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Short Communication

Adenosine Triphosphate Content and Seed Vigor[1]

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ABSTRACT

ATP content in imbibed seeds is significantly correlated with seedling size in fatty, starchy and proteinaceous seeds, and it indicates viability in seed lots. ATP content thus appears to be a useful biochemical index of seed vigor.

Since ATP increases very rapidly during the early stage of seed germination for the energy requirement of biosynthesis (2, 3, 7) and the ATP content and adenylate energy charge are related with the growth potential as well as protein and RNA synthesizing ability of wheat seedlings (4), an attempt was initiated to discern if seedling vigor is correlated with the ATP content of imbibed seeds in different species. I found indeed that ATP content correlates with the viability and the seedling size of ryegrass, rape, and crimson clover seeds.

MATERIALS AND METHODS

Two freshly harvested commercial seed lots each of Dixie crimson clover (*Trifolium incarnatum* cv. Dixie), annual ryegrass (*Lolium multiflorum* L.), and common rape (*Brassica napus* L.) were separated into three size classes by screening or into density classes by blowing with different air currents. The weight of air-dried seeds (7–8% moisture content) was determined on four replications of 10 seeds each. Four replications of 50 seeds each of clover and rape were germinated in paper towels at 20 C in the dark, and ryegrass seeds were germinated on blotters at an alternating temperature of 15 C for 16 hr in the dark and at 25 C for 8 hr in 4000 lux of light. Seedling size and seedling fresh and dry weights were determined at a designated time of germination. Another lot of crimson clover seeds containing 8% moisture and stored for 15 years in hermetically sealed cans at different temperatures was used to elucidate the aging effect on ATP synthesis at the very early stage of germination. In addition to naturally aged seeds, artificially killed seeds were also studied. For that study, one sample each of the large ryegrass and rape seeds was moistened to contain 20% water and then heated at 100 C for 24 hr.

ATP was determined by the luciferin-luciferase method in boiling water extract (9) of seeds imbibed at the germination temperatures. Two replications of 10 seeds each of crimson clover and rape and 50 ryegrass seeds were quickly dropped in 10 ml of boiling glass-distilled water and extracted for 10 min. The extract was cooled in an ice bath. One aliquot of the extract was diluted 2- to 5-fold with a buffer to a final concentration of 25 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, pH 7.5, and 25 mM $MgSO_4$-$7H_2O$. ATP was added to a final concentration of 0.05 μM to another diluted aliquot as an internal standard. The light emission was determined in 0.5 ml of diluted extracts by an Aminco Chem-Glow photometer after adding 0.1 ml of firefly lantern extract containing 50 mM potassium arsenate and 20 mM magnesium sulfate, pH 7.4 (Sigma Chemical Co.). The ATP concentration in the extract was calculated by the formula of St. John (9).

RESULTS AND DISCUSSION

Significant correlations between the ATP content in imbibed seeds and seed weight or seedling size and weight are clearly indicated in Fig. 1 to 3. McDaniel (6) observed in several pure lines of barley that seed weight is correlated with seedling size or vigor and he concluded that the quantity and the biochemical competence of mitochondria are responsible for the seed vigor. Even though mitochondria were not studied here, the results indicated that the quantity and possibly a higher phosphorylation efficiency of mitochondria present in heavier seeds must conserve more ATP in cells. Other possible differential efficiency such as nuclear phosphorylation capacity (1) may also exist in seeds of varied weight though little work has been conducted on plant materials. Nevertheless, this is a new finding that over-all energy supply is higher in heavier seeds at a very early stage of germination, and this high energy supply may extend to seedlings and sustain their higher growth potential (4).

Apparently, the correlation of ATP content and seed weight or seedling size was not affected by the seed chemical composition as crimson clover seeds are proteinaceous, rape seeds fatty, and ryegrass seeds starchy. The need of energy supply for the initiation of germination and maintenance of growth activities (2) is universal, so the chemical nature of seed reserves did not interfere with the energy synthesis at this early stage of germination. The ATP contents per seed, however, are different in these three species even though their weight range is similar. The large portion of endosperm in ryegrass seeds is probably insignificant to energy supply as all the endosperm cells (except aleurone layers) are devoid of mitochondria and contain dissociated nuclei (2). Therefore, the ATP content of ryegrass seeds was only several picomoles per seed. The difference between rape and crimson clover is obvious but difficult to explain.

