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Role of Moisture Content and Controlled Atmosphere in *Citrus* Seed Storage

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ABSTRACT

The survival of *Citrus* seeds was examined under different moisture contents and storage atmospheres. Results demonstrated that grapefruit (*Citus paradisi* Macf.) and rough lemon *(Citrus limon* L.) seeds can be dried upto 7% moisture content without any significant damage and their storage behaviour may be orthodox. Kinnow mandarin (*C. reticulata* Blanco) seeds were intolerant of desiccation and showed recalcitrant storage behaviour. Results suggessted that there is considerable variation in seed storage behaviour of *Citrus* seeds which needs detailed investigation on *Citrus* and other related species. Controlled atmosphere did not have any consistent effect in maintaining *Citrus* seed viability. The role of moisture content and storage environment in *Citrus* seed longevity is discussed.

Key Words: *Citrus limon* L.; *Citrus paradisi* Macf;, *Citrus reticulata* Blanco; Germination; Rough lemon

INTRODUCTION

It has been frequently reported that viability of *Citrus* seeds decrease or seeds are completely killed when moisture content is reduced. Therefore, some workers have suggested that *Citrus* seeds have recalcitrant storage behaviour and it may be advisable to store seeds in a moist or imbibed state (Barton, 1943; Childs, 1948, Childs & Hrnciar, 1959; Richards, 1952; Bacchi, 1958; Zheng, 1980). For short-term maintenance, seed storage at relatively low temperature (2-5°C) with the treatment of suitable fungicide has proved adequate (Mungomery *et al.,* 1967; Button *et al.,* 1971; Soost & Cameron, 1975; Honjo & Nakagawa, 1978; Mobayen, 1980 a, b) but little is known about long-term preservation and storage behaviour of the seeds of many *Citrus* species. Species within the genus differ in storage behaviour and drying may or may not be deleterious to citrus seeds (Barton, 1943; Krishna & Shanker, 1974; Honjo & Nakagawa, 1978; King & Roberts, 1980b). Hong and Ellis (1995) reported that there was considerable interspecific variation in citrus seed storage behaviour.

Mumford and Grout (1979) showed that removal of the seed coat can permit lemon (*C. limon*) seeds to be dried to a low moisture content without any damage. But King and Roberts (1980b) showed that the removal of the seed coat is not necessary, provided sufficient time is allowed during the germination test for intact seeds to germinate. *Citrus* seeds have often been reported as being killed by drying because drying slows down the rate of seed germination, particularly when the seed coat is intact. It therefore seems possible that the storage behaviour of *Citrus* seeds may be recalcitrant (King & Roberts, 1979, 1980 a, b), i.e. their longevity can be increased by lowering the moisture content (at least down to 5%) and temperature. However, it has been shown that seeds of several species can survive considerable desiccation but nevertheless do not show strictly orthodox seed storage behaviour (Ellis *et al.*, 1990; 1991a, b; 1991; Hong & Ellis, 1990). Some other species behave similarly; for example arabica coffee seeds can withstand considerable desiccation but their storage behaviour does not conform to that defined by Roberts (1973) for orthodox seeds (Ellis *et al*., 1990; Hong & Ellis, 1992). This type of storage behaviour has been described as intermediate (Ellis *et al*., 1990).

The nature of the surrounding atmosphere (CO_2, O_2, N_2, vacuum, etc.) may affect seed longevity and much of this evidence has been reviewed by Barton (1961). Several studies showed little or no advantage for seed preservation in inert or nitrogenous atmospheres (Isely & Bass, 1959; Barton, 1960; Bass *et al*., 1962; 1963a; 1963b; Justice & Bass, 1978). Roberts (1972) revealed puzzling discrepancies between the performance of seeds stored in nitrogen and those stored in argon. Red clover seed (*Trifolium pratense*) stored in nitrogen rich atmosphere showed some advantage, whereas white clover (*T. repens*) maintained in the same environment did not show any significant effect (Davies, 1977). Roberts (1961) reported rice seeds to be better preserved in nitrogen at 12% moisture, but not at 13.5% or 14.5%, and Sampietro (1931) claimed that rice retained full germinability over eight years in nitrogen at 5% hydration, but deteriorated completely at 13% hydration.

