Bot. Bull. Acad. Sin. (1996) **37**(4)**:** 229-237

Content of adenylate nucleotides and energy charge in the early stage of germination of orthodox and recalcitrant seeds

Chih-Chang Chao and Tsan-Piao Lin[1]

Tree Seed Laboratory, Taiwan Forestry Research Institute, 53 Nan-Hai Road, Taipei, Taiwan, Republic of China

(Received April 1, 1996; Accepted July 9, 1996)

Abstract. The content of adenosine phosphates and the energy charges of three species of orthodox seeds, i.e. *Phellodendron wilsonii*, *Michelia compressa,* and soybean; three species of recalcitrant seeds, i.e. *Podocarpus macrophyllus*, *Cyclobalanopsis globosa,* and *Machilus thunbergii*; one species of intermediate category, i.e. *Neolitsea parvigemma* at the early stage of germination were analysed to compare the energy concept established for crop seeds. AMP was the major adenylate in the dry state, and the ATP content in dry orthodox seeds was usually lower than 10 nmol g^{-1} d.wt resulting in low energy charge values of around 0.20. The highest increase of ATP levels after incubation compared to the level of dry seeds was 167-fold in soybean and 60-fold in *Ph. wilsonii.* The maximum ATP accumulation coincided with the seed being ready to germinate. A decrease in ATP content was observed after seed germination, and the decline continued throughout the period of radicle emergence. The imbibed dormant seeds of *Ph. wilsonii* synthesized smaller amounts of ATP than non-dormant ones at the early stage of incubation; the ATP was eventually depleted. The ATP content of recalcitrant seeds, including the intermediate species, ranged from 20 to 220 nmol g^{-1} d.wt, thereby reflecting energy charge values above 0.6. The ATP levels in the fresh recalcitrant seeds before and after incubation, however, generally remained similar. This suggests that orthodox crop seeds, i.e. soybean synthesize adenosine phosphates extremely efficiently, unlike most of the tree seeds, which are more or less dormant. *P. macrophyllus* seeds are viviparous and had the highest amount of ATP, ADP and the highest energy charges among the species tested, indicating high metabolic activities in mature seeds. Seeds of *C. globosa*, another type of recalcitrant seed and *N. parvigemma* were characterized by low adenylate and energy charges, no significant changes in adenylate in the early stage of incubation, and slow germination. During an artificial drying process, the degradation of adenylates in recalcitrant seeds seemed to continue indefinitely, resulting in lower energy charges. This process must be different from that of orthodox seeds in which AMP is the major adenosine phosphate to accumulate.

Keywords: Adenylates; Artificial dehydration; Energy charge; Orthodox; Recalcitrant; Seed germination.

Abbreviations: EC, energy charges; **TA,** total adenylate; **MC,** moisture content.

Introduction

Most crop seeds are in the category of orthodox types, which are tolerant of desiccation. The longevity of orthodox seeds is increased by decreasing storage temperature and moisture content (Roberts, 1973). The accumulation and the synthesis of ATP at the early stage of crop seed germination have been documented since the middle of the 1960s. It was found that AMP is the major adenylate accumulating in mature seeds (Obendorf and Marcus, 1974; Barua et al., 1981). In terms of adenylate energy charge, most orthodox seeds exhibited a value lower than 0.2 (Quebedeaux, 1981; Lunn and Madsen, 1981; Hourmant and Pradet, 1981). During the early stage of seed imbibition and germination, a rapid increase occurs in ATP accumulation, and energy charges increase to a range of 0.6 to 0.9 (Pradet and Raymond, 1983), after which a stationary phase in ATP concentration occurs (Ching and Ching, 1972; Moreland et al., 1974; Hourmant and Pradet, 1981). Crop seeds like wheat (Ching and Kronstand, 1972) showed strong oscillations of the changes of adenylates during germination probably indicating different growth events.

Recalcitrant seeds are desiccation sensitive and have a high moisture content, which is necessary to maintain seed viability for a certain period of time. Most (if not all) are probably metabolically active as reported for mangrove species (Farrant et al., 1992). Respiration of seeds represents the gross metabolic activity, normally quite low in mature orthodox seed but in recalcitrant seed probably in the range of 0.5–1.5 mmol O_2 min^{-1} g^{-1} f.wt (Lin and Chen, 1995). The adenylates in the early stage of seed germination have received little attention in recalcitrant seeds. It is reasonable to speculate on the different levels of adenylates in these two seed types, but how great the difference is and its significance are presented in this report.

