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Seed Deterioration in Chickpea (*Cicer arietinum* L.) under Accelerated Ageing

N. Kapoor, A. Arya, M.A. Siddiqui, A. Amir and H. Kumar
Department of Biotechnology, Meerut Institute of Engineering and Technology,
N.H. 58, Delhi-Roorkee Highway, Baghpat Road Bypass Crossing, Meerut-250005, UP, India

Abstract: Accelerated ageing test were made in five chickpea varieties viz., three desi (Pusa 256, Pusa 212 and BGD 72) and two kabuli (Pusa 267 and K551) with the objective of studying sequence and relationship of process of deterioration over short periods. All the varieties were investigated to consider whether there are cultivars differences for tolerance to ageing conditions. In the study, effect of accelerated ageing on seed viability, seedling vigour and biochemical process were explored. The overall results showed that seed quality in chickpea deteriorates following accelerated ageing treatment. All the physiological parameter viz., germination percentage, seedling root length, shoot length and consequently the vigour index in all varieties were significantly decreased. An increase in moisture content was observed during ageing. Decrease in seed viability and germination rate, vigour is also correlated with biochemical changes (decreased soluble proteins and sugar content) associated with seed ageing. The study also exhibited differential responses among varieties with respect to ageing. All the test experiments performed concluded that Pusa 256 is the best variety, followed by K 551 among all five varieties and Pusa 212 is highly sensitive variety with respect to accelerated ageing.

Key words: Desi, Fabaceae, kabuli, pusa, seed germination, stress, vigour index

INTRODUCTION

Chickpea (*Cicer arietinum* L.), a member of family Fabaceae, is an important and inexpensive source of protein in human food and animal feed. After soyabeans, peanuts beans and peas, chickpea is the world's fifth most important grain legume (FAO, 2003). Chickpea are generally grouped into two types, the desi type with small angular, dark colored and rough seeds, cultivated mostly in the Indian subcontinent, Ethiopia, Mexico and Iran and the kabuli type with large, light colored and smooth seeds, cultivated mainly in Southern Europe, Northern Africa, Chile and Afghanistan (Zohary and Hopf, 2000; Kumar and Dua, 2006). They are a staple food in some tropical and subtropical countries.

Chickpea contributes 15% to world's pulse harvest of about 58 million tones. In the world, India covers maximum area under pulses out of which chickpea is grown on approximately 7.29 mha area with 780 kg ha^{-1} productivity which represents, about 32 and 42% of the national pulse acreage and production, respectively (Munirathnam and Sangita, 2009). Notwithstanding its distribution throughout the country, four states, namely, Madhya Pradesh, Rajasthan, Uttar Pradesh and Maharashtra, together contribute 87% of the production from 65% area. Despite significant gains in world pulse production during the past two decades, with an average annual growth rate of 1.9%, chickpea productivity is not high enough to fulfill the requirements of an ever increasing demand (Saxena *et al*., 1996).

There are two major constraints viz., biotic stresses like fusarium wilt, pod borer etc. and abiotic stresses like drought, salinity, heat and cold, hampered chickpea production. Inspite of these constraints another factor which also adversely influences the viability of seed is prolonged storage and ageing. In the tropical and subtropical conditions, under high ambient relative humidity the seeds uptakes moisture from the environment resulting in high ambient temperature, thus resulting changes in genetic integrity leading to deterioration of seeds.

Seed deterioration can be defined as the loss of quality, viability and vigour either due to ageing or effect of adverse environmental factors. The rate of deterioration rapidly increases with increase in either seed moisture content or temperature of storage (Ellis *et al*., 1985). The process of seed deterioration can be understood by test of seed storage potential or vigour potential (vigour test) and accelerated ageing (Deluche and Baskin, 1973). Artificial ageing treatments take advantage of the fact that seed ageing process is determined by the seed moisture level and temperature. Manipulation of these factors, therefore, hastens movement of seed through the pattern of deterioration. The physiological and biochemical

Corresponding Author: M.A. Siddiqui, Department of Biotechnology, Meerut Institute of Engineering and Technology, N.H. 58, Delhi-Roorkee Highway, Baghpat Road Bypass Crossing, Meerut-250005, UP, India

changes during seed ageing have been extensively reviewed (McDonald, 1999; Jatoi *et al*., 2004). Under storage conditions, seed typically lost their viability within a few days or weeks (Murthy and Kumar, 2003).

