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Relationship between the ATP content measured at three imbibition times and germination of onion seeds during storage at 3, 15 and 30 °C

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Abstract

The possibility of using ATP content as an indicator of seed quality was studied in onion seeds (*Allium cepa* cv. Wädenswil). The percentage germination and ATP content of imbibed seeds were compared during 145 weeks of storage at three temperatures (3, 15 and 30 °C). ATP content, which was undetectable in air-dried seeds (moisture content: 9%, w/w), increased rapidly as a function of imbibition time, as did the fresh weight and respiration rate, reaching a steady-state level after about 17 h. After 36 weeks of storage, the rate of ATP formation was greater for the seeds stored at 3 °C than for those kept at 15 and 30 °C. Furthermore, the onset of ATP synthesis was delayed. These phenomena, which are likely to be an expression of seed ageing, are useful indicators, allowing the prediction of the loss of seed viability before the decrease in percentage germination which occurred beyond 36 weeks of storage. In addition, the correlation between ATP content and germination capacity of seeds during 145 weeks of storage was excellent ($r = 0.95$ at 15 °C and 0.97 at 30 °C), provided that a 17 h-imbibition time, specific for onion seeds, was chosen. These results are discussed in terms of the controversy concerning the correlation between the ATP content and germination percentage of seeds.

Key words: *Allium cepa*, ageing, bioenergetic metabolism, seed quality, temperature storage.

Introduction

The quality of a seed is generally estimated by its capacity to germinate. Although the germination method is simple, it is often tedious and provides results only after one or several weeks, depending on the seed species. Thus, several authors have attempted to find a biochemical parameter which could express seed viability more rapidly.

It is well established that seed germination is accompanied by important energy-requiring processes. Therefore, it is expected that adenosine nucleotides, namely ATP, may reflect the capacity of the seed to germinate. This approach has been explored by several investigators, but so far the findings are controversial.

Earlier results by Ching (1973) showed that ageing of several seed species (*Trifolium incarnatum*, *Lolium multiflorum* L. and *Brassica napus* L.) causes a reduced capacity to germinate which is correlated with a decrease in ATP content. Both phenomena are accelerated when the temperature of seed storage is increased. These results have been extended by Lunn and Madsen (1981) who demonstrated, in several *Brassica* species and *Beta vulgaris* seeds, a linear correlation between ATP levels after 7 h of imbibition and the dry matter produced during a 30 d period. Moreover, these authors observed that, during artificial ageing, seed deterioration was reflected in the ATP levels long before loss of viability, as determined by percentage germination throughout the ageing. These results have been confirmed by Zungsontiporn *et al.* (1989) who showed good correlation between ATP content and percentage germination during imbibition and storage of *Echinochloa crus-galli* seeds.

In contrast to the above results, Perl's group (Perl, 1980, 1986, 1987; Mazor *et al.*, 1984) and Styer *et al.* (1980) showed that seed ATP content is not correlated with percentage seed germination or vigour in any of the 12 species studied. These authors point to the conclusion that the amount of ATP measured in seeds is the result

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of the balance between processes of synthesis and utilization. Hence, large amounts of accumulated ATP may be the result of either active ATP synthesis (probably reflecting high-vigour seeds), or impaired ATP-utilizing activities (indicating defective, low-vigour seeds). Thus, in the author's opinion, no clear correlation is to be expected (Perl, 1986).

In the present investigation, we compared percentage germination and ATP content during imbibition of onion seeds throughout 145 weeks of storage (under non-artificial ageing conditions) at three different temperatures (3, 15 and 30 °C) and at 9% seed moisture content. The results reveal quite clearly that, under all conditions tested, a high correlation exists between these two parameters. Some of these results have been presented elsewhere (Douet-Orhant and Siegenthaler, 1991).

Materials and methods

Plant material

Onion seeds (*Allium cepa* cv. Wädenswil), obtained in 1989 from ASPI (Association suisse pour la promotion des semences potagères indigènes), were stored at three different temperatures (3, 15 and 30 °C) for 145 weeks in closed vessels. Their initial water content was measured after drying the samples for 1 h at 130 °C and found to be about 9% (w/w). The moisture content remained constant in these air-dried seeds during the storage period.