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[1] Corresponding author. Fax: 02-3895531.

Materials and Methods

Seed Collection and Seed Germination

Freshly mature seeds of *Podocarpus macrophyllus* D. Don, *Cyclobalanopsis globosa* Lin et Liu, *Neolitsea parvigemma* (Hay.) Kaneh. et Sasak., and *Michelia compressa* (Maxim.) Sarg. were collected from natural forests in Taiwan sometime between June and October 1994, depending on the species. Some of the seeds of *P. macrophyllus* were wet stored (or stratified) at 4°C for 4.5 months. Seeds of *Machilus thunbergii* were collected in June 1995. All fresh mature seeds of the above species were used for the following experiments. Seeds of the soybean cv. "Tainaon no. 15", *M. compressa* and *Phellodendron wilsonii* Hay. et Kaneh. collected in October 1993 with a water content of 6.1%, 6.6%, and 7.0%, respectively, were stored at 4°C for one year before use. The moisture content of seeds was obtained from a triplicate sample dried at 103°C for 17 h and is expressed on a fresh weight basis. The seeds of *P. macrophyllus*, *C. globosa*, and *Ma. thunbergii* are recalcitrant types; and soybean, *Ph. wilsonii* (Lin et al., 1994) and *M. compressa* (Lin and Wu, 1995) are recognized as orthodox types. *Neolitsea parvigemma* was recognized as belonging to an intermediate category (unpublished observation).

The seeds were mixed with moistened clean sphagnum in sealable polyethylene bags (17 × 11.5 cm, 0.04 mm thick), and the water content of the sphagnum was adjusted to about five times the dry mass (83–85% wet basis), Roughly 2/3 of the space inside the bag was occupied by the sphagnum with seeds. Germination was carried out in growth chambers with 12 h of fluorescent light (60–80 $\mu E \cdot m^{-2} \cdot sec^{-2}$) at 30/20°C. Only seeds of *Ph. wilsonii* were incubated at 35/10°C with 8 h of light to break the deep dormancy (Lin et al., 1994).

Artificial Dehydration

Slow drying was achieved by placing seeds in a desiccator over a series of saturated salt solutions: $MgSO_4 \cdot 7H_2O$, RH 89% → $CH_3COONa \cdot 3H_2O$, RH 75% → $MgCl_2 \cdot 6H_2O$, RH 33% → LiCl, RH 13%. Seeds of *Ma. thunbergii* were first placed over the $CH_3COONa \cdot 3H_2O$ solution before proceeding to lower relative humidities. Seeds were transferred to the next saturated salt solution with lower humidity every five days, and the seeds were left over the final salt solution until the desired water content was achieved.

Determination of Adenosine Phosphates

Most of the tree seeds showed a greater or lesser degree of dormancy, taking two weeks to one month to germinate. This probably is associated with the relatively undifferentiated axes in many of the recalcitrant seed species (Berjak et al., 1989). For adenosine phosphates analysis, seeds of three replicates were homogenized with 10% ice-cold perchloric acid. The homogenate was kept on ice for 15 min before being centrifuged (13,000 *g*) for 10 min at 5°C. The supernatant was neutralized with 5M KOH as measured by broad-range pH paper. The salt precipitate was removed by centrifugation (13,000 *g*) at 5°C, and the supernatant was transferred to a fresh, preweighed tube where the final volume of the extract was determined by the weight (Oresnik and Layzell, 1994).

The ATP content was measured by the luciferin-luciferase system with a Lumac biocounter MT 2000 (Chapman et al., 1971). The light signal was integrated for 10 sec and compared with the standard for ATP (2.5 to 250 nM). ADP was converted to ATP with phosphoenolpyruvate and pyruvate kinase (EC 2.7.1.40) (Sigma) by incubation at 37°C for 15 min. AMP was converted to ADP with endogenous ATP by using myokinase (EC 2.7.4.3) (Sigma); the resulting ADP was converted to ATP (Ching and Ching, 1972). EC was calculated according to Atkinson (1969) : EC = ([ATP] + 1/2 [ADP]) / ([ATP] + [ADP] + [AMP]). Firefly lantern extract (FFT from Sigma) was prepared by ammonium sulphate precipitation (Strehler, 1974), but the precipitates were dissolved in 20 mM glycylglycine (pH 7.4), 50 mM $NaAsO_2$, and 20 mM $Mg(CH_3COO)_2$. The limit of sensitivity of this method is 0.1 nM. The conversion rates of AMP to ADP and ADP to ATP were 100%. A known amount of ATP was ground with the sample, and the ATP recovery was generally higher than 90%.