Several studies have been conducted to standardized accelerated ageing test procedure for Brassica (Bedi *et al*., 2006), corn (Woltz and Tekrony, 2001), chick pea (Roy *et al*., 1994; Gil *et al*., 1996), lentil (Fernandez and Johnston, 1995), maize (Bako, 2006), mung bean (Murthy and Kumar, 2003), pea (Jatoi *et al*., 2004), pigeon pea (Kalpana and MadhavRao, 1995) soyabean (Deluche and Baskin, 1973; McDonald and Dranath, 1978; Tekrony, 1993) and weed (El- Keblawy, 2003).

Desi and kabuli chickpea seeds show large variability among genotypes in seed germination, seedling vigour and storability during long term storage (ambient temperature) and accelerated ageing. A broad review of the aspects related to chickpea seed quality with regards to accelerated ageing was also produced by several researchers (Fernandez and Johnston, 1995; Gil *et al*. 1996; Kenghe and Kanawade, 1996). Seed size dependant responses of cultivars of chickpea to accelerated ageing were also reported by Roy *et al*. (1994) and Raje and Khare (1996). In general, small seeds germinated and grew more rapidly compared to medium and large seeds of the same cultivars. A significant positive relationship between seed size and mean germination time and a negative relationship between seed size and germination index, root and shoot length was also reported (Kaya *et al*., 2008).

Thus present investigation was aimed to investigate various physiological and biochemical changes during seed deterioration as a result of accelerated ageing as well as to find out superior variety among five experimental varieties of chickpea.

MATERIALS AND METHODS

Five chickpea varieties viz., three desi (Pusa 256, Pusa 212 and BGD 72) and two kabuli (Pusa 267 and K551) were selected for the experiment. This work was carried out at Department of Biotechnology, Meerut Institute of Engineering and Technology (MIET), Meerut during Jan., 2009-June, 2009. The seeds of all the varieties were obtained from IARI, New Delhi in December 2008 and maintained in the field garden of MIET. The seeds were subjected to accelerated ageing by keeping them at 45°C and 100% relative humidity for 72 h. The varieties were analyzed after 24, 48 and 72 h ageing treatment. All the seeds of different treatment were incubated in a germinator maintained at a constant temperature of 30°C. The germination tests were evaluated after 7 days from planting.

One set of seeds of all the five varieties were maintained as control. Experiments were performed in three replicates. To understand the physiological and biochemical changes taking place in normal and accelerated seeds several tests were performed. For physiological studies, the experiment was performed on the basis of paper towel method. The various physiological parameters screened include moisture content, germination percentage (ISTA, 1993), seedling vigour (Perry, 1981) and vigour index (Abdul-Baki and Anderson, 1973) and biochemical parameters include soluble protein content (Lowry *et al*., 1951) and reducing sugar content (DNSA method).

RESULTS AND DISCUSSION

Chickpea is an appropriate crop for enhancement of bioproductivity and the recovery of marginal lands. The present study was conducted to study the behavior of seeds during accelerated ageing. Varietals differences among desi and kabuli chickpea varieties were also made. All the five varieties exhibited a clear deterioration behavior during accelerated ageing. Both type of chickpea that is desi and kabuli and even as different varieties (Pusa 256, Pusa 212, BGD 72, Pusa 267 and K551) differ greatly in their response to accelerated ageing. The experiments were designed to test the various physiological and biochemical changes which clearly demonstrate the process of deterioration. The various physiological parameters investigated includes moisture content, germination percentage, root length, shoot length and vigour index.

Initial germination percentage ranged between 92-97.3% (control). With respects to time duration of ageing a significant decline in germination percentage was also observed. A maximum decrease were observed in variety Pusa 212 (94.5-32%), followed by BGD 72 (96-34%), Pusa 267 (92-40%) and K 551 (95.8-64%). Pusa 256 was found to be least affected by ageing with respect to germination percentage that is a decline from 97.3 to 67%. These data are shown in Table 1. Such differences in maintenance of seed germination capacity have also been observed by Ellis *et al*. (1992) among the three sub species of rice and also by Kalpana and MadhavRao (1995) in pigeon pea. Progressive lose of seed viability has been reported by number of workers during seed ageing (Fernandez and Johanston, 1995; El-Keblawy, 2003; Jatoi *et al*., 2004).