Seed germination

Samples of 200 seeds were germinated in distilled water on blotting paper at 20 °C for 12 d. The germination percentage was determined by counting normal and abnormal seedlings as well as dead seeds according to the ISTA rules (ISTA, 1985). Seedlings classified as normal must be able to produce normally developed plants and only those were included in the calculation of germination capacity, because all other categories were without interest for agriculture (Lunn and Madsen, 1981).

Preparation of seed extracts

Samples of 50 seeds were weighed (fresh weight), imbibed on filter papers wetted with distilled water at about 18 °C, then weighed again to obtain the relative fresh weight increase. The procedure for preparing seed extracts was adapted from that described for potato tubers (Siegenthaler and Biotto, 1994). Seeds were ground at full speed for 1 min with a polytron type 87 PTA 105 Kinematica (Lucerne, Switzerland) in a solution containing 0.6 N sulphuric acid. The extract was filtered (Schleicher and Schüll 287, Feldbach, Switzerland). Then, 1 ml of the filtrate was mixed with 19 ml of a neutralization solution containing 3.2 mM EDTA-Na_2 (ethylenediaminetetraacetate-Na_2), 3.5 mM $MgCl_2$, 52.6 mM MOPS (3-[*N*-morpholino]propanesulphonic acid) adjusted to pH 9 with ethanolamine. The final pH of the extract was 7.3 ± 0.1.

Determination of ATP content

The content of ATP was measured by bioluminescence: 1 ml of seed extract was mixed in the cuvette of the Skan XP-2000 luminometer with 200 µl firefly lantern extract (FLE 250 from Sigma). After the addition of FLE, the light signal was integrated for 10 s by the luminometer and compared with standards of ATP (0 to 75 nM). All the above operations, including the grinding step, could be carried out with the automatic apparatus (prototype NIVAC 260, Nivarox-Far, Le Locle, Switzerland). Each preparation and ATP measurement was performed in triplicate. Measurements of ATP were made after 4, 7 and 17 h of seed imbibition.

Measurement of seed respiration

Oxygen uptake of onion seeds was measured polarographically with a Clark electrode in a 2 ml, water-thermostatted glass reaction cell maintained at 25 °C. After various times of imbibition, the intact seeds (50 per each measurement) were placed in the reaction cell containing 1% glucose in 0.03 M potassium phosphate buffer (pH 5.5) as described by Moreland *et al.* (1974). Oxygen consumption was linear with time until anaerobiosis occurred. In Neuchâtel, oxygen content of the air-saturated medium was 221 µM at 25 °C.

Statistical analyses

Analysis of the results was carried out with the Excel program, and the curve fitting was performed with the Polarographic program.

Results

Figure 1 shows that before imbibition of fresh seeds (e.g. at the beginning of storage) there was neither ATP present nor respiration. However, the ATP level increased rapidly during the first hours of imbibition (up to about 8 h), then slowly up to 24 h. The patterns of respiration rate and of relative fresh weight were similar. However, the extent of the increase of these two parameters was much smaller than that of the ATP level. Figure 2 illustrates the situation after 36 weeks of storage. When the seeds were imbibed at 18 °C, the onset of ATP production

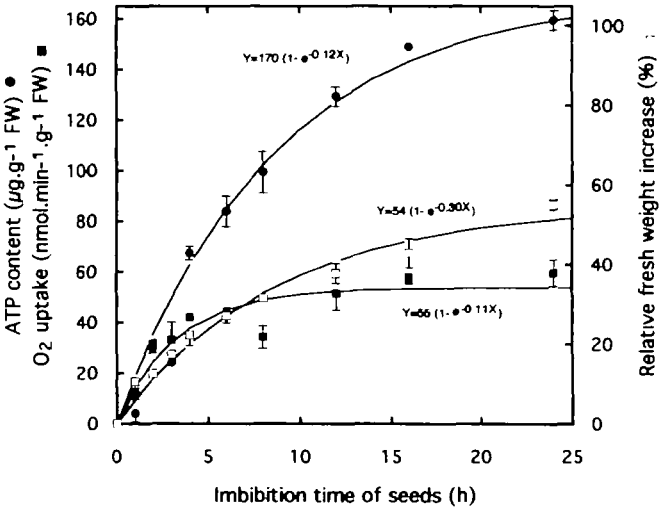

Fig. 1. ATP content, O_2 uptake and relative fresh weight increase of onion seeds as a function of imbibition time at 18 °C These measurements were made at the beginning of the storage period. The weight of 50 seeds, before imbibition, was 155 ± 10 mg. The results are presented as means ± standard deviation of triplicate samples. In certain cases, the standard deviation is included within the symbols

