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RELATIONSHIP BETWEEN ENDOGENOUS LEVELS OF MALIC ACID AND DORMANCY IN GRAIN OF *AVENA FATUA* L.

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Abstract—The endogenous level of malic acid in grain of *Avena fatua* L. is inversely correlated with the dormancy of the population. Induction of secondary dormancy by anaerobiosis results in a decrease in the level of malic acid. Imbibing seed incorporate $^{14}CO_2$ into malic acid before showing visible signs of germination. Inhibition of germination by continuous white light decreases such $^{14}CO_2$ fixation.

INTRODUCTION

CARBON dioxide is known to affect the germination of certain seeds[1,2] and in *Avena fatua* L. (wild oat) is involved in the negation of a dormancy mechanism in which light inhibits germination.[3] Besides possible effects on such environmental factors as, for example, pH, or on the seed coat, carbon dioxide could be directly involved in the metabolism of the germinating seed through carboxylation of pyruvic or phosphoenolpyruvic acids. The latter possibility is investigated in this paper. There seems to be some correlation between the percentage germination of samples of this grain and their malate content. Imbibing seeds fixed $^{14}CO_2$ into malate and related acids in the dark; the $^{14}CO_2$ fixation was markedly less in the light.

RESULTS AND DISCUSSION

Organic Acid Content

Unimbibed seed from populations showing different degrees of dormancy were assayed for malic and succinic acid. Table 1 lists the acid levels of the several populations tested, together with the corresponding germination percentages. In wild oat, the level of dormancy was inversely correlated with malic acid content. The level of succinic acid was relatively constant. The fact that the ratio of malic to succinic acid in cultivated oats approached that of the most dormant wild oat sample suggests that it is the level of malic acid which is related to germination rather than the ratio between these two acids.

A condition known as secondary dormancy can be induced in wild oats by imbibing the seed in anaerobic atmospheres.[4,5] Table 2 shows the changes in malic acid and succinic acid levels which accompanied the induction of dormancy under an atmosphere of argon. As

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[4] J. M. NAYLOR and L. A. CHRISTIE, *Proc. Western Sec. Natl Weed Comm., Canada* **10**, 56 (1956).
[5] J. R. HAY and B. C. CUMMING, *Weeds* **7**, 34 (1956).

TABLE 1. RELATIVE AMOUNTS OF MALIC AND SUCCINIC ACIDS IN DRY SEED FROM SAMPLES OF OATS DIFFERING IN DEGREES OF DORMANCY

Treatment	Germination (%)	μg acid per seed*		Malate/ succinate
		Malate	Succinate	
Cultivated oat (*A. sativa*)	95	23·1	11 8	1·96
Wild oat (*A. fatua*) 24 month post harvest	65	8·8	2·7	3·26
Wild oat (*A. fatua*) 12 month post harvest	45	7·0	2·9	2 41
Proximal grains (*A. fatua*) 1 month post harvest	25	4·3	2·5	1·72
Distal grains (*A. fatua*) 1 month post harvest	15	3·0	2·7	1·10

* Error associated with the organic acid determinations was 1·2–1·7 per cent of the μg values per seed.

TABLE 2. CHANGES IN LEVELS OF MALIC AND SUCCINIC ACIDS DURING DORMANCY INDUCTION UNDER ARGON

Time under dormancy inducing conditions* (hr)	Germination (%)	μg acid per seed		Malate/ succinate
		Malic	Succinic	
0	80	10·2	3·1	3·32
20	62	1·7	4·7	0·36
54	50	1·1	5·6	0·36
100	30	0·8	6·8	0 12
7 days	15	1·5	large†	—

* The degree of dormancy induced in the population was measured by germinating the treated samples under standard conditions.

† Not determined, but greater than 20μg/seed.

dormancy became imposed on an increasingly larger proportion of the population, the malic acid level fell while that of succinic acid rose. Examination of the paper on which the seeds were imbibed showed that malic acid was not leached from the seed during imbibition.

Tracer Studies

The ability of imbibing seeds to incorporate $^{14}CO_2$ into malic acid was investigated. In Table 3, a comparison is made between seeds imbibed in darkness and under continuous white light of an intensity which inhibits germination. After 40 hr imbibition under 1 per cent CO_2 (500 μC $^{14}CO_2$) i.e. before radicle appearance at 48 hr, there was marked fixation of carbon dioxide with 2–3 times more incorporation in darkness than in light. Thin layer chromatography of the organic acid fraction (Amberlite eluate) followed by autoradiography showed labelled acids to be malic, fumaric, citric (and/or isocitric) and one other corresponding to α-ketoglutaric or glycolic. It is noteworthy that no measurable activity was found in the amino acid fraction (Zeocarb eluate). Substantial activity was present in the neutral fraction (Amberlite effluent), presumably due to labelling of carbohydrates via reverse glycolysis.[6]

[6] H. BEEVERS, *Respiratory Metabolism in Plants*, Row, Peterson, New York (1961).