Carbon dioxide has occasionally been used as a seed storage medium. In seeds maintained at high moisture levels, carbon dioxide may play some role as metabolic repressor (Rao & Achaya, 1969). Bennici *et al*. (1984) stored durum wheat (*Triticum durum*) in carbon dioxide atmosphere and found it to be beneficial. Lettuce seeds (5-6% moisture) declined in viability more slowly in CO_2 than air (Harrison, 1966). Sayre (1940) sealed maize grains in air, oxygen, carbon dioxide and nitrogen and showed that loss of viability was more pronounced in oxygen and nitrogen atmospheres compared to air or carbon dioxide. Oxygen is considered important to seeds with elevated hydration levels capable of sustained respiration (Ibrahim *et al*., 1983). Roberts and Abdalla (1967) showed that oxygen has a deleterious effect on the viability of barley, beans, and peas, and that deleterious effect was more pronounced at high moisture contents. Barton (1960) found that pure oxygen to be detrimental to *Lobelia cardinalis* seeds stored at room temperature, whereas at 5°C seeds declined in viability at about the same rate in air, in carbon dioxide, in nitrogen, and under vacuum as they did in oxygen. An atmosphere of oxygen can enhance the free radical processes, which may shorten the life of some type of seeds under certain conditions-especially those of unfavourable moisture and temperature (Ohlrogge & Kernan, 1982).

This study was designed to explore the role of seed moisture and controlled atmosphere on seed longevity in *Citrus*. A further aim of this study was to investigate seed storage behaviour of three contrasting *Citrus* species, and to find out whether their long-term seed conservation is likely to practicable.

MATERIALS AND METHODS

Plant material. Freshly harvested seeds of *Citrus* species, grapefruit (*Citrus paradisi* Macf.), kinnow mandarin (*Citrus reticulata* Blanco) and rough lemon (*Citrus limon* L.) were obtained from the Experimental Fruit Garden, Department of Horticulture, University of Agriculture, Faisalabad, Pakistan.

The seeds were extracted with a knife and forceps and were thoroughly washed in order to remove the pulp and mucilage. The seeds were sterilised using 10% sodium hypochlorite solution for 10 min (Mumford & Grout, 1979) and rinsed thoroughly in distilled water. Prior to use seeds were held in sterilised distilled water with added Thiram (Agrichem Limited) fungicide ($3.3 mlL^{-1}$), at 4±1°C in darkness for one day.

Seed treatment, storage and germination. Testas were removed from half the seeds and two lots of intact and decoated seeds were dried slowly over silica gel in 6 L desiccators to obtain 7 and 15% moisture at room temperature. The gel was replaced frequently to ensure continuous drying. The seeds were weighed at intervals of 4 or 5 h, during the drying process. When they had reached the expected weight, the seeds were removed, counted, and 100 seeds were packed in each 5 mm nylon mesh (Netlock) bag for storage experiments. Seed moisture content was determined by low constant temperature oven method for tree seeds, as described by (ISTA, 1993) and expressed on a fresh weight basis.

Table I. Differences in moisture content of *Citrus* seeds (*C. reticulata* Blanco, *C. paradisi* Macf., and *C. limon* L.) at the time of storage and harvest