appeared first after 2 h, then after 3 and 4 h for the seeds which were stored at 3, 15 and 30 °C, respectively. Although the patterns of ATP increase were similar for all three samples, the rate of synthesis was correspondingly as high as the storage temperature was low. However, after 20 h of imbibition, the ATP content of all three seed samples was the same, as attested by the standard deviation values shown in Fig. 2, and remained constant to the end of the 25 h experimental period (see results and equations of the curves in Fig. 2).

The effect of ageing on onion seed germination at three different temperatures can be seen in Fig. 3. At the beginning of the experiment, the germination capacity, which is considered to be an indicator of ageing, was about 90%. When the seeds were stored at 3 °C under the conditions described in Materials and methods, this high percentage remained constant for 145 weeks. Raising the temperature of storage to 15 and 30 °C resulted in a diminution of the germination capacity which started after 45 (Fig. 3B, E, H) and 35 (Fig. 3C, F, I) weeks of storage, respectively. In both cases, the incapacity to germinate, occurred after about 105 and 95 weeks, respectively.

For the seeds stored at 3 °C (Fig. 3A, D, G), the level of ATP, as well as the germination percentage, remained constant for 145 weeks, regardless of the imbibition time. However, the ATP values were less reliable after 4 and 7 h hydration.

In contrast, when the seeds were stored at higher temperatures (15 and 30 °C), the ATP level decreased (Fig. 3B, C; E, F; H, I). In 17 h-imbibed seeds, the ATP content began to diminish after 45 weeks of storage at 15 °C (Fig. 3H). After 110 weeks, the seeds died and the level of ATP was zero (Fig. 3H). This pattern was very similar to that of germination capacity. When seeds stored at 30 °C were imbibed for 17 h (Fig. 3I), the level of ATP started to decrease after 35 weeks as did the germination capacity. However, the decrease was slow and, even after 145 weeks, about 25% of the initial ATP content was present in the seeds (Fig. 3I). In the seeds which were imbibed for shorter times (7 and 4 h), the ATP level started to decrease earlier and the rates of decrease were much faster than for the 17 h-imbibed seeds (Fig. 3B, C; E, F). Raising the storage temperature from 15 to 30 °C accelerated both changes.

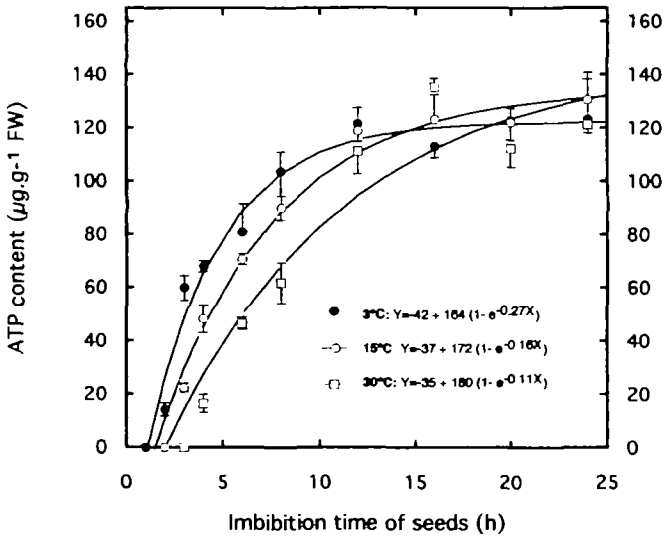

Fig. 2. ATP content of onion seeds as a function of imbibition time at 18 °C. Seeds were stored for 36 weeks at 3, 15 and 30 °C, respectively The results are presented as means ± standard deviation of triplicate samples.

