Phytochemistry, 1975, Vol. 14, pp. 329–331. Pergamon Press. Printed in England.

PURINE AND PYRIMIDINE BASES AND NUCLEOSIDES OF GERMINATING *TRITICUM AESTIVUM* SEEDS

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(*Revised Received* 20 *June* 1974)

Key Word Index—*Triticum aestivum*; Gramineae; wheat; germination; purines; pyrimidines; nucleosides; adenine; guanine; uracil.

Abstract—Adenine, guanine, uracil and the corresponding nucleosides were identified in mature wheat grain. On germination, contents of these compounds changed in a regular pattern. Amounts of the free bases rose immediately and remained at the increased levels for a short period (3–6 hr). Nucleoside content decreased at the initial phase and increased sharply 12 to 48 hr after germination.

INTRODUCTION

While the nucleotide metabolism in germinating plant seeds has been extensively studied, data concerning the behaviour of nucleosides and free bases are scant. Brown [1] has shown that adenosine and uridine content decreases in pea seeds 16 hr after imbibition starts and he was unable to detect free bases at any stage of germination. Other studies [2–10] concerned with the acid-soluble fraction have given no indication for the presence of free bases in either dry or germinating seeds. We have previously [11] isolated adenine and uracil from the ethanol-soluble fraction of wheat grain. However, the use of ethanol for extraction has made these results questionable after it was found [12] that ethanol may serve as a water analogue for some hydrolytic enzymes surviving the alcohol treatment. Indirect evidence, based on the measurements of adenine- [13] and uracil- [14] phosphoribosyltransferase activities, does, however, indicate that both free adenine and uracil are present in wheat seeds. Moreover, extensive degradation and synthesis of RNA during early germination may be expected to proceed via release and reutilization of the free bases.

The present studies have demonstrated the occurrence of free bases in mature wheat grain. The levels of adenine, guanine, uracil and the corresponding ribonucleosides have also been found to change markedly during the investigated period of germination (0–48 hr).

RESULTS

Adenine, guanine, uracil and the corresponding ribonucleosides were identified in the $HClO_4$ extract of ungerminated wheat seeds. Neither cytosine nor cytidine could be found in the same material. Difficulties were experienced in the isolation of adenine, guanine and uracil, when the ethanol treatment of crude $HClO_4$ extract (see Experimental) was omitted.

The amounts of free bases and nucleosides isolated from wheat grain lots of various crop years are given in Table 1. While the nucleosides occurred in all the samples analysed at a comparable level, the amounts of free bases were different for grains harvested in various years. The levels of adenine, guanine and uracil were also influenced by the storage time and for this reason all the data reported are for grains stored for 3–4 months prior to the analysis.

Independently of the level of free bases in ungerminated grain, their content underwent regular changes after germination started. A considerable increase in the amounts of adenine, guanine and uracil could be observed after 3 hr imbibition (Fig. 1). The increased level was maintained for the next 3 hr, to drop markedly 12 hr after the beginning of imbibition. Further germination again resulted in an increase of these compounds. No similar pattern could be observed for nucleosides (Fig. 2). The content of adenosine, guanosine and uridine decreased somewhat at the onset of imbibition,

Table 1. Amounts of purine and pyrimidine bases and nucleosides in wheat grain (*Triticum aestivum*) lots from different crop years

Crop year	μmol/500 seeds*					
	Adenine	Guanine	Uracil	Adenosine	Guanosine	Uridine
1970	0·11	0·17	0·02	1·14	0·09	0·60
1971	0·01	0·01	0·01	1·12	0·11	0·65
1972	0·12	0·09	0·02	1·30	0·08	0·60
1973	0·15	0·16	0·12	0·80	0·14	0·73

* Weight of the 500 wheat seed sample was, on average, 23·6 g; moisture content, 12%.

remained at a relatively constant level for the next 6 to 9 hr and increased sharply at the end of the investigated period.

Changes in the fresh and dry wt of the investigated grain samples were similar to those reported previously [14] for the same germination period (0–48 hr). The leaching of free bases and nucleosides to the surrounding medium was negligible.

DISCUSSION

The variable amounts of free bases in apparently the same material may be a reason for the shortage of information on their occurrence in plant seeds. Moreover, the omission of ethanol treatment of $HClO_4$ extracts could have made the previous attempts [1] to isolate free bases unsuccessful. The ethanol treatment removes the bulk of the interfering substances and, apparently, prevents losses by surface adsorption or coprecipitation.

The rapid increase in the amount of free bases in the initial stages of seed germination is surprising. The observed changes seem to indicate that nucleosides are degraded to the corresponding bases immediately after imbibition starts. The increased level of the free bases is maintained in the seeds up to the 12th hr of germination, when ribosomal RNA synthesis is known [15] to be triggered. These observations confirm the suggestion of Brown [1] that nucleosides may represent a reserve of mononucleotides in mature seeds. Enzymes necessary to catalyse the reutilization of free bases, PRPP synthetase [13, 16], adenine [13] and uracil [14] phosphoribosyltransferases are known to be active from the beginning of germination.