Storage time (days)	Intact/ Decoated	Moisture content (% f.wt.)		
		C. paradisi Macf	*C. reticulata* Blanco	*C. limon L.*
Pre-storage	Intact	35.9±1.4	40.0±1.9	30.7±0.5
75	Intact	33.5±3.6	38.0±1.8	28.7±1.4
180	Intact	31.6±1.9	36.3±0.7	27.2±0.8
Pre-storage	Decoated	42.5±1.4	46.8±1.0	33.3±1.2
75	Decoated	39.2±3.2	41.0±0.1	31.4±3.3
180	Decoated	38.7±1.0	38.3±3.1	29.5±0.9
Pre-storage	Intact	14.9±0.3	15.1±0.1	15.1±0.1
75	Intact	14.8±0.2	14.9±0.5	14.9±2.8
180	Intact	14.7±0.3	14.3±1.9	14.7±0.8
Pre-storage	Decoated	14.8±0.1	15.0±0.1	14.9±0.1
75	Decoated	14.6±0.5	14.8±1.4	14.6±0.6
180	Decoated	14.5±0.4	14.6±0.2	14.1±0.3
Pre-storage	Intact	7.1±0.0	7.0±0.0	7.0±0.1
75	Intact	7.1±0.1	7.0±0.1	7.0±0.1
180	Intact	7.0±0.1	6.9±0.1	7.0±0.3
Pre-storage	Decoated	6.9±0.1	7.1±0.0	7.0±0.1
75	Decoated	6.9±0.3	7.0±0.1	7.0±0.4
180	Decoated	6.8±0.2	7.0±0.1	6.9±0.3

The dried and moist *Citrus* seeds, intact or decoated, were stored in different controlled atmospheres of air, 50 kPa oxygen, 5 kPa oxygen and 1 kPa carbon dioxide (hereafter gases pressure called '%`) at 4°C in the dark. Each treatment was replicated three times. The seeds were stored in sealed 10 L capacity desiccators. Storage atmospheres were maintained by purging the jars twice a week with desired gases. The flow and amount of gas was provided by a double set of precision gas mixing pumps (Wöstoff, Bochum, Germany). Every storage jar was purged for 40 min at the flow rate of 850 $mL\ min^{-1}$.

Table II. The moisture content of citrus seeds during desiccation

Time (h)	Moisture content (%)					
	C. paradisi Macf		*C. reticulate* Blanco		*C. limon*	
	Intact	Decoated	Intact	Decoated	Intact	Decoated
0	35.9	39.9	46.7	30.7	33.3	
4	33.2	40.8	37.2	44.7	28.5	29.9
8	31.1	37.9	33.4	41.8	25.3	23.5
12	29.5	26.57	31.7	33.7	23.4	18.9
16	27.0	22.3	30.0	26.9	22.7	17.9
20	26.1	21.1	26.5	19.1	19.6	15.1
25	24.2	19.4	20.5	14.9	17.3	14.8
29	20.8	17.2	18.8	11.9	15.1	9.9
33	18.5	14.8	16.4	9.6	13.9	7.9
37	17.9	13.2	15.0	8.9	12.1	7.0
41	16.5	10.4	12.9	7.1	9.4	
45	15.1	8.3	11.2	8.5		
50	14.9	7.8	8.5	7.0		
54	12.8	6.9	8.1			
58	11.6	7.0				
62	9.7					
66	8.6					
70	7.1					

Seed respiration as CO_2 released into the storage jars was monitored regularly using a gas analyser (PP, Systems EGM-1). All three species were stored together in the same jars, so our respiration data are unable to distinguish differences between species.

Germination and radicle expansion were measured after 75 and 180 days storage. Germination tests were performed in Petri dishes on Whatman No 1 filter papers, which were moistened regularly with deionized water. Percentage radicle emergence was recorded at 25°C (Edwards & Mumford, 1985) and 12 h photoperiod. Germinated seeds were counted every day for six weeks or more. The radicle length of first 20 germinated seeds was recorded 7 days after germination. Germination tests were replicated three times using 20 seeds per replicate. Analysis of variance tests were performed on angular transformed percentage germination data.

RESULTS AND DISCUSSION

Moisture contents. *Citrus* seed moisture content at pre-storage and at the time of harvests are presented in Table II. Little change in moisture content was observed over the period of 180 days storage.

