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RESPIRATION AND SEEDLING VIGOUR OF *BRASSICA OLERACEA* UNDER NATURAL AND ARTIFICIAL AGEING CONDITIONS

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Respiration and seedling vigour of naturally and artificially aged seeds of *Brassica oleracea* were studied to elucidate the nature of deteriorative changes under these two types of conditions. Results indicate that the ageing conditions had a variable effect on oxygen uptake and seedling vigour and metabolic changes associated with artificial ageing may differ from those during natural ageing.

Key words : Respiration, seedling vigour, *Brassica oleracea*, ageing

The respiratory characteristics of stored seeds have received particular attention from investigators. Oxygen uptake of seeds have been found to have a bearing on the inherent storability characteristics of species (Vertucci and Leopold, 1987). Low rates of O_2 uptake have been observed during imbibition of aged seeds. This has usually been ascribed to mitochondrial deterioration.

Studies on seed ageing have often used techniques to accelerate the ageing process, thereby shortening the time necessary for study. Exposure to high temperature and high moisture conditions (typically 40-50°C and 100% RH) is a standard method used for accelerated ageing of seeds in the laboratory (Powell and Mathews, 1977). The effect of artificial ageing conditions of high temperature and low humidity on seed processes has not been studied. Similar conditions could be encountered in some situations in the drying room or in the seed store when power fails. Of particular interest are the differences between accelerated ageing at high temperature and high relative humidity and deterioration under less stressful conditions.

This investigation was conducted to evaluate changes in respiration and seedling vigour of *Brassica oleracea* seeds during deterioration under (1) controlled conditions of storage at –18°C and 6 per cent seed moisture content and (2) artificial ageing conditions of high temperature, high relative humidity and high temperature, low relative humidity.

MATERIALS AND METHODS

Seeds of two lots of *B. oleracea* var. capitata cv. Early Round Dutch, stored for different durations under controlled conditions of –18°C were obtained from the seed bank at National Seed Storage Laboratory, Fort Collins, Colorado. Lot 1 was stored since 1961 and lot 2 was stored under same conditions since 1981. Germination test showed 36% and 92% viability respectively. These were considered naturally aged seeds. Another seed lot stored at 10°C for five months was used to get artificially aged material. The germination value of this lot was 93 percent. The experiment was started in Dec. 1989. Seeds were aged under two different environments. One lot was held at 45°C and near 100 per cent relative humidity (RH) for 3 days. Another lot was placed over concentrated sulphuric acid in an airtight container at 35°C for 12 weeks. These two treatments provided seeds aged under high humidity and desiccating conditions respectively. Oxygen uptake as a function of seed moisture was measured manometrically at 25°C in dark using Gilson differential respirometer. 1.5g seed weight was used per sample flask. The well contained 0.1 ml of 60% KOH solution. Different seed moisture contents were obtained by equilibrating seeds over saturated salt solutions, sulphuric acid solution or by adding calculated amounts of water in a sealed petridish and equilibriating the seeds overnight at room temperature. Moisture contents are expressed on a dry weight basis. The dry weights were determined immediately after O_2 uptake experiment by drying seeds at 95°C in a forced draught oven for 5 days. Measurement at each moisture content were replicated three times.

Standard germination test was conducted for all four seed lots. Four replicates of 25 seeds each were germinated at 25°C. Radicle lengths were measured after 72 hrs. Germination Index (GI) was calculated as a product of radicle length and germination percentage.

The rate of leakage of electrolytes from seeds within first hour of soaking was measured using an ASAC-100 conductivity meter. Three replicates of 5 seeds each were used. Seeds were prehumidified overnight at saturating humidity to avoid possible leakage due to imbibitional damage.

RESULTS AND DISCUSSION

Ageing conditions had a variable effect on the seedling vigour expressed as germination index. When seeds were accelerated aged at 45°C and near 100 per cent relative humidity for 3 days seedling vigour declined drastically as in the 29 years old "Naturally" aged seed (Table 1). In comparison the decline in GI was not so drastic when the same seed was artificially aged over H_2SO_4, (1%RH) at 35°C for 12 weeks. The rate of leakage of electrolytes was 1.5 to 3.0 fold higher in the artificially aged seed lots (Table 1). Rate of leakage was more in the lot aged at 45°C and 100% RH which is a more stressful treatment. These results show deteriorative changes in the membrane integrity as measured by conductivity in response to artificial ageing treatments and that the damage to membrane integrity is greater as a result of artificial ageing treatment than due to "natural" or slow ageing of seeds. Therefore, impaired membrane semipermeability is suggested as a critical lesion caused by artificial ageing in seeds both under high humidity and desiccating conditions. Increase in solute leakage from seeds after accelerated ageing (45°C, 100% RH) were also documented in soybean. The amount of leachate collected was positively correlated with the degree of accelerated ageing. Solute leakage is notably increased even before reduced performance is detectable (Powell and Mathews, 1977). A similar trend was observed in seed aged over H_2SO_4 at 35°C, where the decline in GI was not concomitant to increase in conductivity indicating that leachate conductivity is not closely correlated with seedling vigour (Table 1).

Table 1. Germination index and rate of leakage of electrolytes in seed lots stored/aged in different environments.