EC values in the early stage of seed germination generally ranged from 0.8 to 0.9 in non-limited supply of oxygen (Rasi-Caldogno and De Michelis, 1978; Mocquot et al., 1981). The slightly lower EC measured in this study, for example, >0.7 for soybean axes, 0.56–0.74 for *Ph. wilsonii*, 0.55 for *M. compressa* and 0.78 for *N. parvigemma* is due probably to partial oxygen deprivation in the polyethylene bags. In a separate experiment, we found that the germination percentages of the seeds incubated in polyethylene bag were the same as in a germination box, even though the germination process may be more or less delayed in the bag. Also the EC of the seeds incubated in the PE bag was generally 0 to 0.1 lower than that obtained in the germination box.

Results

Orthodox Seeds

The changes of ATP, ADP, AMP, TA, and EC in orthodox seeds during the germination process are presented in Tables 1 to 3. Mature seeds have only an embryo in soybeans and *Ph. wilsonii* (exalbuminous), but a tiny embryo surrounded by endosperm in *M. compressa* (albuminous). Seeds of *Ph. wilsonii* are markedly smaller than soybeans, thereby making it difficult to dissect into cotyledon and embryonic axis. The levels of ATP and EC were very low in the dry soybean seeds (Table 1). The axis had 39 pmol ATP and an EC of 0.24; the cotyledon had 426 pmol ATP and an EC of 0.19. Adenylates and EC in the axis and cotyledon of soybean increased to a maximum probably at two days of incubation; with an average of 5792 pmol, 4466 pmol, 6580 pmol for ATP, ADP and

Table 1. Contents of ATP, ADP, AMP, and total adenylates (TA) and energy charge (EC) in the axis and cotyledon of soybean seeds. Each value is the average of three replicates. Standard errors are shown in parentheses. Seeds began germination between 1 and 2 days incubation. Seeds reached 100% germination within 3 days.

Incubation (day)	ATP	ADP	AMP	TA	EC
			pmol / axis		
0	39 (3)c*	193 (25)c	330 (13)c	562 (42)c	0.24 (0.01)c
1	2957 (102)b	1217 (260)b	836 (75)c	5010 (83)b	0.71 (0.01)a
2	5792 (1400)a	4466 (558)a	6580 (787)b	16838 (1221)a	0.48 (0.04)b
3	2156 (102)bc	4265 (111)a	11677 (964)a	18097 (928)a	0.24 (0.02)c
			pmol / cotyl		
0	426 (44)d +	2203 (143)c	5496 (474)b	8125 (370)c	0.19 (0.02)c
1	56678 (1658)b	32834 (4641)b	14859 (5872)b	104370 (12170)b	0.70 (0.04)a
2	71212 (3237)a	56288 (1693)a	42409 (5092)a	169908 (3548)a	0.58 (0.03)b
3	46759 (4929)c	60028 (5123)a	45292 (1086)a	152079 (1280)a	0.50 (0.02)b

Mean separation for axis (*) or cotyledons (+) within rows by Duncan's multiple range test, 5% level.

Table 2. Contents of ATP, ADP, AMP, and total adenylates (TA) and energy charge (EC) in seeds of *Phellodendron wilsonii* which were incubated either at 35/10°C or 22°C. Each value is the average of three replicates. Standard errors are shown in the parentheses. Seeds began germination on the 15th day of incubation at 35/10°C. Seeds reached 60% germination after 7 weeks incubation at 35/10°C and 8 h light.