A rise in moisture content was observed in all the five varieties of chickpea. These data are shown in Table 1. The seed moisture content among varieties were ranged from 8.92-9.71, 11-13.27, 13.22-15.54 and 14.2-19.11 at 0 (control), 24, 48 and 72 h of ageing respectively. This rise

Table 1: Effect of accelerated ageing (45°C and 100% RH) on various physiological parameters of five chickpea varieties

Varieties	Moisture content (%)	Germination (%)	Root length (cm)	Shoot length (cm)	Vigour index
Control					
Pusa 256	9.14	97.3	10.40	9.10	1897.35
Pusa 212	8.98	94.5	7.19	7.06	1346.25
BGD 72	9.71	96	9.20	12.40	2073.60
Pusa 267	8.92	92	6.33	9.46	1452.68
K 551	9.29	95.8	6.71	12.50	1744.518
24 h					
Pusa 256	11.00	90	8.13	7.60	1415.70
Pusa 212	12.53	80	2.80	4.75	604.00
BGD 72	11.47	82	4.58	8.10	1039.76
Pusa 267	13.18	81	3.50	4.38	638.28
K 551	13.27	88	4.75	8.75	1188.00
48 h					
Pusa 256	13.22	87	5.30	6.15	996.15
Pusa 212	15.54	64	1.10	1.08	139.52
BGD 72	14.66	70	3.29	6.00	650.30
Pusa 267	15.08	78	1.50	2.40	304.20
K 551	14.99	82	3.40	5.60	738.00
72 h					
Pusa 256	14.20	67	3.25	4.24	501.83
Pusa 212	18.50	32	0.30	0.54	26.88
BGD 72	17.48	34	2.10	3.20	180.20
Pusa 267	19.11	40	0.60	1.80	96.00
K 551	16.58	64	2.10	4.80	441.60

in moisture content is attributed to the absorption of moisture by the seed samples when they were subjected to 100% relative humidity during accelerated ageing. These results are in harmony with those obtained by Perez and Arguello (1995), Gil *et al.* (1996) and Kenghe and Kanawade (1996).

The accelerated ageing treatment significantly resulted in reduction of root length in all the varieties. These data are shown in Table 1. In the control, variety Pusa 256 has maximum root length followed by BGD 72 and Pusa 212 and than K 551, while Pusa 267 has least value. After accelerated ageing treatment also variety Pusa 256 has maximum root length followed by K 551 and then BGD 72. With respect to ageing the maximum decline in root length was observed in variety Pusa 212. Similar reduction pattern was also observed with respect to shoot length. In the control, among three desi varieties BGD 72 has maximum shoot length (12.4 cm) and Pusa 212 has minimum (7.06 cm) and between kabuli varieties K 551 has maximum (12.5 cm) and Pusa 267 has minimum (9.46 cm) shoot length. After accelerated ageing K 551 has maximum shoot length (4.8 cm) followed by Pusa 256 (4.24 cm) and minimum shoot length was observed in Pusa 212 (0.54 cm) followed by Pusa 267 (1.8 cm). Similar results of decrease in germination percentage and seedling length was also reported by Roy *et al.* (1994) in chickpea, Kalpana and MadhavRao (1995) in pigeon pea, Perez and Arguello (1995) in peanuts and Basra *et al.* (2003) in cotton. This decline is attributed to DNA degradation with ageing which leads to impaired transcription causing incomplete or faulty enzyme synthesis essential for earlier stages of germination.

The seedling growth is the morphogenetic expression of genetic programming. This morphogenetic expression leads to elongation of radical and plumule at more or less defined rate under a particular environmental condition. Under the congenial environmental conditions the growth of the seedlings takes on a defined temporal pattern, which is the manifestation of the sum, total of activities increasing cell number, cell expansion, fresh and dry weight. Alterations of the environmental condition can modify the growth of the seedlings in different ways affecting various components of the growth.

