

Endogenous Growth Substances And Sugar Levels in Wheat Grains During Germination

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ABSTRACT

Wheat (*Triticum aestivum* L.) grains of the cultivar 'Sidi Misri 1' were germinated in petri dishes at 25°C for seven days. Samples were taken daily to evaluate the level of reducing and non-reducing sugars and the type of growth substances present in the seeds during germination. After 24 hours of germination, a predominant presence of growth promoting substances were found in the extract of seeds. A growth inhibiting activity, possibly due to salicylic acid and/or coumarin, was seen on the third, fourth and fifth day of germination. No promotive or inhibitory activity was detected on the seventh day of germination. Reducing sugars were steadily increased, however, the daily increment was significant only until the fourth day of germination. The non-reducing sugars increased on the second day of germination but disappeared afterwards.

INTRODUCTION

Freshly harvested grains of wheat and barley do not germinate well, possibly due to the presence of germination inhibitors. However, few months of dry storage had significantly improved the percentage of germination (6). Germination inhibitors are formed in the pericarp, endosperm, testa, and embryo of wheat grain and in the husks of barley (6, 9). The effect of these growth inhibitors could be offset by changes in the outer layers of pericarp during dry storage (16). A germination inhibitor, extracted from the husks of barley, was identified as coumarin. The application of coumarin to wheat and other seeds inhibited its germination (9). A growth inhibitor was also found in the hulls of wild oats (*Avena fatua* L.). This inhibitor had to be leached out before germination (16). Chilling treatment had been reported to be effective in breaking the dormancy of some seeds through the breakdown of inhibitors present in the seeds (15) or through the activation of some germination promoters in the embryo (16). A major germination inhibitor, abscisic acid (3, 14), was found to block the natural gibberellin mediated germination response. The effect of this inhibitor was antagonized by cytokinins, thereby, allowing gibberellins to function (8,13).

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During seed germination, starch is hydrolyzed into sugars which are translocated to the growing points (5). The breakdown of starch in rice grains took place four days after germination (10,11). Glucose was the predominant product of starch hydrolysis which was later converted into other forms of sugar (10,11,12). Both reducing and non-reducing sugars were present during germination of seeds. However, the concentration of reducing sugars was higher than non-reducing sugars (9,11).

This experiment was initiated to study the types of growth regulators present in wheat grains during germination and to associate this with the levels of reducing and non-reducing sugars.

MATERIALS AND METHODS

Four batches of 100 grains of wheat cultivar 'Sidi Misri 1' were germinated in petri dishes placed in a germinator at 25°C for seven days. Samples of the grains were taken in three replicates every 24 hours. These grains (with radicle and plumule removed, if outside the pericarp) were used for growth regulator and sugar analysis.

Endogenous growth regulators

Ten grams of wheat grains were crushed in a porcelaine mortar and extracted three times with absolute methanol, each for half an hour. The combined extract was evaporated to complete dryness under vacuum at 40°C using a flash evaporator and dissolved in 5 ml of absolute methanol. A volume of 500 microliters of this extract was streaked on 20 by 20 cm thin layer chromatography plates covered with 0.75 mm film of silica gel. These plates were developed in isobutanol — methanol — water (80:5:15 v/v) solvent system till the solvent rose to 10 cm. Sections of the different Rf zones were scraped off and bioassayed using cress (*Lepidium sativum* L.) seed germination test (1). For identification of endogenous growth regulators, some synthetic growth regulators were also developed as above. Their Rf values were determined using UV light and Salkowski's color reagent (7). The Rf values of these chemicals were: Indol 3-YL Acetic Acid = 0.70, Y-(3-indol YL) Butyric acid = 0.75–0.80, Kinetin = 0.65–0.70, Thiourea = 0.85–0.90, Salicylic acid = 0.85–0.90, and coumarin = 0.80.

Reducing and non-reducing sugars

Wheat grains (10.5 g) were crushed in a porcelaine mortar and extracted with 5 ml ethanol. Analysis of reducing and non-reducing sugars were carried out as given in the cereal laboratory methods (2).

RESULTS AND DISCUSSION

Endogenous growth substance

Data on the effect of extracts from wheat grains during germination on the germination of cress seeds are given in Table 1. After 24 hours of wheat grain germination, the predominant presence of growth promoting substance were found in the extract of grains at Rf 0.1, 0.2, 0.6, 0.7, and 0.9 as the percentage of germination was significantly

Table 1 Effect of extracts from wheat grains germinated for different days on the germination of cress seeds.