Incubation (temp. day)	ATP	ADP	AMP	TA	EC
35/10°C		pmol / seed			
0	8.9 (0.9)d*	41.4 (4.2)c	98.7 (2.1)b	149 (3.0)c	0.20 (0.02)c
3	139 (24)c	82 (25)c	28 (4)b	249 (45)c	0.72 (0.02)a
9	175 (7)c	76 (8)c	36 (0)b	287 (9)c	0.74 (0.01)a
15	531 (34)a	438 (66)a	365 (34)a	1334 (91)a	0.56 (0.02)b
24	437 (25)b	317 (20)b	315 (69)a	1069 (114)b	0.56 (0.03)b
22°C		pmol / seed			
0	8.9 (0.9)c+	41.4 (4.2)c	98.7 (2.1)a	149 (3.0)b	0.20 (0.02)d
3	85 (5)a	71 (8)ab	64 (11)bc	220 (25)a	0.55 (0.02)b
9	69 (5)a	77 (2)a	77 (7)b	223 (9)a	0.48 (0.00)c
15	74 (2)a	59 (7)b	51 (0)c	184 (10)ab	0.56 (0.00)ab
24	38 (8)b	31 (2)c	22 (6)d	91 (15)c	0.59 (0.01)a

*Mean separation for 35/10°C (*) or 22°C (+) treatment within rows by Duncan's multiple range test, 5% level.

AMP in the axis and 71212, 56288, and 42409 pmol in the cotyledon, respectively.

Similar results for ATP, ADP, TA, and EC were found in *Ph. wilsonii* seeds (Table 2). ATP, ADP, and AMP in dry seed were 8.9, 41.4, and 98.7 pmol, respectively, with an EC of 0.20. When incubated at alternating temperatures, ATP and ADP increased up to the 15th day, at which time the embryo broke the seed coat and germination became observable. Only the level of AMP decreased in the beginning of incubation. Seeds of *Ph. wilsonii* maintained dormancy if they were incubated at 22°C and the energy metabolism was less dynamic than seeds incubated at 35/10°C. ATP and ADP increased initially but decreased continuously later on. AMP decreased slightly in the first several days and became significant at the 24th day of incubation.

Michelia compressa seeds (Table 3) were different from those of soybean or *Ph. wilsonii*. The seeds had 302, 809, and 1367 pmol of ATP, ADP, and AMP, respectively. ATP increased at the beginning then decreased. ADP and AMP decreased from the beginning, thus causing an increase of EC from 0.28 to 0.55. Once these seeds started germinating at around the 25th day of incubation, the second peak of adenylate was observed in the endosperm. On the 28th day of incubation, the sizes of the embryo and endosperm of germinating seeds were equal to each other. By the 35th day of incubation, the endosperm was almost exhausted.

Recalcitrant Seeds

ATP was the predominant form in the gametophyte of *P. macrophyllus* (Table 4) with an average of 113 nmol, as compared with ADP at 37 nmol, and AMP at 16 nmol. Another characteristic of the gametophyte was a high EC value of 0.79. During the incubation period, ATP decreased slightly in the early stage of seed germination. ATP remained roughly similar when radicle emergence occurred on the 10th day of incubation. ATP increased again to 98 nmol on the 20th day of incubation while the cotyledon was beginning to open up from the hypocotyl. In the embryo, ATP content increased significantly after

Table 3. Contents of ATP, ADP, AMP, and total adenylates (TA) and energy charge (EC) in seeds of *Michelia compressa.* The seeds were stored at 4°C with water content of 6.6%. Seeds began germination at about the 25th day of incubation and were dissected into the embryo and endosperm at 28 days. Each value is the average of three replicates. Standard errors are shown in parentheses. Seeds reached 41% germination after 7 weeks incubation.

Incubation (day)	ATP	ADP	AMP	TA	EC
pmol / seed					
0	302 (31)bc*	809 (134)a	1367 (315)a	2478 (467)a	0.28 (0.02)d
7	449 (96)a	536 (15)b	603 (10)bc	1588 (121)b	0.45 (0.03)b
14	279 (10)bc	424 (42)bc	630 (35)b	1333 (87)bc	0.37 (0.00)c
21	258 (28)c	225 (24)de	191 (2)d	674 (54)de	0.55 (0.02)a
pmol / embryo					
28	105 (3)d	102 (25)e	85 (4.6)d	292 (27)e	0.53 (0.02)a
35	231 (21)c	322 (36)cd	225 (18)cd	778 (3)cde	0.50 (0.00)a
pmol / endosperm					
28	391 (30)ab	457 (3)bc	757 (5)b	1605 (32)b	0.38 (0.01)c
35	73 (8)d	140 (7)e	443 (53)bcd	656 (68)de	0.22 (0.00)e

*Mean separation within rows by Duncan's multople range test, 5% level.

