is considered as an important tool to evaluate the seed vigour, since it indirectly assesses the cell membrane degradation degree by determining the amount of electrolytes released in the seed soaking solution

Table 1. Influence of modified atmospheric storage conditions on total dehydrogenase activity (OD value) of pigeonpea seeds during storage

Treatments			Months of storage (July-2012 to April 2013)				
			2	**4**	**6**	**8**	**10**
T_1	:	Control	0.691	0.572	0.455	0.357	0.211
T_2	:	70% N_2 + 20% O_2 + 10% CO_2	0.700	0.603	0.485	0.367	0.220
T_3	:	60% N_2 + 20% O_2 + 20% CO_2	0.706	0.616	0.490	0.371	0.223
T_4	:	40% N_2 + 20% O_2 + 40% CO_2	0.719	0.627	0.492	0.379	0.226
T_5	:	20% N_2 + 20% O_2 + 60% CO_2	0.755	0.630	0.494	0.386	0.229
T_6	:	80% N_2 + 10% O_2 + 10% CO_2	0.761	0.639	0.498	0.387	0.232
T_7	:	70% N_2 + 10% O_2 + 20% CO_2	0.768	0.645	0.504	0.394	0.234
T_8	:	50% N_2 + 10% O_2 + 40% CO_2	0.764	0.657	0.516	0.398	0.237
T_9	:	30% N_2 + 10% O_2 + 60% CO_2	0.765	0.660	0.524	0.401	0.240
T_{10}	:	90% N_2 + 00% O_2 + 10% CO_2	0.766	0.662	0.549	0.405	0.245
T_{11}	:	80% N_2 + 00% O_2 + 20% CO_2	0.768	0.664	0.551	0.408	0.250
T_{12}	:	60% N_2 + 00% O_2 + 40% CO_2	0.772	0.671	0.556	0.429	0.271
T_{13}	:	40% N_2 + 00% O_2 + 60% CO_2	0.777	0.677	0.561	0.432	0.276
T_{14}	:	Vacuum	0.770	0.669	0.553	0.425	0.269
		Mean	0.748	0.642	0.516	0.395	0.240
		S.Em±	0.010	0.014	0.007	0.006	0.006
		CD (5%)	0.031	0.043	0.022	0.019	0.019

Table 2. Influence of modified atmospheric storage conditions on electrical conductivity (dSm^{-1}) of pigeonpea seeds during storage

Treatments			Months of storage (July-2012 to April 2013)				
			2	**4**	**6**	**8**	**10**
T_1	:	Control	0.639	0.906	1.272	1.861	2.207
T_2	:	70% N_2 + 20% O_2 + 10% CO_2	0.632	0.877	1.266	1.796	2.153
T_3	:	60% N_2 + 20% O_2 + 20% CO_2	0.627	0.859	1.263	1.792	2.106
T_4	:	40% N_2 + 20% O_2 + 40% CO_2	0.623	0.853	1.257	1.777	2.098
T_5	:	20% N_2 + 20% O_2 + 60% CO_2	0.620	0.850	1.233	1.771	2.084
T_6	:	80% N_2 + 10% O_2 + 10% CO_2	0.619	0.811	1.221	1.767	2.077
T_7	:	70% N_2 + 10% O_2 + 20% CO_2	0.615	0.801	1.216	1.759	2.072
T_8	:	50% N_2 + 10% O_2 + 40% CO_2	0.613	0.791	1.192	1.741	2.070
T_9	:	30% N_2 + 10% O_2 + 60% CO_2	0.610	0.789	1.187	1.739	2.062
T_{10}	:	90% N_2 + 00% O_2 + 10% CO_2	0.609	0.786	1.183	1.735	2.059
T_{11}	:	80% N_2 + 00% O_2 + 20% CO_2	0.607	0.785	1.182	1.733	2.057
T_{12}	:	60% N_2 + 00% O_2 + 40% CO_2	0.598	0.768	1.170	1.726	2.042
T_{13}	:	40% N_2 + 00% O_2 + 60% CO_2	0.597	0.765	1.165	1.721	2.029
T_{14}	:	Vacuum	0.602	0.772	1.180	1.728	2.045
		Mean	0.615	0.815	1.213	1.760	2.083
		S.Em±	0.011	0.013	0.016	0.018	0.024
		CD (5%)	NS	0.037	0.049	0.053	0.072

NS – Non significant

(Maristela and Roberval 2007).

