

BIOLUMINESCENCE FOR DETERMINING ENERGY STATE OF PLANTS

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The bioluminescence produced by the luciferin-luciferase system is a very sensitive assay for ATP content in extracts of plant materials. With two additional enzymes, pyruvate kinase (E.C. 2.7.1.40) and adenylate kinase (E.C. 2.7.4.3), and an excess of phosphoenolpyruvate, the ADP and AMP in tissue extracts can be quantitatively converted to ATP and then assayed with the luciferase system (Ching and Ching, 1972). This simple, rapid, and sensitive procedure has been used for determining the energy state of plant materials for metabolic studies (Ching and Ching, 1972; Ching et al., 1974) under different environmental stresses,* in cultivars of varied genetic capabilities (Ching and Kronstad, 1972), and in seeds of different viability and vigor (Ching, 1973; Ching and Danielson, 1972). The experimental results from our laboratory and others indicate that the bioluminescence test is a very useful tool for (a) predicting viability of seeds and pollens in a time period of one hour instead of the usual days or weeks required by growth tests; (b) screening cultivars for high growth potential and productivity without long-term and labor-demanding field tests; (c) detecting the incipient damage of environmental pollutants and stresses so that reparative measures can be implemented; and (d) discerning the role of ATP concentration, total content of adenosine phosphates, ATP/ADP ratio, and energy charge [EC, $EC = ([ATP] + 1/2 [ADP])/([ATP] + [ADP] + [AMP]) - (7)$] in metabolic control of enzymes, organelles, tissues, and organisms. All these possibilities will be discussed in the following sections in more detail and particular applicational requirements will be mentioned.

ATP TEST FOR SEED AND POLLEN VIABILITY AND VIGOR

ATP is needed for biosynthesis of nucleotides, nucleic acids, proteins, lipids, amino acids, and carbohydrates (Henderson and Paterson, 1973). ATP also modifies existing enzymes in cells and tissues, thus it activates, inactivates,

* Barlow, E. W. R., "Physiological Effects of Water Stress on Young Corn Plants," Ph.D. thesis, 1974, Oregon State University.

or amplifies the enzyme activity, depending on the condition (Holzer and Duntze, 1972). In seeds as well as pollens, ATP is usually low and often limiting for the early metabolic events of germination (Ching, 1972; Obendorf and Marcus, 1974). If more ATP can be synthesized, the metabolic wheel will be turned faster, which eventually leads to more growth. Figure 1 shows such correlation in rape seeds. Further indication of high ATP and energy charge associated with high synthetic ability of ribonucleic acid (RNA), protein, and lipids in wheat embryos or seedlings are summarized in table 1. In pollen of douglas fir (*Pseudotsuga menziesii* Franco), the ATP contents were found to be 50, 136, 209, 292, 456, and 562 n moles per one gram of fresh weight for lots having 0, 9, 16, 30, 57, and 91 percent germination, respectively. Again, the data indicate a high correlation of ATP content and germinability (unpublished data from our laboratory). All these data show that among the seed or pollen lots of one crop or cultivar, ATP content appears to be a good biochemical index of vigor and viability. In order to be of practical use for seed testing or cross pollination work, a standard curve depicting the relationship of ATP content and growth potential or germinability should be established for each cultivar of a crop. At present, several