Grapefruit *(C. paradisi Macf.):* The effects of moisture content and gaseous storage on grapefruit seed viability (Fig. 1). All intact and decoated grapefruit seeds germinated well initially. Moist or dried intact seeds stored under controlled atmospheres showed considerable variation in germination over the 180 days storage period. The general trend was a slow decline in viability over the 180 days storage period. The general trend was slow decline in viability over 180 days storage period. Gaseous atmosphere did not show any consistent effect on loss of viability. However, the effects of seed moisture content ($P < 0.001$), presence or absence of seed coat ($P < 0.005$) and an interaction ($P < 0.005$) were significant after both storage

Fig. 1. The viability of intact or decoated grapefruit (*C. paradisi* Macf.) seeds after storage in air, O_2 50%, O_2 5%, and CO_2 1% for up to 180 days) at different moisture contents

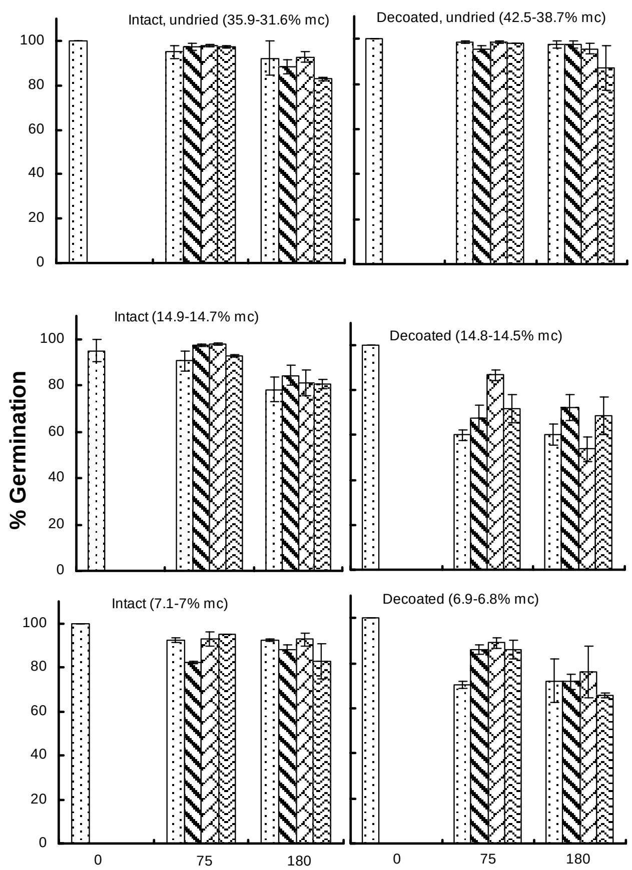
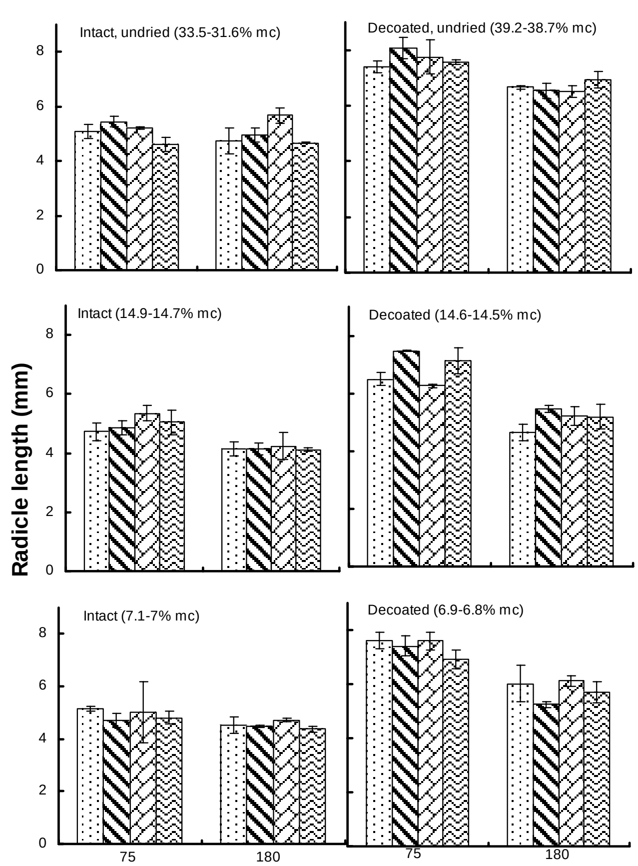


Fig. 2. The radicle length (mm), 7 days after emergence, of intact or decoated grapefruit (*C. paradisi* Macf.) seeds after storage in air, O_2 50%, O_2 5%, and CO_2 1% for up to 180 days) at different moisture contents



Storage time (days)

periods. The moist grapefruit seeds, either intact or decoated, retained better viability than dried seeds in all storage atmospheres. Loss of viability was more rapid in dried decoated seeds than intact seeds.