Storage/ageing environment	Germination Index (Radicle length × Percent germination)	SE	Rate of leakage (µmhos $min^{-1} g^{-1}$)	SE
"Naturally" aged Seed: Stored at –18°C, 6% MC				
9 year old lot	38.54	1.53	463.89	11.72
29 year old lot	1.52	0.18	753.16	17.62
Artificially aged seed :				
45°C, 100% RH, 3 days	3.36	0.73	1471.12	563.53
35°C, 1% RH, 12 weeks	29.18	2.03	1113.25	226.83

These observations support previous suggesting that membrane dusruption is one of the factors responsible for drying damage (Herter and Burris, 1989). Studies have also shown that majority of cellular membranes remain physically intact following a lethal desiccation stress but their biophysical and biochemical properties are dramatically altered (Mckersie *et al.*, 1988). This might explain the increased rate of leakage of electrolytes in seeds aged over H_2SO_4 with no concomitant change in the GI.

Seed deterioration is usually associated with one or more aspect of respiratory metabolism. Respiratory oxygen uptake as a fuction of seed moisture content was observed in naturally and artificially aged seeds at 25°C in dark. At specific moisture contents ranging from 0.10 to 0.20g H_2O /g dry weight, oxygen uptake increases sharply with increase in moisture contents (Fig. 1). Below these threshold moisture levels the O_2 consumption was lower. Oxygen uptake at low moisture contents (i.e. < 0.10 to 0.15 g/g dw) is higher in the artificially aged seeds, more so in the seeds aged at 35°C over H_2SO_4. At high MC (> 0.10 to 0.20 g/g dw) the trend is reversed i.e. uptake at higher MC is lower in the accelerated aged seeds than in the naturally aged (9 year old) seeds, however O_2 uptake was slowest in the 29 years old lot reflecting the high degree of deterioration.

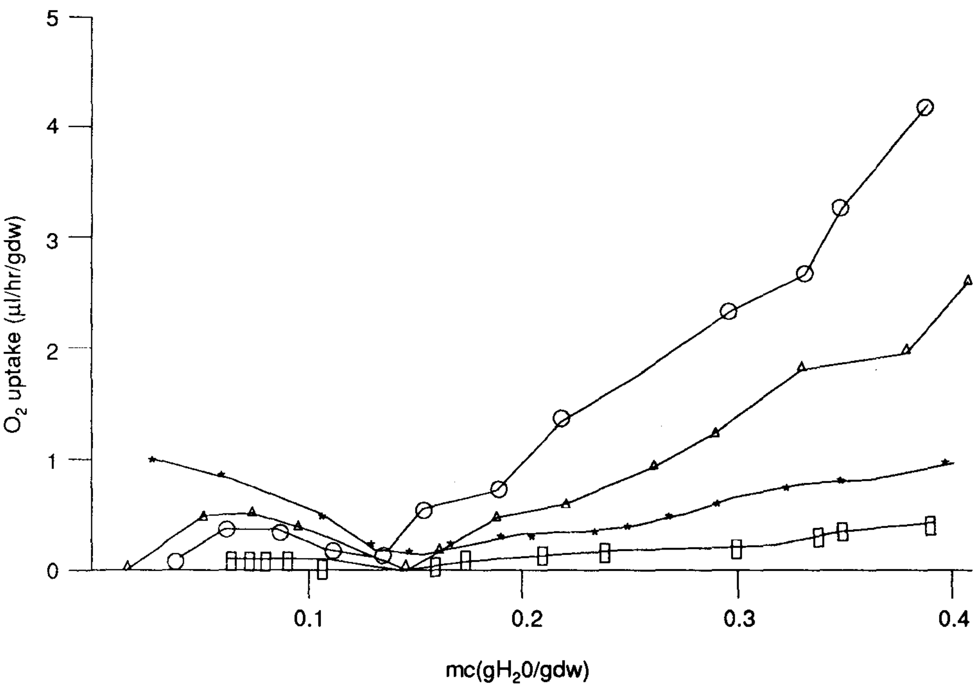

Fig. 1. The relationship between moisture content and oxygen uptake for the 29 year old "naturally aged" seed (□), 9 year old "naturally aged" seed (o), Artificially aged, 35°C, over H_2SO_4, seed (x) and artificially aged 45°C, 100% RH seed (Δ). Each point represents the mean of four replicates.

These trends suggest that oxidative reactions at low moisture level are predominant in the artificially aged seeds whereas the respiratory O_2 uptake at high moisture gets depressed. The decline in mitochondrial function begins early during seed deterioration and apparently can progress significantly before changes in whole seed vigour are observed. Correlation between respiration and seedling vigour are therefore not universal. The nature of oxidative reactions responsible for the slow rates of O_2 uptake at low water contents is not clear but it is suggested that oxidative reaction at these low water contents have deleterious effects on seed viability (Ibrahim and Roberts 1983). The increased O_2 uptake at low water contents in artificially aged seeds indicates enhanced level of these oxidative reactions in response to the stressful conditions prevailing during the ageing treatment.

Among the accelerated ageing treatments, seeds aged at high humidity showed higher respiratory oxygen uptake than the seeds aged at low humidity (Fig. 1).

Studies have shown that the mechanism of ageing change with hydration level and that in very dry seeds autooxidations are largely responsible for ageing (Wilson and McDonald, 1986; Flood and Sinclair, 1981). Artificial ageing techniques introduce damage processes not present in naturally ageing seeds and/or alter the relative rates of damage processes responsible for ageing. Reports on whether the mechanism of natural and artificial ageing are similar or not are conflicting (Mathews, 1987; Priestley and Leopold, 1983). The results presented here, do however, support the reservation of Priestley and Leopold (1983) and Petruzzelli and Taranto (1984) that the metabolic changes associated with artificial ageing may differ from those during natural ageing.

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