Table 4. Contents of ATP, ADP, AMP, and total adenylates (TA) and energy charge (EC) of the embryo and gametophyte in seeds of *Podocarpus macrophyllus.* Each value is the average of three replicates. Standard errors are shown in parentheses. Seeds without radicle emergence when mature were selected for the experiment. Seeds began germination on the 10th day of incubation. Seeds reached 92% germination within 21 days.

Incubation (day)	ATP	ADP	AMP	TA	EC
nmol / embryo					
0	1.78 (0.30)b*	1.11 (0.32)bc	1.54 (0.08)b	4.43 (0.48)b	0.53 (0.03)b
3	1.69 (0.15)b	1.12 (0.17)bc	1.41 (0.53)b	4.22 (0.41)b	0.53 (0.07)b
6	3.77 (0.26)b	0.96 (0.36)c	0.55 (0.28)b	5.28 (0.82)b	0.81 (0.06)a
15	2.59 (0.02)b	1.75 (0.02)b	0.88 (0.28)b	5.22 (0.07)b	0.66 (0.05)b
20	9.77 (2.16)a	4.56 (0.23)a	4.99 (1.49)a	19.32 (3.89)a	0.62 (0.01)b
nmol g^{-1} f.wt gametophyte					
0	113 (13)a +	37 (12)ab	16 (2)ab	166 (26)a	0.79 (0.01)a
3	91 (1)ab	17 (14)b	21 (2)ab	129 (10)a	0.77 (0.07)ab
6	99 (9)ab	31 (3)ab	13 (1)b	143 (11)a	0.80 (0.01)a
15	84 (5)b	30 (5)ab	30 (2)a	144 (1)a	0.68 (0.03)b
20	98 (2)ab	48 (4)a	25 (5)ab	171 (11)a	0.71 (0.02)ab

*Mean separation for embryo (*) or gametophyte (+) within rows by Duncan's multiple range test, 5% level.

Table 5. Contents of ATP, ADP, AMP, and total adenylates (TA) and energy charge (EC) in seeds of *Cyclobalanopsis globosa.* Each value is the average of three replicates. Standard errors are shown in parentheses. Only seed germinating by the 15th day were further dissected into the axis and cotyledon. Seeds reached 58.7% germination after 17 weeks incubation.

Incubation (day)	ATP	ADP	AMP	TA	EC
nmol / seed					
0	3.80 (0.59)a*	4.77 (0.91)a	1.57 (0.24)ab	10.14 (1.23)a	0.61 (0.02)b
3	7.16 (2.84)a	3.65 (0.94)a	1.39 (0.44)ab	12.20 (2.44)a	0.74 (0.06)ab
6	4.75 (1.22)a	4.43 (0.44)a	2.20 (0.56)a	11.38 (1.97)a	0.61 (0.02)b
nmol / axis					
15	1.43a	0.36b	0.15b	1.93b	0.83a
nmol / cotyl					
15	7.66a	3.21a	0.84ab	11.71a	0.79a

*Mean separation within rows by Duncan's multiple range test, 5% level.

the seeds had been incubated for 20 days. The quantities of ADP and AMP ranged from 1.11 to 4.56 and 1.54 to 4.99 nmol, respectively, during the 20 days of incubation. EC values varied from 0.53 to 0.81.

The axes of the other two species of recalcitrant types were too small to be dissected from their cotyledons, so the whole seeds were used for analysis. ATP, ADP, and AMP of *C. globosa* seeds up to the 15th day of germination (Table 5) maintained roughly the same levels during the germination period. On the 15th day of incubation, EC values increased from an initial 0.61 to 0.83 and 0.79 in the axis and in the cotyledon, respectively.