TABLE 3. ^{14}C-FIXATION BY WILD OAT GRAINS IMBIBING IN 1% $^{14}CO_2$ FOR 40 hr IN LIGHT OR DARKNESS

Material	Activity (cpm)			
	Light		Dark	
	Total	1 grain	Total	1 grain
Alcohol extract	$2{\cdot}0 \times 10^5$	$1{\cdot}14 \times 10^3$	$2{\cdot}81 \times 10^5$	$1{\cdot}61 \times 10^3$
Zeocarb effluent	$8{\cdot}38 \times 10^4$	$4{\cdot}75 \times 10^2$	$1{\cdot}35 \times 10^5$	$7{\cdot}95 \times 10^2$
Zeocarb eluate (amino acid fraction)	No activity		No activity	
Amberlite effluent (neutral fraction—carbohydrates)	$3{\cdot}9 \times 10^4$	$2{\cdot}23 \times 10^2$	$4{\cdot}2 \times 10^4$	$2{\cdot}4 \times 10^2$
Amberlite eluate (organic acid fraction)	$7{\cdot}37 \times 10^3$	$4{\cdot}21 \times 10^1$	$2{\cdot}67 \times 10^4$	$1{\cdot}52 \times 10^2$
Malic acid/grain		1·2 μg		2·6 μg
Activity of malic acid cpm/μg.	7·95		17·95	

Attempts to assess the importance of malic acid in the system by studying the utilization of an exogenous supply of the acid were unsuccessful due to lack of uptake. Caryopses which had been imbibed at 20° in darkness for 40 hr were incubated for 4 hr at 25° in a medium containing labelled malic acid (5 ml phosphate buffer pH 7·2, 1 ml 10^{-2} M malic acid, ^{14}C specific activity 20 μc/ml, 18 kernels per treatment). Autoradiograms of chromatograms of the organic acid fraction disclosed only labelled malic acid in trace amounts. Further evidence that this represents malic acid superficially bound to the seed arises from the fact that analysis by gas liquid chromatography showed no difference in the endogenous levels of acids before and after incubation in malic acid.

The inverse correlation noted here between dormancy and malate level suggests that the early observation[7] in *Avena fatua* L. of an increase in "seed acidity" with loss of dormancy during storage, may have been due to a rise in the level of malic acid.

The oxidation of succinate to fumarate and then to malate is a sequence common to both the tricarboxylic and glyoxylic acid cycles. Dehydrogenase activity, as indicated by the tetrazolium test, was not observed in dormant wild oat seed but was present in non-dormant seed.[8] In lettuce seed, succinic dehydrogenase was not detected in extracts from dry seed but became detectable as germination progressed.[9] The production of malic by carbon dioxide fixation could circumvent a block to respiratory synthesis of this acid.

EXPERIMENTAL

Germination

Avena fatua L. grain was obtained through Hasler and Co. (Dunmow, England) and used directly or grown for seed under natural conditions at Glasgow. Standard germination tests were carried out in 9 cm petri dishes, on Whatman Seed Test paper moistened with 5 ml water per replicate of 50 seeds, at 20° in darkness or under continuous incandescent irradiation of 6 μW/cm². In darkness, radicles first appear at 48 hr; appearance of further radicles is essentially over by 5 days.

Secondary dormancy was induced in after-ripened populations by imbibing grains under argon atmospheres in conical flasks After treatment the organic acid content of a portion of each sample was assayed, the remainder being used to estimate the degree of induced dormancy in the populations by germination tests under standard conditions in darkness.

[7] W. M. ATWOOD, *Botan. Gaz.* **57**, 386 (1914).
[8] J. R. HAY, *Can. J. Botany* **40**, 191 (1962).
[9] A. M. MAYER, A. POLJAKOFF-MAYBER and W. APPLEMAN, *Physiol. Plantarum* **10**, 1 (1957).

Radiotracer studies were performed on seed imbibing in 1 l. conical flasks with ground glass joints. The germination flasks were connected to a 50 ml flask containing enough Na_2CO_3 to generate a concentration of 1% CO_2 throughout the apparatus upon the addition of HCl. $NaH^{14}CO_3$ was added such that the level of activity was not less than 500 μc.

Extraction

Samples (2 g, i.e. 100 seeds) of imbibed seeds were plunged into 100 ml boiling 80 per cent ethanol for 10 min and then ground in a mortar and pestle. The brei was returned to the 80 per cent ethanol and further extracted for 6–8 hr at 20°. Unimbibed samples were first milled and the flour similarly extracted in 80 per cent ethanol.

The plant debris was removed by centrifugation and the supernatant fractionated by ion exchange chromatography,[10] using Zeocarb 225 (H^+ form) and Amberlite IR-4B (OH^- form) in 15 cm × 0·5 columns. The organic acid fraction was eluted from the Amberlite column with 2 N ammonium hydroxide. A solution of the free organic acids was obtained by a further passage through the cation exchange column.

Chromatography

(*a*) *Gas–liquid system. In vacuo* dried aliquots of the organic acid fraction were methylated with freshly prepared CH_2N_2 in ether or with methanolic BF_3. The methyl ester derivatives were taken up in $CHCl_3$ for chromatography against similarly treated standards in a Pye Argon Chromatograph, equipped with a preheater. The stationary phase was 10 per cent polyethylene glycol adipate on 85–100 GasChrom No. S. Operating conditions were: gas inlet pressure—10 lb/in^2; gas flow rate—50 ml/min: column temperature—125°; detector voltage—1500; chart speed—15 in/hr.

For quantitative determinations of specific organic acids by gas liquid chromatography an internal standard of nonadecane was included in each sample.[11,12] Standard curves for each acid were prepared by adding a similar amount of nonadecane to known amounts of the acids.

(*b*) *Thin-layer system.* Chromatograms were run on silica gel plates developed in 96 per cent EtoH: 20 per cent NH_4OH (100:40) or *n*-butanol:acetic acid:water (120:30:50). Bromocresolgreen was used as locating reagent. Labelled acids were located by autoradiography using Ilford Medical X-ray film, suitable autoradiograms being obtained after 7 days.

Further characterization of the organic acids was obtained by elution of the R_f region of a specific acid from a thin layer plate and again identifying the suspected acid by gas–liquid chromatography. A number of reconstruction experiments gave recovery values of malic acid of 65–71 per cent. Error associated with the processing of the extracts was low: 6 replicate extractions of a single population gave values of $12{\cdot}5 \pm 0{\cdot}2$ μg malic acid per seed.

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