Aging stress imposed during a long period of storage had reduced the ATP content in imbibed seed as shown by the data in Table I. Killing seeds by high temperature at high

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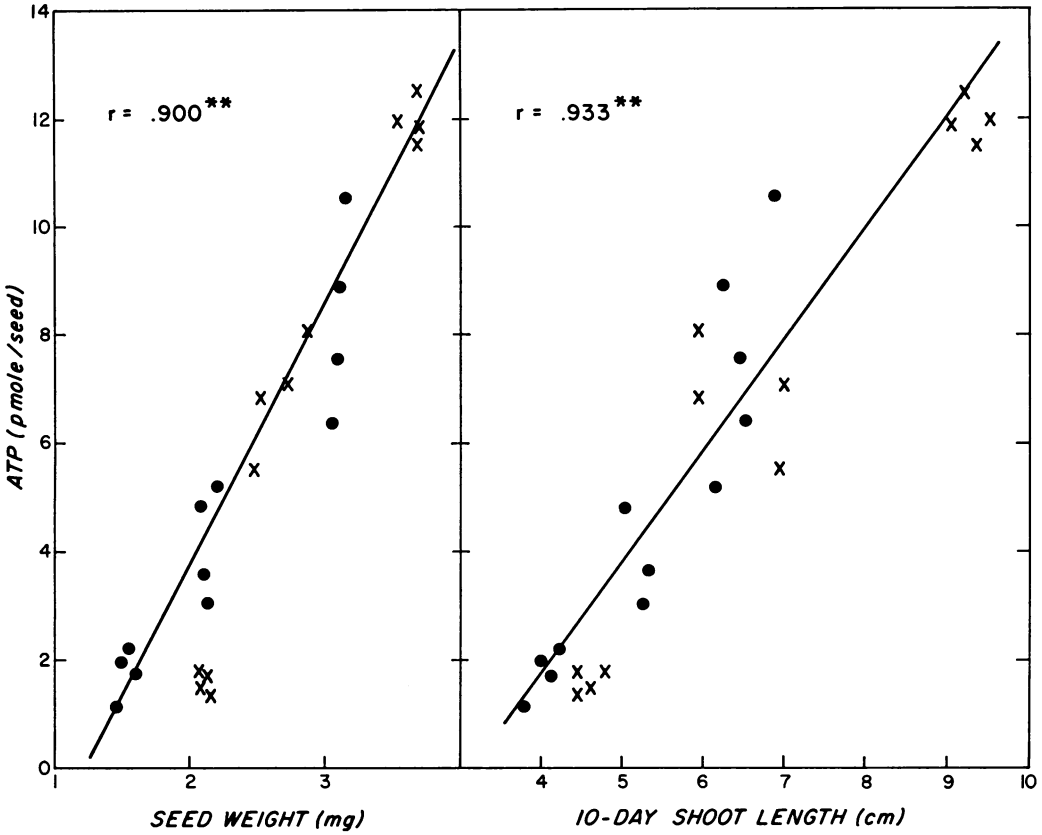

FIG. 1. Correlation of ATP content in 4-hr imbibed seeds and seed weight and 10-day shoot length of annual ryegrass seeds. X, ●: Different lots; r: correlation coefficient; **: significantly correlated at 1% level.