Marked differences in radicle length of grapefruit seeds were observed, where decoated seeds showed generally faster growth compared to intact seeds (Fig. 2). Analysis of variance showed a significant effect of seed moisture content ($P < 0.001$ and presence or absence of seed coat ($P < 0.001$) on radicle expansion. No other treatment was significant.

Rough lemon *(C. limon L.):* The results for rough lemon were very similar to those for grapefruit (Fig. 3 & 4). Analysis of variance indicated significant effects of presence or absence of seed coat ($P < 0.005$), moisture content ($P < 0.001$) and their interaction on seed longevity over both periods of storage. There was again no consistent effect of gaseous atmosphere. There was no significant loss of viability in moist intact or decoated seeds, but loss of viability was pronounced when seeds were dried to 15 or 7% moisture content, particularly when decoated.

Moisture ($P < 0.001$) and presence or absence of seed coat ($P < 0.001$) had significant effects on radicle length after 75 days of storage. After 180 days storage there was a significant effect of the presence or absence of seed coat ($P < 0.001$), whereas all other treatments had no significant effect on radicle growth. Gaseous atmosphere had no effect on seed radicle expansion after either storage period. Decoated seeds again showed more rapid radicle elongation than intact seeds (Fig. 4).

Kinnow mandarin *(C. reticulata Blanco):* The results for kinnow mandarin germination are shown in Fig. 5. There was a very marked decline in final percentage germination between harvests. After 75 days storage there was a

Fig. 3. The viability of intact or decoated rough lemon (*C. limon* L.) seeds after storage after storage in air, O_2 50%, O_2 5%,and CO_2 1% for up to 180 days) at different moisture contents

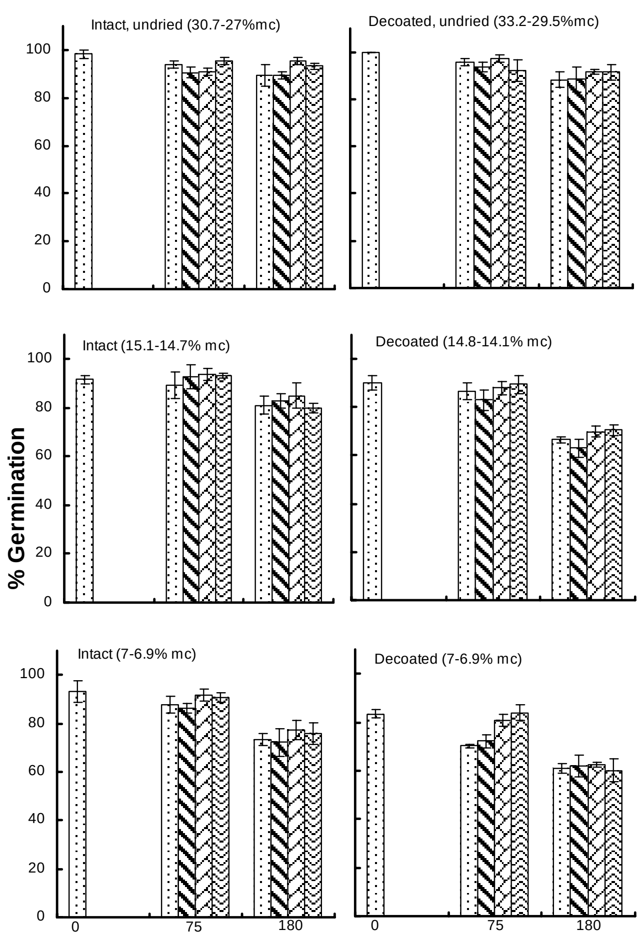
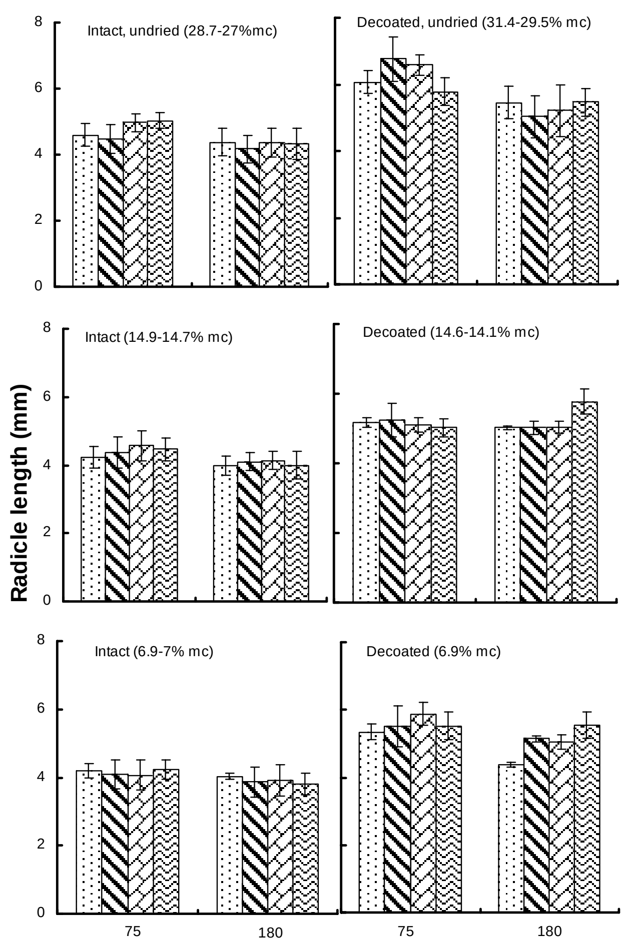