With the decrease in germination percentage and seedling length in the entire five varieties vigour index also showed a decline pattern during accelerated ageing. These data are shown in Table 1. Thus, it is clear that the germination percentage showed a positive and significant correlation with vigour index. Maximum vigour index was observed in variety BGD 72 followed by Pusa 256 in control and Pusa 256 followed by K 551 after ageing. Pusa 212 exhibited minimum vigour index before and after accelerated ageing among all the five varieties. Thus it was found to be having least germination growth potential in natural as well as ageing conditions. This is in contrast to the previous studies made by Abdul-Baki and Anderson (1973) and Roy *et al.* (1994). Differences in seedling vigour have been noted in many other species, such as cotton, lentil, bean, pea and also sunflower. (Fernandez and Johnston, 1995; Pallavi *et al.*, 1995; Jatoi *et al.*, 2004). The present maintained damage associated with reduction in the various growth parameters of artificially aged chickpea varieties seedlings can be considered as a reflection of biochemical and hormonal imbalances.

The physiological results are coinciding with the biochemical parameters including proteins and reducing sugars estimation. It has been observed that there is continuously decline in total soluble protein content in each variety after successive accelerated ageing time periods (Table 2). The maximum protein content was found in Pusa 267 followed by Pusa 256, Pusa 212 and K 551 under normal conditions. Maximum decline in protein content after accelerated ageing were observed in Pusa 212 followed by BGD 72. The decline in protein content in the varieties can be due to the denaturation of protein during the process of ageing. Gidrol *et al.* (1998) also reported the effect of accelerated ageing on protein synthesis in two legume seeds and they concluded that on ageing the rate of protein synthesis is decreased.

Table 2: Effect of accelerated ageing (45°C and 100% RH) on various biochemical parameters of five chickpea varieties

Varieties	Protein content (mg g^{-1} fw)				Sugar content (mg g^{-1} fw)			
Time duration (h)	Control	24	48	72	Control	24	48	72
Pusa 256	8.8	6.8	5.8	4.6	1.92	1.45	1.30	1.20
Pusa 212	8.6	5.4	2.2	0.8	1.55	0.84	0.21	0.10
BGD 72	7.5	3.6	2.4	1.2	1.70	1.32	0.88	0.38
Pusa 267	10.0	6.7	5.1	3.8	2.28	1.24	0.98	0.61
K551	8.0	5.8	5.6	4.2	2.13	2.11	1.70	1.34

Pallavi *et al*. (1995) observed a decline in protein content in sunflower seeds upon ageing over fresh seeds. Gil *et al*. (1996) also studied certain changes in physiological and chemical characters in desi and Kabuli chickpea and concluded that process of ageing led to deterioration of both germinability and seed viability.

The effect of accelerated ageing on soluble reducing sugar content (mg g^{-1} fw) of five chickpea varieties revealed that the sugar content as estimated by DNSA method shows a declined in all cultivars, with accelerated ageing. This is due to the inhibition of photosynthesis which is associated with decline in pigment contents resulted from the reduction in leaf area or due to decrease in leaf organic acid or due to less stomatal openings in leaf due to artificial ageing. With respect to sugar content, variety K 551 is least affected during process of ageing followed by Pusa 256. Maximum and minimum sugar content was observed in Pusa 267 and Pusa 212, respectively under control conditions and K 551 and Pusa 212, respectively under accelerated ageing conditions (Table 2). Similar results were reported by Gil *et al.* (1996) in chickpea and Pallavi *et al*. (1995) in sunflower. Bedi *et al.* (2006) also reported a significant increase in the level of starch and total sugar while protein content decreases in *Brassica* seeds.

CONCLUSIONS

Five varieties of chickpea namely three desi (Pusa 256, Pusa 212 and BGD 72) and two kabuli (Pusa 267 and K 551) were subjected to accelerated ageing treatment by keeping them at 45°C for 72 h. The parameters studied included the germination percentage, moisture content, seedling root length, shoot length, vigour index, soluble protein content and sugar content. The overall results showed that seed quality in chickpea deteriorates following accelerated ageing treatment. Accelerated ageing decreases the germination percentage, seedling length and consequently the vigour index in all the five varieties. Deteriorated seeds had decreased protein and sugar content. The study also showed that the varieties of chickpea also exhibit a differential responses with reference to physiological and biochemical changes occurring due to seed ageing.

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