Table 6 displays the ATP, ADP, and AMP contents of *N. parvigemma* seeds at 4.37, 3.63, and 2.67 nmol, respectively. An increase in ATP and total adenylate levels in the beginning of incubation was not found. ATP decreased to 1.03 nmol per seed on the 6th day of incubation and increased to 9.27 nmol in the cotyledon on the 31st day of incubation, which coincided with seed germination. ADP and AMP decreased after an initial increase for 3 days of incubation. EC values increased from an initial 0.58 to 0.78 for the axis and 0.70 for the cotyledon by the 31st day of incubation.

The contents of adenylates on a dry weight basis in various fresh recalcitrant and dry orthodox seeds are summarized in Table 7. The orthodox species had moisture contents in the range of 6.1% to 7.0%, and EC values varied from 0.20 to 0.28. The ATP level was markedly lower

Table 6. Contents of ATP, ADP, AMP, and total adenylates (TA) and energy charge (EC) in seeds of *Neolitsea parvigemma.* Each value is the average of three replicates. Standard errors are shown in parentheses. Only seeds germinating by the 31st day were further dissected into the axis and cotyledon. Seeds reached 93.3% germination after 15 weeks incubation.

Incubation (day)	ATP	ADP	AMP	TA	EC
			nmol / seed		
0	4.37 (1.70)b*	3.63 (0.68)ab	2.67 (1.20)ab	10.67 (3.36)ab	0.58 (0.06)bc
3	3.38 (0.63)b	4.23 (0.58)a	6.07 (1.77)a	13.68 (2.94)ab	0.40 (0.02)cd
6	1.03 (0.22)b	2.27 (0.48)bc	5.31 (0.82)a	8.61 (0.25)bc	0.25 (0.06)d
15	2.62 (1.05)b	2.76 (0.62)b	3.11 (0.47)ab	8.49 (1.22)b	0.47 (0.0.9)c
			nmol / axis		
31	1.63b	1.04c	0.07b	2.74c	0.78a
			nmol / cotyl		
31	9.27a	4.16a	2.70ab	16.13a	0.70ab

* Mean separation within rows by Duncan's multiple range test, 5% level.

Table 7. Comparison of ATP, ADP, AMP, total adenylates (TA), energy charge (EC) and moisture content (MC) in various seeds. Each value is the average of three replicates. Standard errors are shown in parentheses.

	ATP	ADP	AMP	TA	EC	MC (%)
1. Orthodox seeds			nmol g^{-1} d.wt			
Soybean						
Axis	3.3 (0.2)	16.8 (0.9)	38.2 (1.2)	58.3 (0.5)	0.19 (0.02)	6.1
Cotyl	9.9 (0.9)	48.5 (6.8)	83.1 (4.1)	141.5 (11.7)	0.24 (0.01)	6.1
Phellodendron wilsonii						
Seed	1.3 (0.2)	6.3 (0.7)	15.0 (0.1)	23.6 (0.7)	0.20 (0.02)	7.0
Michelia compressa						
Seed	10 (1)	28 (3)	47 (8)	85 (12)	0.28 (0.02)	6.6
2. Recalcitrant seeds			nmol g^{-1} d.wt			
Podocarpus macrophyllus						
Fresh seed						
Embryo	243 (43)	149 (32)	213 (21)	605 (27)	0.53 (0.03)	61.5
Gametophyte	220 (27)	73 (24)	31 (4)	324 (51)	0.79 (0.01)	48.5
Stratified seed						
Embryo	491 (62)	114 (14)	78 (13)	642 (38)	0.80 (0.04)	61.5
Gametophyte	272 (6)	48 (6)	38 (9)	385 (9)	0.83 (0.03)	48.9
Machilus thunbergii						
Fresh seed	91.1 (18.2)	42.7 (6.7)	9.2 (0.3)	143.0 (24.6)	0.79 (0.02)	44.7
Cyclobalanopsis globosa						
Fresh seed	23.6 (2.6)	29.7 (3.9)	9.9 (1.8)	63.2 (3.5)	0.61 (0.02)	49.0
3. Intermediate category						
Neolitsea parvigemma						
Fresh seed	23 (6)	19 (2)	14 (5)	56 (11)	0.58 (0.06)	26.0

Table 8. Contents of ATP, ADP, AMP, and total adenylates (TA) and energy charge (EC) in seeds of *Machilus thunbergii* during artificial dehydration. Each value is the average of three replicates. Standard errors are shown in parentheses. Percentage of germination (G) was calculated after 35d of incubation at fluctuating temperatures of 30/20°C, and 8 h light.