Fig. 4. The radicle length (mm), 7 days after emergence, of intact or decoated rough lemon (*C. limon* L.) seeds after storage in air, O_2 50%, O_2 5%,and CO_2 1% for up to 180 days) at different moisture contents



Storage time (days)

significant effect of moisture and presence or absence of seed coat (both $P < 0.001$) on seed germination capacity. As a single treatment gaseous atmosphere showed no significant effect whereas several treatment interactions like gaseous atmosphere and presence or absence of coat ($P < 0.005$), presence or absence of seed coat and moisture ($P < 0.001$), and gaseous atmosphere and presence or absence of coat and moisture ($P < 0.05$) were significant . Reduction in kinnow mandarin seed viability continued progressively over 180 days storage. After 180 days storage, all three major treatments, gaseous atmosphere ($P < 0.05$), presence or absence of coat ($P < 0.001$), and moisture content ($P < 0.001$) showed significant effects on seed viability. Some treatment interactions were also significant.

Reduction in seed moisture content to 15 and 7% severely reduced longevity in both intact or decoated seed material. However, this loss of viability was significantly greater in decoated seeds, where no dried seeds survived 180 days storage Intact or decoated moist seeds stored under different gaseous atmosphere showed no significant decline in viability. Although the effect of gaseous atmosphere, alone or in combination, was sometimes significant, no consistent effect on seed viability could be discussed.

Moist seeds, intact or decoated were capable of better extension in radicle growth than dried seed material (Fig. 6). After 75 days storage seed moisture content was the only significant main effect ($P < 0.001$). Radicle expansion after 180 days storage was significantly reduced and analysis of variance showed a significant effect of all major treatments although the effect of gaseous atmosphere again showed no consistent trend. Many confidence intervals were rather large owing to small sample size.