Additional data concerning the germination of abnormal seedlings are illustrated in Fig. 4. At 3 °C, the percentage of abnormal seedlings (11%) remained constant during the whole of the storage period. At 15 °C, it increased from 50 to 100 weeks. During this storage period, the percentage germination capacity decreased drastically as shown in Fig. 3H, whereas the percentage of dead seeds, obtained by subtraction, increased progressively. Beyond 100 weeks of storage at 15 °C, the percentage of abnormal seedlings decreased down to zero which corresponded to the death of all seeds. As seen in Fig. 4, the changes in the percentage of abnormal seedlings occurring at 30 °C were similar to those observed at 15 °C, except that their onset began earlier (at about 30 weeks). Moreover, although the percentage of abnormal seedlings remained rather low during storage, it stayed at about 10% even after 145 weeks of storage.

The correlation between ATP content and percentage germination of onion seeds is shown in Fig. 5, under the conditions considered to be optimal, e.g. after 17 h of imbibition. At 3 °C, all the data, representing the germination percentage as a function of ATP content, appeared as a cluster of points centred at 140 μg ATP g^{-1} FW and 90% of germination (results not shown). The two parameters were linearly correlated when the results obtained at 15 and 30 °C were presented simultaneously. When the germination percentage of normal seedlings was considered (Fig. 5A) the correlation coefficient was 0.95 compared to 0.92 for normal plus abnormal seedlings (Fig. 5B). Although the correlation was very good in both cases, the line intercepted the abscissa closer to the origin in normal plus abnormal, than in normal seedlings. When each fitting function was calculated for the two storage temperatures (15 °C: $y=0.67x+9$; 30 °C: $y=0.97x-28$) a deviation occurred especially at low ATP contents (Fig. 5B). This was probably due to errors made in ATP as well as in germination percentage determinations, especially at the lowest values.

Discussion

It is known that, during prolonged storage periods, most seeds deteriorate and lose their germinability and vigour.