Rf values	0.1	0.2	0.3	Germination % of check							LSD .05
				0.4	0.5	0.6	0.7	0.8	0.9	1.0	
Days 1	120	122	106	105	117	122	140	111	122	115	18
2	85	91	85	95	119	84	79	87	92	101	
3	84	86	95	93	93	84	93	88	72	87	
4	91	105	101	94	111	106	100	92	73	84	
5	92	96	97	93	84	90	97	66	86	86	
6	88	83	96	87	99	84	92	90	86	84	
7	88	94	100	96	93	92	96	93	94	90	

increased above the check, being 120, 122, 122, 140, and 122, respectively. In general, there was a trend towards growth inhibition starting the second day of germination of all Rf values except at Rf 0.5 on the second day. The Rf values of 0.6, 0.7 and 0.9 seem to be especially significant, because the Rf values of all the synthetic growth substances tried in this study fell between 0.65 and 0.90. It is likely that kinetin, indoleacetic acid, and thiourea might have been among the growth promoting substances released by wheat grains on the first day of germination. The dominant presence of these growth promoters on the first day of germination have probably outweighed or antagonized any possible inhibiting activity in the early stage of germination (8, 13). The inhibitory effect which was obtained later on the third, fourth, and fifth day at Rf 0.8 and 0.9 might have been due to coumarin and/or salicylic acid. This was also reported by other workers (9). At the end of seven days when most of the seeds had germinated, no promotive or inhibitory effect was detected, probably indicating the breakdown or leaching out of all the endogenous growth substances.

Reducing and non-reducing sugars

Reducing sugars steadily increased from a very low level on the first day to about twelve times that on the seventh day. The increment on each subsequent day was significant from the first until the fourth day of germination. There was no significant increase in reducing sugars from the fourth to the seventh day (Fig. 1). Reducing sugars have been reported to be the major product of starch hydrolysis during germination of seeds (5, 10, 11, 12). Hydrolysis of starch in germinating wheat grains seems to start very early (after about 24 hours). The high concentration (4.5%) of reducing sugars attained at four days was approximately maintained until seven days when most of the seeds had germinated (Fig. 1). Hydrolysis of starch in rice grains had been reported to start after about four days (10, 11).

The percentage of the non-reducing sugars in wheat grains showed an increase during the second day of germination, then completely vanished on the third and the following days (Fig. 1). Perhaps, due to their hydrolysis into reducing sugars (4).

A relationship seems to exist between the endogenous growth substances and sugars during germination of wheat grains (Fig. 1). The dominant presence of growth promoters only 24 hours, after placing wheat grains in the germinator, seems to stimulate a steady and significant development of reducing sugars. The increment of reducing sugars was unchanged on the fourth day of germination, possibly due to the presence

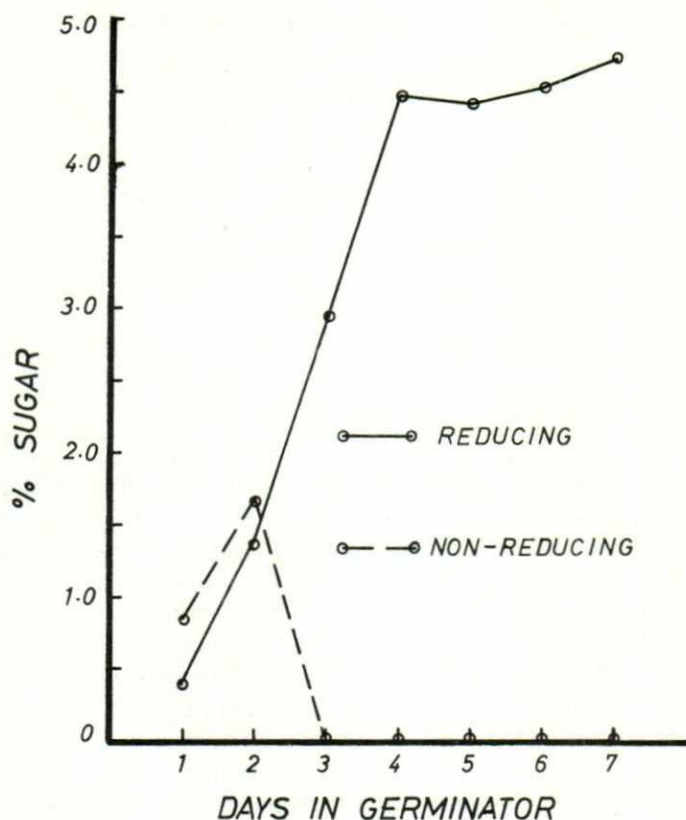


Fig. 1. The levels of reducing and non-reducing sugars in grains of wheat CV. Sidi Misri 1 during germination (LSD .05 = 1.24% Reducing sugars).

of growth inhibiting substances at Rf 0.8 and 0.9 on the third, fourth, and fifth days of germination. When most of the seeds had germinated on the seventh day, no promotive or inhibitory activity could be detected, indicating that growth promoters and inhibitors were released by wheat grains to stimulate or inhibit the development of reducing sugars.

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