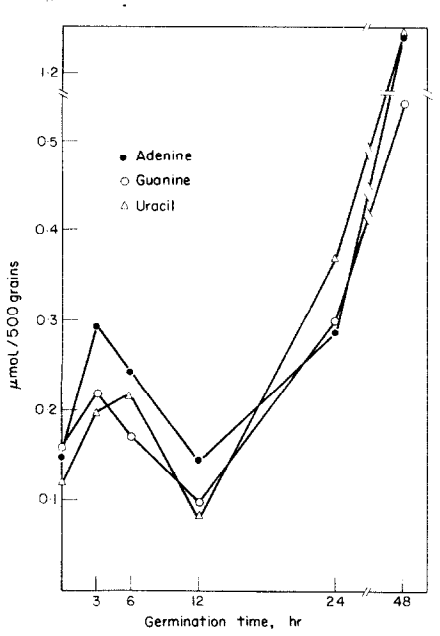

Fig. 1. Changes in adenine, guanine and uracil contents of wheat grain (*Triticum aestivum*) during germination.

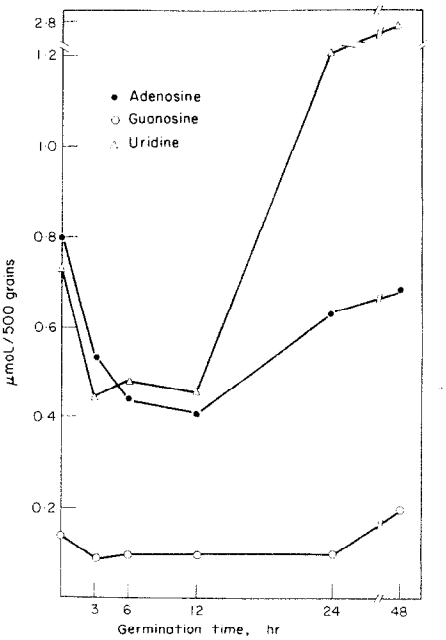

Fig. 2. Changes in adenosine, guanosine and uridine contents of wheat grain (*Triticum aestivum*) during germination.

EXPERIMENTAL

A sample of 500 wheat grains (*Triticum aestivum*), av 23·6 g, was surface-sterilized with 1% NaOCl and germinated at 22° in the dark for 0–48 hr. The whole sample was then homogenized with ice-cold 0·4 M $HClO_4$. The crude $HClO_4$ extract (*ca* 100 ml) was treated with 3 vol. of 96% EtOH to ppt. carbohydrate and other interfering materials. The supernatant was neutralized with KOH and, after removing the $KClO_4$ ppt., evaporated to dryness at red. press. The dry residue was dissolved in 20 ml of ice-cold 0·1 M $HClO_4$. Purine and pyrimidine derivatives were adsorbed from the soln on activated charcoal (1 g) and recovered with a warm Me_2CO–0·1 M NH_3 mixture [17]. The eluate was conc under red. press. (until all traces of Me_2CO and NH_3 were removed) and applied to a Dowex 1 × 1 (Cl^- form) column (1 × 20 cm). Purine and pyrimidine bases and nucleosides were eluted from the column with 250 ml H_2O. The H_2O effluent was conc and examined by PC in H_2O-satd *n*-BuOH as solvent system [18]. Partial separation of the individual compounds was achieved at this stage. Guanine plus guanosine, adenosine plus uridine, and adenine plus uracil were eluted from the corresponding areas of the chromatogram and finally separated by TLC. Guanine and guanosine were separated from each other on cellulose F plates using 0·01 M HCl as the solvent [19]. Similarly, adenosine, uridine, adenine and uracil were obtained as individual spots using silica gel F_{254} plates and *tert*-BuOH–MeCOEt–H_2O–NH_3 (4:3:2:1) solvent system [20]. The separated products were quantitatively eluted from the TLC plates as described by Wong [21].

The purified products were identified by spectrophotometric examination in acid, neutral and alkaline solutions. The measurements at λ_{max} in 0·1 M HCl were taken to calculate the amounts of the isolated products. ^{14}C-Labelled adenine, guanine, uracil, adenosine, guanosine and uridine could be re-isolated with 87, 60, 75, 73, 70 and 72% recoveries, respectively, when added to the crude $HClO_4$ extract. Uncorrected values are reported in Table 1 and Figs. 1 and 2. When [^{14}C]AMP and [^{14}C]UMP were added to wheat grains during homogenization with $HClO_4$ no significant radioactivity could be found in the corresponding nucleosides and free bases.

Acknowledgements—This work was supported by The Polish Academy of Sciences within the project 09.3.1 and by grant No. FG-Po-262 from The United States Department of Agriculture, Agricultural Research Service.

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