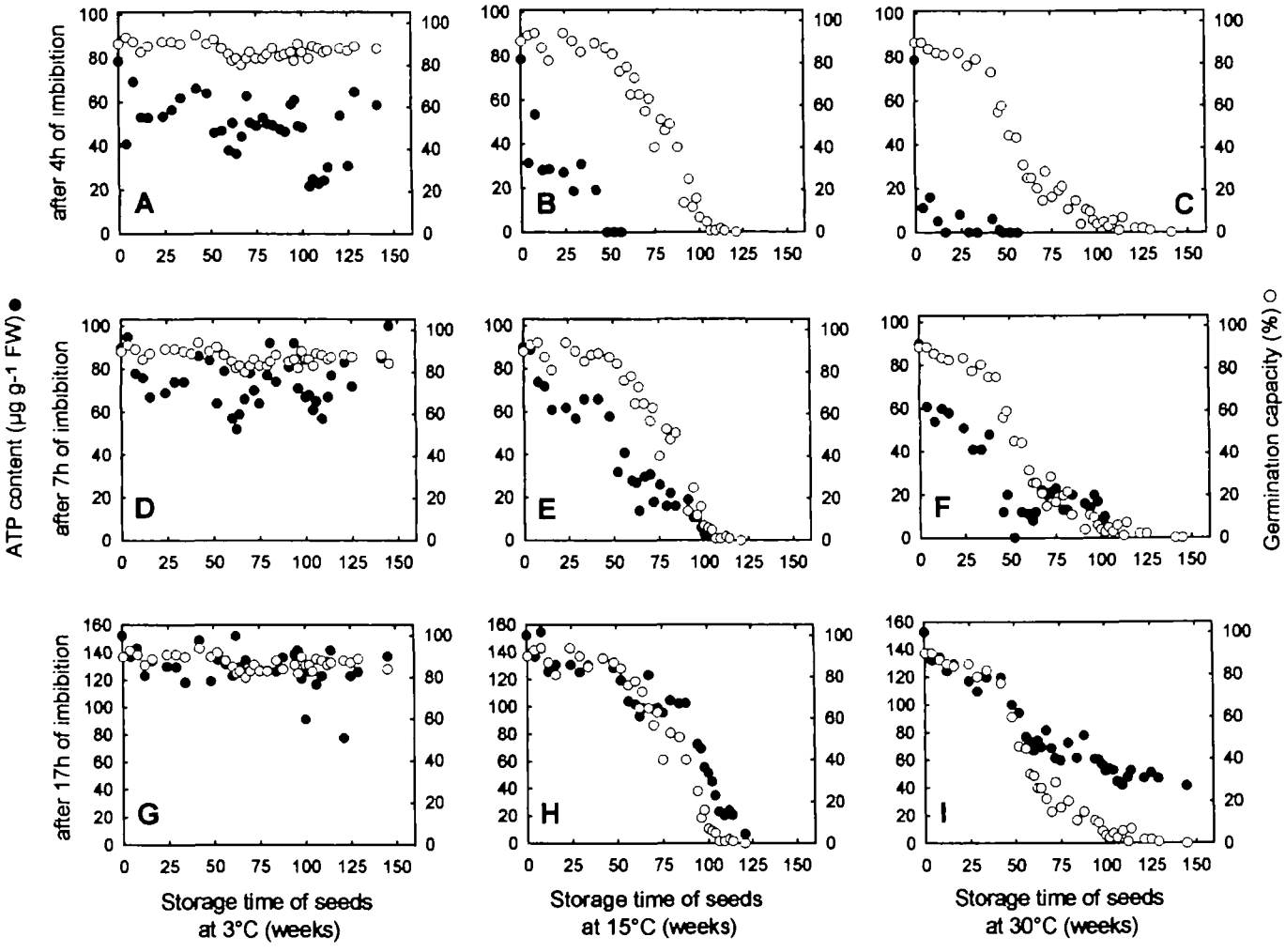

Fig. 3. Changes in ATP content and germination capacity during storage at 3 °C (A, D, G), 15 °C (B, E, H) and 30 °C (C, F, I). For each storage time, 50 onion seeds were imbibed at 18 °C for 4 h (A, B, C), 7 h (D, E, F) and 17 h (G, H, I).

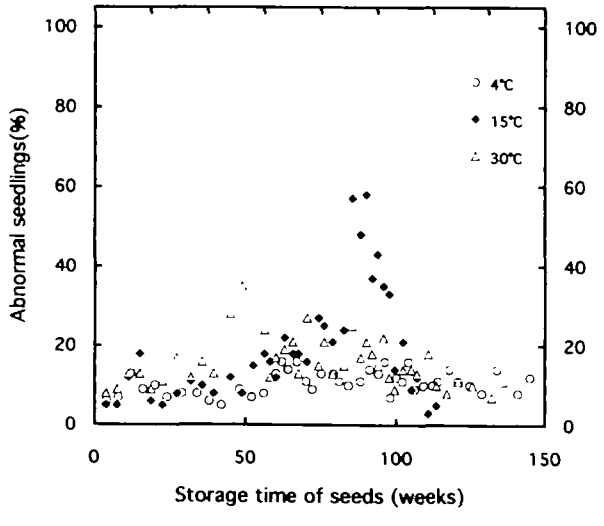

Fig. 4. Changes in percentage of abnormal seedlings during storage at 3, 15 and 30 °C. Conditions are described in Materials and methods.

This has great economical consequences. Therefore, several investigators have attempted to explain how environmental factors (temperature, relative humidity, etc.) and internal parameters (accumulation of toxic compounds, degradation of various biomolecules, etc.) may influence seed ageing (Ellis and Roberts, 1981; Powell, 1988; Gidrol *et al.*, 1989, 1990; Ellis, 1991). The improved viability equation was proposed by Ellis and Roberts (1980) to quantify the relationship between seed longevity and storage environment in order to predict the viability of seed lots under a wide range of storage conditions.

In most studies, the so-called accelerated ageing model, in which seed storage is carried out under wet and hot conditions, has been adopted (Styer *et al.*, 1980; Ellis and Roberts, 1981; Lunn and Madsen, 1981; Gidrol *et al.*, 1988, 1989, 1990). However, in this experimental system, drastic conditions of temperature and relative humidity may have induced accelerated biochemical degradations which are undesirable and irreversible and which do not mimic the conditions under which seeds are generally