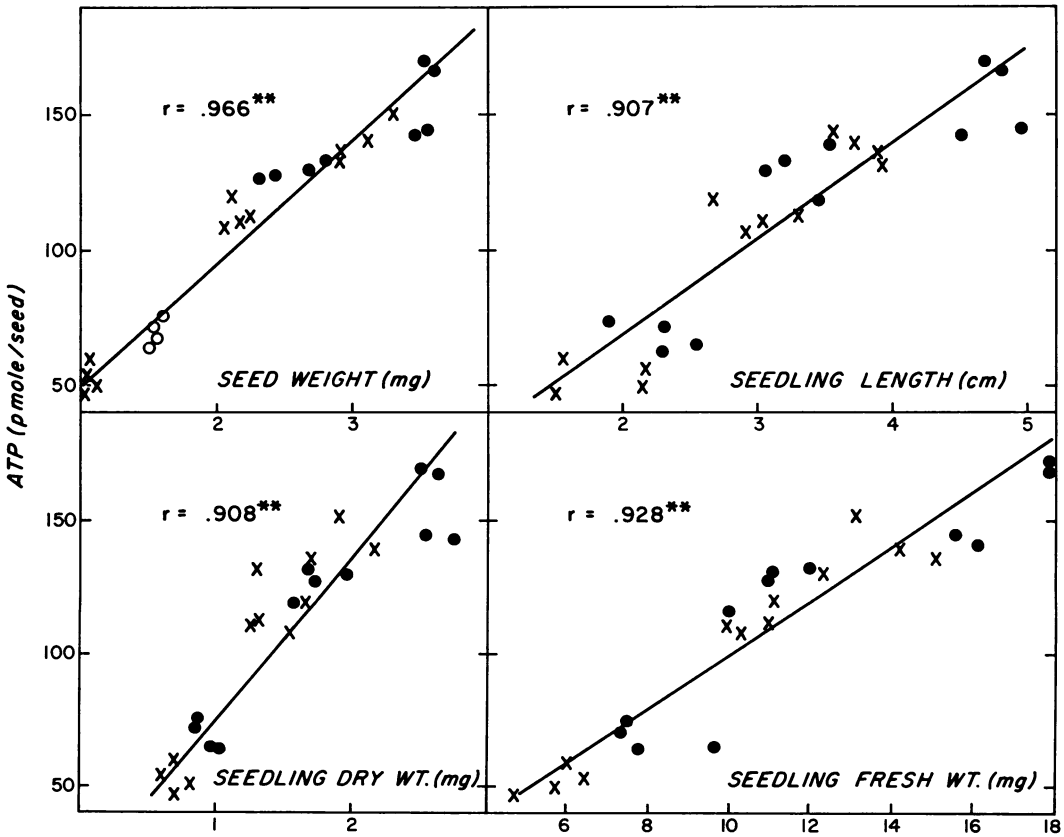

FIG. 2. Correlation of ATP content in 4-hr imbibed seeds and seed weight, 4-day seedling length, 4-day seedling fresh weight, and 4-day seedling dry weight of rape seeds. X, ●: Different lots; r: correlation coefficient; **: significantly correlated at 1% level.

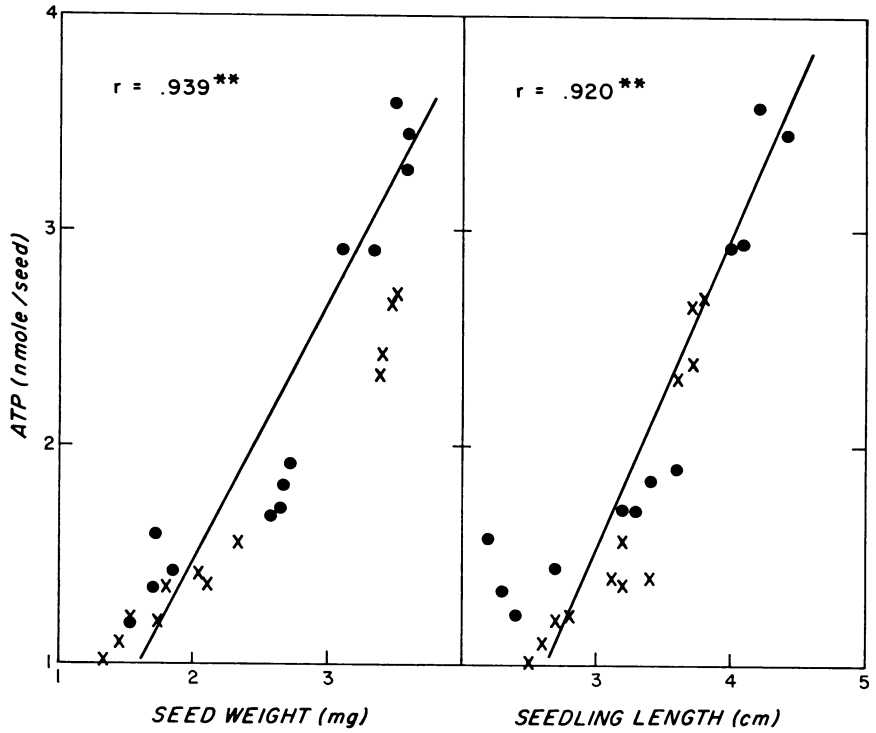

FIG. 3. Correlation of ATP content in 4-hr imbibed seeds and seed weight and 3-day seedling length of Dixie crimson clover seeds. X, ●: D if ferent lots; r: correlation coefficient; **: significantly correlated at 1% level.

Table I. *Aging Stress on ATP Content in Imbibed Seed, Germination Percentage, and Seedling Size of Crimson Clover*

The seeds were imbibed in water for 4 hr at 20 C. The data are the average of two replications.

	Seed Age			
	6 months	15 years		
	22 C	3 C	22 C	38 C
Seed weight, mg	2.9	3.8	3.8	3.2
ATP, nmole/seed	2.28	2.16	0.92	0.02
Germination percentage	98	96	69	0
4-Day seedling length, cm	4.4	3.0	0.9	0

moisture content also decreased ATP content from an average of 11 to 2 pmoles per ryegrass seed and from 150 to 26 pmoles per rape seed.

The level of ATP in plant tissue appears to be a very sensitive index to environmental and developmental changes. It is rapidly reduced upon chilling in cotton seedlings (8), under anaerobiosis in imbibed lettuce seeds (7), and in pea roots exposed to saline media (5). It increases in concert with the synthesis of organelles and enzymes in nondividing tissues (3, 7), and it also rises in pace with cell numbers and size of growing organs (3, 4, 7). Data reported in this paper add another dimension of the monitoring value of ATP content in plant materials, and they indicate a possibility of predicting seedling vigor without a long period of germination and growth.

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