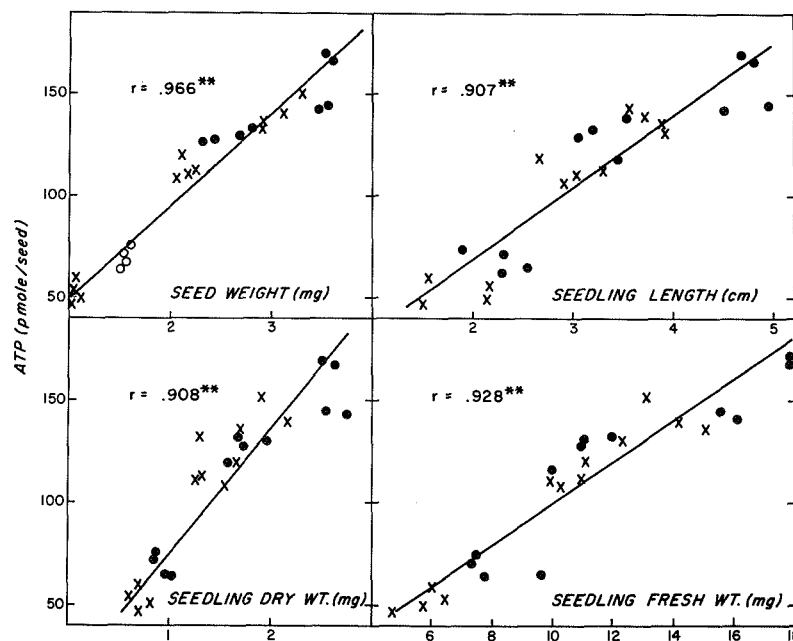


Figure 1. Correlation of ATP content in 4-hour imbibed seeds of rape (*Brassica napus* L.) and seed weight, 4-day seedlings length, 4-day seedling fresh and dry weight. X, O = different lots; r = correlation coefficient; ** = significant at 1 percent level (Ching and Ching, 1973).

Table 1

Contents of ATP and Total Adenosine Phosphates (TAP), Energy Charge (EC), Protein, RNA, and Lipid Synthesizing Ability and Growth Rate of the Embryo or Seedling of Wheat Cultivars, Hyslop (C.I. 14565) and Yamhill (C.I. 14563) (Ching, 1972)

	Hyslop	Yamhill
Embryo dry weight, mg	1.19 \pm 0.13	1.26 \pm 0.14
4-hours embryo: ATP, pmole	232 \pm 29	290 \pm 35
TAP, pmole	504 \pm 56	620 \pm 41
EC	0.71	0.72
2-day seedling: ATP, pmole	2985 \pm 315	4800 \pm 360
TAP, pmole	4819 \pm 501	6656 \pm 702
EC	0.81	0.86
UL- ¹⁴ C-amino acid incorporation (cpm/5 seedlings): 30 hours old	1526 \pm 184	2138 \pm 329
44 hours old	4701 \pm 301	7626 \pm 513
5- ³ H-uridine incorporation (cpm/5 seedlings): 20 hours old	1060 \pm 172	1259 \pm 140
36 hours old	2438 \pm 313	5897 \pm 647
UL- ¹⁴ C-glucose incorporation into lipids (cpm/5 seedlings):		
24 hours old	1415 \pm 152	1760 \pm 154
48 hours old	3229 \pm 289	6242 \pm 344
7-day seedlings: fresh weight mg	157 \pm 12	201 \pm 15
dry weight mg	18 \pm 1	21 \pm 1

standard curves have been established for cultivars of lettuce seeds (Ching and Danielson, 1972). More standard curves for different crops are therefore needed for this test.

PREDICTOR FOR HIGH GROWTH POTENTIAL AND PRODUCTIVITY IN NEW CROSSES AND SELECTIONS OF BREEDING MATERIALS

The data in table 1 indicate that between cultivars of the same crop, differences exist in synthetic ability, and that a positive correlation of high ATP content, total adenosine phosphates, and energy charge with high synthetic ability is clearly demonstrated. A time curve showing the fluctuation of ATP, ADP, and AMP pools in the wheat embryo or seedling further depicts

the dynamic aspect of energy metabolism in germinating seeds (figure 2). It shows clearly that three oscillations of synthesis and utilization of adenosine phosphates occurred in the wheat embryo and seedlings during the first 48 hours of germination. Each dip on the curve coincides with major metabolic and morphological events. The first one associates with protein and RNA synthesis (Ching, 1972; Mazus' and Buchowicz, 1973); the second one follows DNA synthesis (Ching and Kronstad, 1972) and root emergence; and the third dip relates with the shoot emergence and the myriad of synthetic activities that followed. One trend shows clearly that the cultivar Yamhill with higher growth potential had higher ATP utilization and synthesis ability than Hyslop (table 1). Furthermore, the rate of synthesis exceeded utilization much more in Yamhill, resulting in a larger pool of ATP and ADP and a higher energy charge in Yamhill seedling. The larger pool and high energy charge in turn facilitates more biosynthesis and faster growth which eventually could lead to more productivity under optimum conditions. This speculation was born out by a cooperative study with Dr. R. G. McDaniel at the University of Arizona on nine newly developed Mexican wheat cultivars. In the study we found that the efficiency of oxidative phosphorylation of isolated mitochondria and the adenylate energy charge of germinating seedlings are positively significantly correlated with grain yield (correlation coefficients of +0.729* and +0.687*, respectively). Therefore, we have concluded that both biochemical parameters appear to be useful tools for plant breeders to