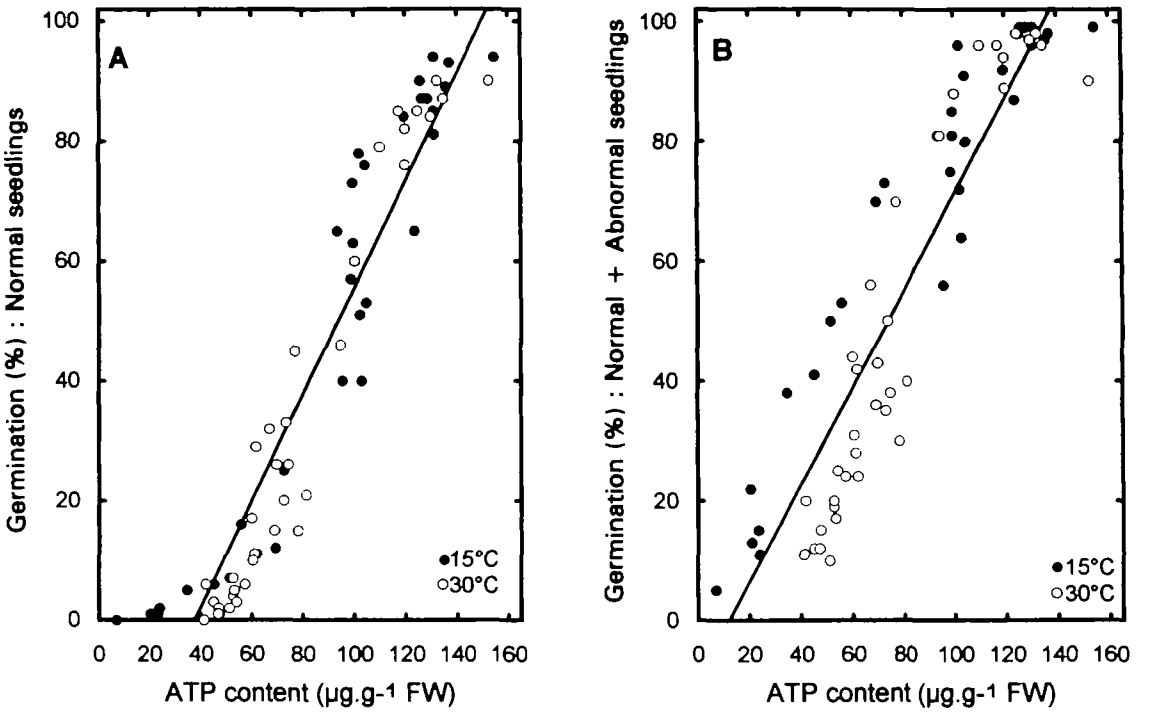

Fig. 5. Correlation between the percentage of germination of normal (A) and of normal plus abnormal (B) seedlings and the ATP content of onion seeds stored at 15 and 30 °C. Seeds were imbibed at 18 °C for 17 h, and tested for ATP level and germination percentage. Correlation coefficients (r) were: 0.95 in (A) and 0.92 in (B).

stored. In this investigation, air-dried seeds have been used (9% of moisture content) and three different storage temperatures (3, 15 and 30 °C). Under our experimental conditions, an excellent correlation was found between ATP content after 17 h of imbibition and percentage seed germination determined by 76 measurements made over a period of 145 weeks (Fig. 5).

In air-dried seeds, ATP content was undetectable. Upon imbibition, not only ATP content, but respiration rate and relative fresh weight increased progressively to a steady-state level (Fig. 1). Although these patterns are in agreement with previous reports, it is obvious that the time period to reach the plateau may be different depending on the species considered (Moreland *et al.*, 1974; Lunn and Madsen, 1981; Zungsontiporn *et al.*, 1989). The increase in ATP level in seeds during the early stages of germination (e.g. imbibition) is considered by several authors to be due mainly to the onset of respiration and its concomitant oxidative phosphorylation as soon as seeds are hydrated (Raymond *et al.*, 1983; Attucci *et al.*, 1991).