Dehydration (day)	ATP	ADP	AMP	TA	EC	MC (%)	G (%)
			nmol g^{-1} d.wt				
0	91a*	43a	9.2a	143a	0.79a	44.7	99.0
	(18)	(6.7)	(0.3)	(25)	(0.02)		
10	48b	34a	16a	98ab	0.66b	39.3	56.7
	(3)	(4)	(4)	(9)	(0.04)		
19	19bc	31ab	13a	63bc	0.55c	31.3	3.0
	(6)	(10)	(1)	(17)	(0.02)		
29	13c	12b	13a	38c	0.50c	22.5	0.0
	(1)	(1)	(4)	(4)	(0.04)		

*Mean separation within rows by Duncan's multiple range test, 5% level.

Table 9. Contents of ATP, ADP, AMP, and total adenylates (TA) and energy charge (EC) of the embryo and gametophyte in seeds of *Podocarpus macrophyllus* during artificial dehydration. Each value is the average of three replicates. Standard errors are shown in parentheses. Percentage of germination (G) was calculated after 21d of incubation at fluctuating temperatures of 30/20°C, and 8 h light.

Dehydration (day)	ATP	ADP	AMP	TA	EC	MC (%)	G (%)
			nmol / embryo				
0	3.59a*	0.85a	0.56a	5.00a	0.80a	–	99.0
	(0.61)	(0.06)	(0.40)	(0.95)	(0.04)		
14	1.95ab	0.67a	0.45a	3.07b	0.74ab	–	–
	(0.37)	(0.02)	(0.09)	(0.26)	(0.06)		
20	1.32b	0.91a	0.80a	3.03b	0.59b	–	0.0
	(0.19)	(0.09)	(0.14)	(0.42)	(0.00)		
			nmol g^{-1} d.wt gametophyte				
0	272a +	48b	38a	358a	0.83a	48.9	
	(6)	(6)	(10)	(9)	(0.03)	(1.4)	
14	177b	54b	27a	258b	0.79a	39.7	
	(1.4)	(23)	(2)	(2)	(23)	(0.02)	
20	168b	119a	31a	318ab	0.72a	33.9	
	(2.1)	(25)	(34)	(12)	(21)	(0.07)	

* Mean separation for embryo (*) or gametophyte (+) within rows by multiple range test, 5% level.

than ADP and AMP. *Phellodendron wilsonii* seeds were especially low in adenylates. The moisture contents of recalcitrant seeds ranged from 44% to 49%, except for the embryo of *P. macrophyllus* (61.5%). The quantities of adenylates were different in various species and the TA varied from 63 nmol g^{-1} d.wt in *C. globosa* to 324 nmol g^{-1} d.wt in *P. macrophyllus*. EC values varied from 0.61 in *C. globosa* to 0.79 in *P. macrophyllus* and *Ma. thunbergii.* ATP was the major form of adenylates with the exception of ADP in *C. globosa*.

Artificial Dehydration

The correlation between water content and adenylates in seeds of *Ma. thunbergii* and *P. macrophyllus* was presented in Tables 8 and 9. After drying treatment, the water content of *Ma. thunbergii* seeds decreased from 44.7% to 22.5%. Diminishing quantities of ADP and especially ATP resulted in the decline of EC values from 0.79 to 0.50 and TA values from 143 to 38 nmol g^{-1} d.wt. AMP remained the same. The germination percentage of seeds dehydrated for 19 days decreased from 99% to 3%. The water content of *P. macrophyllus* seeds declined from 48.9% to 33.9% during the 20 days of dehydration. Germinability was totally lost at the 20th day of dehydration. ATP decreased from 272 to 168 nmol, ADP increased from 48 to 119 nmol, and AMP maintained similar levels. These factors resulted in a slight decline of TA and EC values.

Discussion

The maximum ATP accumulation in orthodox seeds coinciding with seed germination agrees with previous investigations (Ching and Ching, 1972; Moreland et al., 1974; Hourmant and Pradet, 1981). However, the time period required to reach the maximum ATP may be different depending on the species considered (Moreland et al., 1974; Lunn and Madsen, 1981). The increase in the quantity of ATP in tree seeds may not be as large as that observed in soybeans. The slow germinating seeds of *M. compressa* exhibited a pattern dissimilar to soybeans. The total adenylates in the process of germination were lower than those in the seeds before incubation. Further investigation is needed to disclose the energy metabolism of this type of orthodox seed.