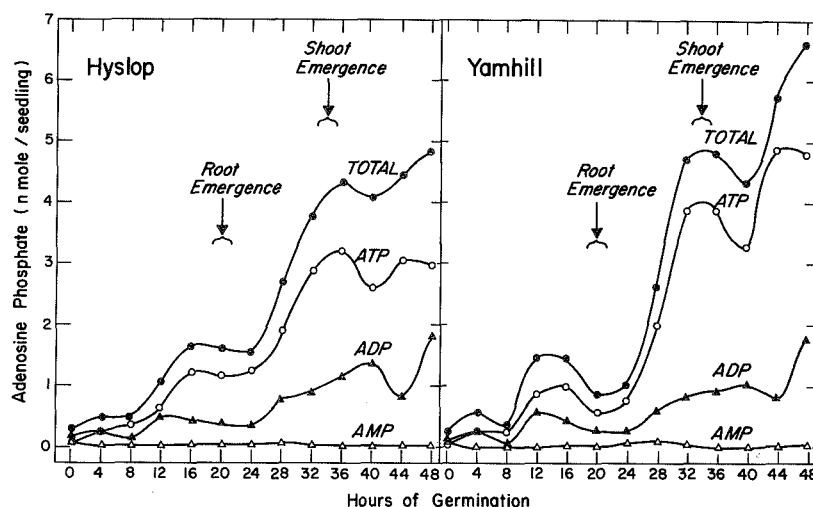


Figure 2. Changes in the content of ATP, ADP, AMP, and total adenosine phosphates in the embryo and the seedling of Hyslop and Yamhill wheat seed germinated at 293 K (20° C) in the dark (Ching and Kronstad, 1972).

* Significantly correlated at the 5 percent level.

screen for high producers at an early stage of a breeding program to reduce the time- and money-consuming field trials. More information is needed in the application of this concept in actual programs.

INDICATOR FOR ENVIRONMENTAL QUALITY AND STRESSES

Salts in soil reduced the growth and the ATP content in pea roots (Hasson-Porath and Paljakoff-Mayber, 1971). Cold temperature decreased ATP in cotton seedlings and germinating douglas fir seeds (Stewart and Guinn, 1969; Ching and Ching, 1973). Herbicides (for example, chloropropham) lowered the ATP level and inhibited RNA synthesis in soybean hypocotyls (Gruenhagen and Moreland, 1971), and atmospheric pollutants (for example, ozone) reduced ATP content and energy charge in douglas fir seedlings.* Water stress or drought conditions caused a reduction of ATP content in corn leaves† and germinating douglas fir seeds (Ching and Ching, 1973). From all these reports, one may develop a procedure with which to monitor the environmental influences and estimate the degree of damage or benefit so that reparative measures can be implemented.

STUDIES ON METABOLIC REGULATION

The regulation of carbohydrate metabolism by adenine nucleotides was known in 1964. Subsequently, Atkinson developed the concept of energy charge as an overall measure of the energy state of cells (Atkinson, 1969). Based on experimental data, Atkinson and his colleagues observed that when the energy charge is greater than 0.5, ATP-utilizing systems increase their activities, and, at an energy charge lower than 0.5, ATP-regenerating sequences become dominant. Therefore, the energy charge could modulate biosynthesis in cells and tissues.