Effect of seed moisture and controlled atmosphere on respiration. Seed respiration was measured at intervals over

Fig. 5. The viability of intact or decoated kinnow mandarin (*C. reticulata* Blanco) seeds after storage in air, O_2 50%, O_2 5%, and CO_2 1% for up to 180 days) at different moisture contents

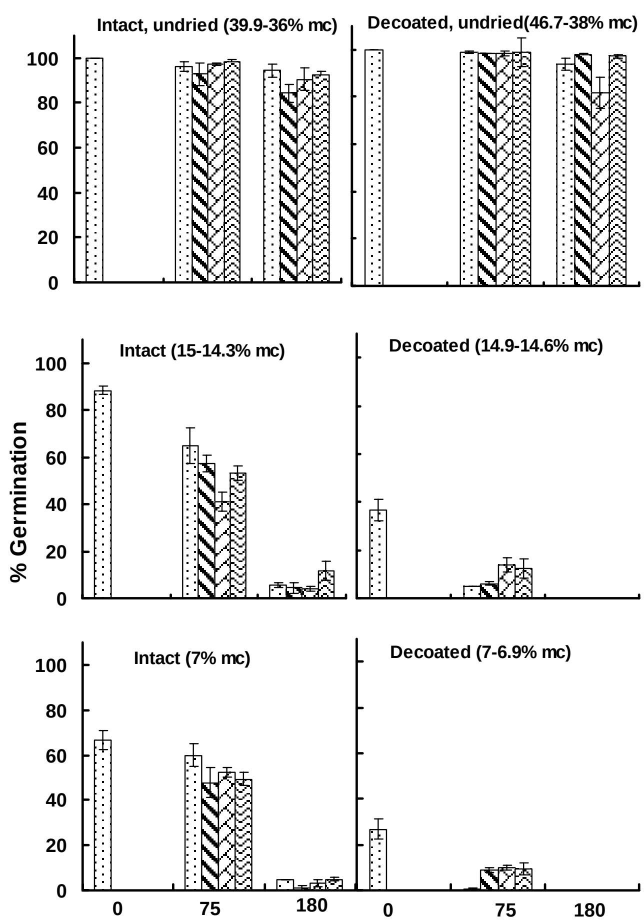
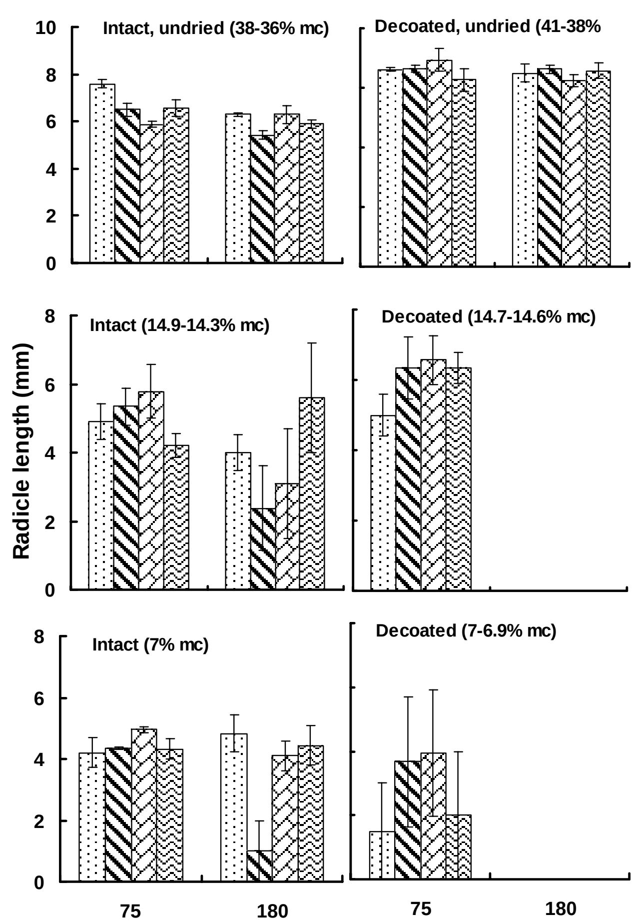


Fig. 6. The radicle length (mm), 7 days after emergence, of intact or decoated kinnow mandarin (*C. reticulata* Blanco) seeds after storage in air, O_2 50%, O_2 5%, and CO_2 1% for up to 180 days) at different moisture contents



Storage time (days)