Non-optimal temperatures could not stimulate the germination of seeds, but metabolism of reserves, including synthesis of adenylates, could be induced (Ching, 1975). The imbibed dormant seeds of *Ph. wilsonii* accumulated smaller amounts of ATP than non-dormant seeds. This may be a result of decreasing ATP synthesis and the level of adenylates during incubation eventually becoming depleted. The initial increase in ATP during incubation is definitely not related to seed germination as has been observed in wild oat seeds (Adkins and Ross, 1983).

An increase of ATP and TA in the cotyledon of soybean and the endosperm of *M. compressa* indicated biosynthesis of adenylates in the storage tissue as has been reported in the endosperm of germinating rice grain (Palmiano and Juliano, 1972) and in the gametophyte of germinating ponderosa pine seeds (Ching and Ching, 1972). This is a temporal rise of the energy supply to provide for the needs of enzyme production in the catabolic breakdown of reserves. A decrease in ATP content was found following seed germination and was continuous throughout the period of radicle emergence, an energy-consuming process (Ching and Ching, 1972).

High moisture content in freshly mature seeds characterizes recalcitrant species. The generally high content of ATP and ADP in mature recalcitrant seeds are due in part to the moderately high rate of respiration (Lin and Chen, 1995). Seeds of *P. macrophyllus* are viviparous, and roughly 25% of the mature seeds show an emergence of radicles, indicating a continuous metabolic activity. ATP was the most abundant energy component as reflected in a high EC value of about 0.8 in mature seeds. However, stratified *P. macrophyllus* seeds produced even more total adenylates, ATP, and energy charge than mature seeds (Table 7). This is similar to recalcitrant seeds, *Quercus rubra*, in which the seeds before and after stratification had a energy charge of 0.5 and around 0.8, respectively (Hopper et al., 1985). High adenylates in mature seeds of *P. macrophyllus* are probably sufficient for the consumption of germinating seeds, especially as no higher rate of adenylate production was observed as in other recalcitrant seeds.

Seeds of *C. globosa* may be considered another type of recalcitrant seed in terms of energy metabolism. *Cyclobalanopsis globosa* and also *N. parvigemma* both were characterized by much lower ADP and ATP contents and EC in mature seeds (Table 7), and slower germination than *P. macrophyllus*. It took 16 weeks and 15 weeks to complete germination of the seeds of *C. globosa* and *N. parvigemma*, respectively. The slight increase (if it exists) in the levels of total adenylates in seeds during the early stage of incubation, as in orthodox seeds, is not directly related to seed germination.

Differences in ATP contents between orthodox and recalcitrant seeds are obvious. The ATP contents in orthodox seeds are normally lower than 10 nmol g^{-1} d.wt while in recalcitrant seeds they range from 20 to 220 nmol g^{-1} d.wt depending on the species. ATP increased 167-fold in the first 2 days of incubation in soybean seeds, and almost 60-fold in 15 days of incubation in *Ph. wilsonii* seeds. The ATP quantities before and at seed germination in recalcitrant seeds remain similar. This finding indicates that recalcitrant tree seeds do not have to change the energy balance significantly as the orthodox crop seeds do. The increase in ATP levels in orthodox seeds during the early stages of germination is considered to be due to the onset of respiration and concomitant oxidative phosphorylation in the mitochondria as soon as seeds are hydrated (Hourmant and Pradet, 1981; Attucci et al., 1991). Recalcitrant seeds have not been examined in terms of energy metabolism in the early stage of seed germination.

During an artificial drying process, the degradation of adenylates, especially ATP, resulted in a decrease in total adenylates and energy charge in recalcitrant seeds. Whether the degradation of adenylates resulted from either enzymatic or non-enzymatic regulation remains unclear. Since AMP did not obviously accumulate in the seeds of *Ma. thunbergii* and *P. macrophyllus* during the drying process as has been observed in orthodox seeds (Obendorf and Marcus, 1974; Barua et al., 1981), a different mechanism for the degradation of ATP from that of orthodox seeds must be involved.

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