In plant materials (Davies, 1973), information regarding the regulation by adenine nucleotides are very scanty. ATP and citrate inhibited phospho-fructo-kinase in carrot, corn scutellum, and peas, whereas ADP and AMP were relatively ineffective in modulating the response to ATP. The plant isocitrate dehydrogenase responded neither to individual adenosine phosphates nor to energy charge, but the decarboxylation of alpha oxoglutarate was stimulated by AMP in the mitochondria of pea and cauliflower. Recently, the first positive evidence of energy charge controlling the activity of 3-phosphoglyceric acid kinase from pea leaves was reported (Pacold and Anderson, 1973). The ribulose-5-phosphate kinase of the same tissue, however, was

* Tingey, D., National Ecol. Res. Lab., U.S. Environmental Protection Agency, Corvallis, Oregon, private communication.

† Barlow, E. W. R., "Physiological Effects of Water Stress on Young Corn Plants," Ph.D. thesis, 1974, Oregon State University.

not controlled by the energy charge. The difference is difficult to resolve and awaits future experimentation.

The data in table 1 indeed indicate a modulation of synthesis by energy supply and energy charge. At different times of seed germination and maturation, such positive correlations do not exist, however, particularly at the peak of cell division (Ching and Ching, 1972) and the peak of synthesis of nucleotides and nucleic acids (Ching et al., 1975) (figure 3). Apparently a temporal control of development overrides the energy charge regulation or simply because the end product inhibition does not occur in the biosynthetic pathways of nucleotides (Henderson and Paterson, 1973). In tissues with stabilized cell numbers, such as the gametophyte of ponderosa pine seeds, an increased ATP content, total adenosine phosphates, and energy charge preceded the biogenesis of enzymes and organelles and then all reduced in concert after the function of these enzymes and organelles was fulfilled (Ching, 1970) (figures 4,5).

It is clearly shown from this discussion that many regulatory mechanisms are operative in the complex systems of eukaryotes, but few are absolutely established yet. The bioluminescence assay of adenosine phosphates will definitely facilitate the exploration of these mechanisms.

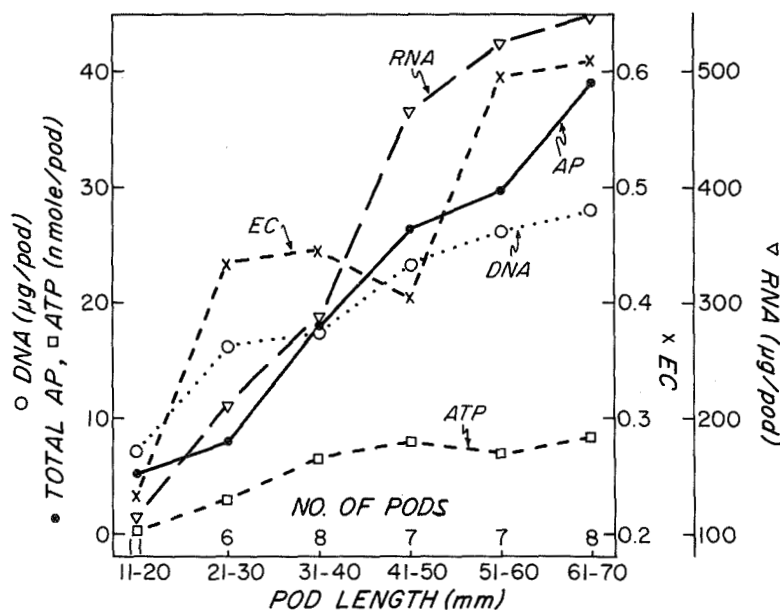


Figure 3. Contents of ATP, total adenosine phosphates (AP), DNA and RNA, and energy charge in whole 8-day-old pods of rape (Ching, et al., 1974).