Electrical conductivity of seed leachates (EC) increased with the advancement of storage period. The mean value of seed leachates (EC) increased from 0.513 dSm^{-1} at the beginning to 2.083 dSm^{-1} after ten months of storage. At the end of ten months of modified atmospheric storage period, lowest electrical conductivity of seed leachate was recorded in T_{13}(40% N_2 + 0% O_2+60%CO_2) (2.029 dSm^{-1}), which was preceded by T_{12}-(60% N_2 + 0% O_2+40%CO_2) (2.042 dSm^{-1}) and this might be due to the better maintenance of membrane integrity while, the highest electrical conductivity was recorded in T_1control (2.207 dSm^{-1}). Increase in electrical conductivity with the storage period may be due to increased membrane permeability and decreased integrity of seed coat resulted in excess release of electrolytes which caused higher electrical conductivity (Table 2). Similar findings of Electrical conductivity of seed leachates (EC) were also observed by Shivappa (2011) in onion and Shrishail (2011) in groundnut.

Seed protein declined with the advancement of storage period. The mean protein content decreased from 20.90% at the initial stage to 19.03% after ten months of storage higher protein content of seed has been found to favour maintenance of vigour and viability during storage (Hewrton, 1994 and Ching and Schoolcraft, 1968). In present investigation, at the end of ten months of modified atmospheric storage period highest protein content was recorded in T_{13}(40% N_2 + 0% O_2+60%CO_2) 19.33% followed by T_{12}-(60% N_2 + 0% O_2+40%CO_2) 19.26% while the lowest protein content was recorded in T_1 control (18.13%). However, de-

Table 3. Influence of modified atmospheric storage conditions on protein content (%) of pigeonpea seeds during storage

Treatments		Months of storage (July-2012 to April 2013)				
		2	4	6	8	10
T_1	: Control	20.03	19.92	19.72	18.23	18.13
T_2	: 70% N_2 + 20% O_2 + 10% CO_2	20.06	19.98	19.79	19.00	18.93
T_3	: 60% N_2 + 20% O_2 + 20% CO_2	20.11	20.02	19.84	19.20	18.98
T_4	: 40% N_2 + 20% O_2 + 40% CO_2	20.13	20.04	19.86	19.33	19.00
T_5	: 20% N_2 + 20% O_2 + 60% CO_2	20.17	20.07	19.88	19.36	19.02
T_6	: 80% N_2 + 10% O_2 + 10% CO_2	20.22	20.08	19.93	19.44	19.07
T_7	: 70% N_2 + 10% O_2 + 20% CO_2	20.25	20.10	19.94	19.50	19.08
T_8	: 50% N_2 + 10% O_2 + 40% CO_2	20.28	20.12	19.96	19.52	19.10
T_9	: 30% N_2 + 10% O_2 + 60% CO_2	20.29	20.13	19.98	19.54	19.12
T_{10}	: 90% N_2 + 00% O_2 + 10% CO_2	20.30	20.14	20.00	19.56	19.14
T_{11}	: 80% N_2 + 00% O_2 + 20% CO_2	20.32	20.16	20.01	19.58	19.16
T_{12}	: 60% N_2 + 00% O_2 + 40% CO_2	20.43	20.22	20.05	19.63	19.26
T_{13}	: 40% N_2 + 00% O_2 + 60% CO_2	20.59	20.35	20.08	19.65	19.33
T_{14}	: Vacuum	20.34	20.20	19.99	19.60	19.19
	Mean	20.25	20.10	19.93	19.36	19.03
	S.Em±	0.406	0.050	0.037	0.246	0.121
	CD (5%)	NS	0.147	0.108	0.714	0.352

NS – Non significant

crease in the protein content was observed in all the treatments as the storage period advanced (Table 3). Similar findings of seed protein were also observed by Shehata *et al*. (2009) The cowpea seeds treated with gases mixture containing 80 % CO_2 + 4 % O_2 + 16 % N_2 showed highest total crude protein , while untreated seeds showed the lowest total crude protein.

Conclusion

It is evident from present study that the seeds stored in polyethylene bags without gases shows higher electrical conductivity of seed leachates (EC), lower dehydrogenase activity and protein content where, as seeds stored in gaseous combination of higher concentration of carbon dioxide and zero percentage of oxygen shows lower EC, higher dehydrogenase activity and protein content. The correlation of biochemical parameters correlated with the vigour and viability of pigeonpea seeds showed that the electrical conductivity of seed leachates (EC) increases, vigour and viability of pigeonpea seeds decreases and high dehydrogenase activity and seed protein content was found to favour maintenance of vigour and viability during storage. Pigeon pea seeds packed with gaseous combination of higher concentration of carbon dioxide (60%) and zero percentage of oxygen enhances the seed longevity with higher seed quality parameters.

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