After 36 weeks of storage, the germination percentages of seeds stored at 3, 15 and 30 °C were almost identical to their initial value and, therefore, did not reveal any signs of ageing. However, the onset of ATP formation in seeds was delayed and its rate during the early stage of imbibition was diminished progressively when the storage temperature was raised (Fig. 2). Although no quantified data are available, we observed a delay of radicle emergence and seedling growth which was as long as the storage temperature was high. It is well established that the mean germination time increases within a seed population as ageing proceeds (Guy, 1982; Dell'Aquila, 1987). This was the case when the storage temperature was raised from 3 °C to 15 °C or 30 °C. Interestingly, Moreland's group has studied the variation of ATP content over a longer period of imbibition than here and found that the beginning of radicle emergence coincided with a further increase in ATP content (Moreland *et al.*, 1974). Altogether, these results indicate that the kinetics of ATP synthesis during imbibition might be a good indicator of the loss of viability of seeds beyond 36 weeks of storage. This hypothesis was further verified by the observation that seeds stored at 30 °C lost their ATP content and germination capacity more rapidly than those stored at 15 °C, whereas those maintained at 3 °C remained intact (Fig. 3). This was true not only for 17 h- (Fig. 3G, H, I) but also for 7 h- (Fig. 3D, E, F) and 4 h- (Fig. 3A, B, C) imbibed seeds. Another possibility of anticipating seed ageing is based on the observation that the extent of decrease in ATP level was much greater in seeds imbibed for 4 h and 7 h than in those imbibed for 17 h. In the two former cases (Fig. 3B, C; E, F), the onset of the decrease in ATP level occurred much earlier than that of the percentage in germination capacity. Altogether, these observations indicate that one or several

enzyme systems involved in the bioenergetic pathways, namely in oxidative phosphorylation, are likely to be better preserved at low (3 °C) than at higher storage temperatures (15 and 30 °C).

It is noteworthy that in 17 h-imbibed seeds, the diminution of both germination capacity and ATP level was simultaneous for storage at 15 °C (Fig. 3H), whereas at 30 °C, the decrease of these two parameters was not concomitant after 60 weeks of storage, the level of ATP always remaining greater than 40 $\mu g\ g^{-1}$ FW (Fig. 3I). This difference can be explained by the presence of abnormal seedlings in the samples stored at 30 °C whereas at 15 °C, all the seeds were dead at the end of storage (Fig. 4).

In view of the controversy about the correlation existing between ATP content and seed germination capacity (see Introduction), the results shown in Fig. 3 illustrate quite clearly that the time of imbibition at which such a correlation can be established is critical. Table 1 shows that for onion seeds, this correlation was excellent only in seeds imbibed for 17 h. These results may explain why Styer *et al.* (1980) claimed that, in 4 h-imbibed onion seeds, there was no correlation between ATP content and germination capacity. This assertion is certainly true for 4 h- but not for 17 h-imbibed seeds.

The correlation between the ATP level and percentage germination of normal (Fig. 5A) and normal plus abnormal (Fig. 5B) seedlings was excellent. Although the correlation was very good in both cases, the straight line expressing the correlation did not intercept the origin. When the germination capacity was zero (Fig. 5A), the amount of ATP was 38 $\mu g\ g^{-1}$ FW. This may be explained by the fact that a seed developing an abnormal seedling contains a certain amount of ATP after 17 h of imbibition, probably as much as a seed giving a normal seedling. However, when all the seeds were dead, the percentage of germination of normal plus abnormal seedlings was zero (Fig. 5B) and the amount of ATP was still 12 $\mu g\ g^{-1}$ fresh weight, indicating that, though seeds were unable to germinate, synthesis of ATP occurred during imbibition. Thus, the contribution of dead seeds in ATP was close to zero.

In conclusion, these results show that in onion seeds, there is an excellent correlation between ATP content and germination capacity, provided that a representative imbibition time (here 17 h), specific for each species, is chosen. In our opinion, however, any correlation between ATP content at an early stage of seed imbibition and its vigour has to be considered with extreme caution, because ATP level does not necessarily represent the ATP in seeds which, upon planting, will result in a rapid and uniform production of healthy seedlings.

Table 1. *Correlation coefficients between germination capacity and ATP content of 4-, 7- and 17 h-imbibed onion seeds stored at 15 and 30 °C*

Imbibition time (h)	Correlation coefficients in seeds stored at			
	15 °C	d.f.[a]	30 °C	d.f.[a]
5	0.51	10	0.42	11
7	0 83	26	0.81	27
17	0.95	31	0 97	35

[a] d.f.: degree of freedom.

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