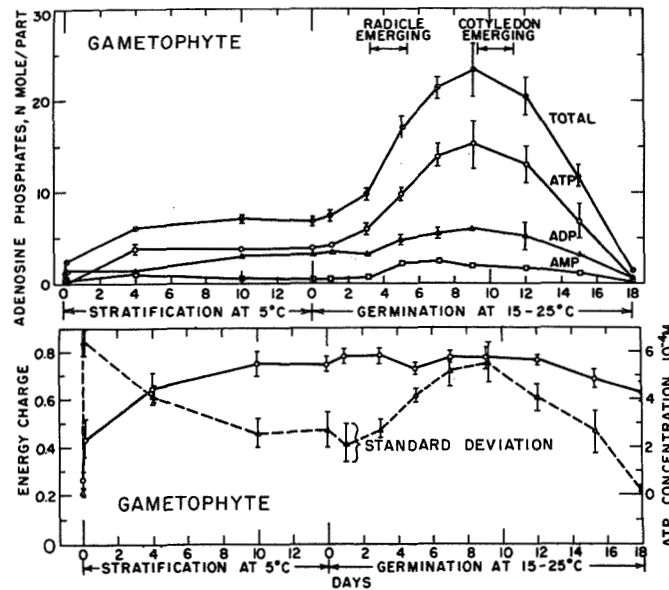


Figure 4. Changes in the content of ATP, ADP, AMP, and total adenosine phosphates (upper) and changes in adenylate energy charge (—) and ATP concentration (---) (lower) in the gametophyte of germinating ponderosa pine seeds (Ching and Ching, 1972).

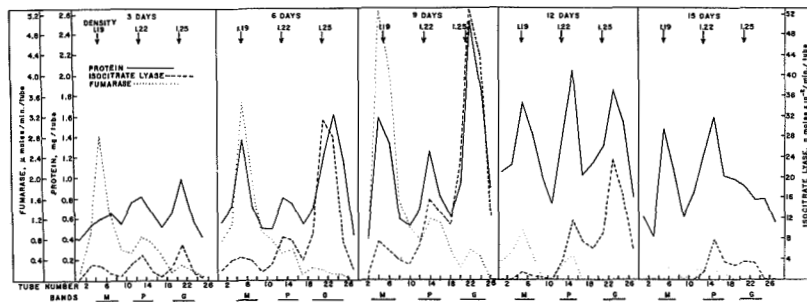


Figure 5. Changes in protein content and activity of fumarase and isocitrate lyase with germination days in fractions isolated by sucrose density gradient centrifugation of 10,000-g pellet from 250 gametophytes of germinating ponderosa pine seeds. M = mitochondria; P = mixture; G = glyoxysomes (Ching, 1970).

REFERENCES

- Atkinson, D. E., *Annu. Rev. Microbiol.*, **23**, 1969, p. 47.
- Ching, T. M., *Plant Physiol.*, **46**, 1970, p. 475.
- Ching, T. M., in *Seed Biology*, II, T. T. Kozlowski, ed., Academic Press, 1972, p. 103.
- Ching, T. M., *Plant Physiol.*, **51**, 1973, p. 400.
- Ching, T. M. and K. K. Ching, *Plant Physiol.*, **50**, 1972, p. 536.
- Ching, T. M. and K. K. Ching, in *International Symposium on Dormancy in Trees*, Polish Academy of Sciences, Kornik, 1973, p. 13.
- Ching, T. M. and R. Danielson, *Proc. Assoc. Off. Seed Analysts.*, **62**, 1972, p. 116.
- Ching, T. M. and W. E. Kronstad, *Crop Sci.*, **12**, 1972, p. 785.
- Ching, T. M., D. L. Stamp, and J. M. Crane, *Plant Physiol.*, **54**, 1974.
- Davies, D. D., in *Biosynthesis and its Control in Plants*, B. V. Milborrow, ed., Academic Press, 1973, p. 2.
- Gruenhagen, R. D. and D. E. Moreland, *Weed Sci.*, **19**, 1971, p. 319.
- Hasson-Porath, E. and A. Paljakoff-Mayber, *Plant Physiol.*, **47**, 1971, p. 109.
- Henderson, J. F. and A. R. P. Paterson, *Nucleotide Metabolism*, Academic Press, 1973, p. 54.
- Holzer, H. and W. Duntze, in *Biochemical Regulatory Mechanisms in Eukaryotic Cells*, E. Kun and S. Grisolia, eds., Wiley-Interscience, 1972, p. 115.
- Mazus', B. and J. Buchowicz, *Phytochem.*, **12**, 1973, p. 271.
- Obendorf, R. L. and A. Marcus, *Plant Physiol.*, **53**, 1974, p. 779.
- Pacold, I. and L. E. Anderson, *Biochem. Biophys. Res. Comm.*, **51**, 1973, p. 139.
- Stewart, J. M. and G. Guinn, *Plant Physiol.*, **44**, 1969, p. 605.