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Do Phytohormones Influence the Uptake and Metabolism of Sucrose in Spikelets of Wheat?

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Summary

ASTHIR B., KAUR A. & BASRA A. S. 1998. Do phytohormones influence the uptake and metabolism of sucrose in spikelets of wheat? – Phyton (Horn, Austria) 38 (2): 293–299. – English with German summary.

Effects of GA_3, ABA and kinetin on ^{14}C-sucrose uptake and invertase activity were studied in three cultivars of wheat *(Triticum aestivum* L.), namely HD 2329, PBW 343 and Kalyansona, using detached ears cultured in a liquid medium containing phytohormones for 6 h (short culturing) or 3 days (long-culturing). During long culturing, addition of GA_3 and kinetin at 100 μM increased the accumulation of free sugars in all the cultivars, but ^{14}C-sucrose uptake and its conversion to starch was enhanced only in Kalyansona, suggesting differential hormonal responsiveness of the cultivars. Similarly, invertase activity especially that of neutral invertase was enhanced by GA_3 and kinetin. ABA inhibited both sucrose uptake, starch and invertase activity in all the cultivars. These results suggest that gibberellin and cytokinin are positive modulators of grain sink activity, whereas ABA is a negative modulator. Hormonal stimulation of sink activity was reflected in increased dry matter accumulation and the starch content of grains.

Zusammenfassung

ASTHIR B., KAUR A. & BASRA A. S. 1998. Beeinflussen Phytohormone die Aufnahme und den Stoffwechsel von Rohrzucker in den jungen Ähren von Weizen? – Phyton (Horn, Austria) 38 (2): 293–299. – Englisch mit deutscher Zusammenfassung.

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Es wurde der Einfluß von GA_3, ABA und Kinetin auf die Aufnahme von ^{14}C-Rohrzucker und die Invertaseaktivität in drei Weizensorten *(Triticum aestivum* L.), HD2329, PBW 343 und Kalyansona untersucht. Dazu wurden abgeschnittene Ähren in einem Flüssigmedium, welches Phytohormone enthielt, 6 Stunden (Kurzkultur) oder drei Tage lang (langdauernde Kultur) kultiviert. Bei langdauernder Kultur führte die Zugabe von GA_3 und Kinetin bei 100 µM in allen Sorten zu einer zunehmenden Akkumulierung von freien Zuckern, aber die ^{14}C-Rohrzuckeraufnahme und seine Umwandlung in Stärke war nur bei der Sorte Kalyansona größer. Dies weist auf eine unterschiedliche hormonelle Reaktion der einzelnen Sorten hin. Gleicherweise wurde die Invertaseaktivität, in besonderem die neutrale Invertase, durch GA_3 und Kinetin erhöht. ABA inhibierte sowohl die Rohrzuckeraufnahme, als auch Stärke und die Invertaseaktivität bei allen Sorten. Diese Ergebnisse legen die Gedanken nahe, daß Giberellin und Cytochinin die Aktivität des Verbrauches bei den Getreidekörnern positiv modulieren, ABA hingegen negativ. Die hormonelle Stimulierung des Verbrauches spiegelt sich in der Trockensubstanzproduktion und dem Stärkegehalt der Getreidekörner wider.

Introduction

The role of phytohormones in grain-filling processes, especially carbon uptake and metabolism is still unclear in cereals (MICHAEL 1980). The content of auxins, gibberellins and cytokinins changes considerably during grain growth in wheat (WHEELER 1972), each following a different time course. Cytokinins are highest at about anthesis and grain set, the gibberellin peak during mid grain development, and auxins towards the end of grain growth. The intrinsic capacity of the cereal grain to accumulate dry matter (kernel sink capacity) is possibly affected by both environmental and hormonal factors (CHEIKH & JONES 1994, LEE & al. 1997). Application of ABA to ears of wheat (DEWDNEY & McWHA 1979) and barley (TIETZ & al. 1981) stimulated ^{14}C assimilate transfer from the flag leaf to the ear. However, KING & PATRICK 1982 observed no stimulation of ^{14}C transport to wheat ears after injection of ABA into the grains. Studies with other phytohormones are, however, scarcely available.

Invertases are involved in the immediate metabolism of sucrose, the major translocated assimilate in higher plants and hence they are linked to assimilate distribution processes (HO 1988). The activity of invertases (both acid and neutral) has been associated with developmental processes of sinks (LOVELL & al. 1989) and they appear as potential targets for hormonal regulation. Indeed, an increase in acid invertase activity has been associated with the stimulation of stem growth induced by gibberellins (MORRIS & ARTHUR 1985) affecting the pattern of carbohydrate distribution as well (MORRIS 1982).

In the present study, the culturing of detached wheat ears in liquid media, mimicking near in vivo conditions, was used for studying the effects of phytohormones on the uptake and metabolism of sucrose in developing grains.

Material and Methods

Three cultivars of wheat *(Triticum aestivum* L.), namely HD 2329, PBW 343 and Kalyansona, were raised in the field under recommended agronomic practices.

Liquid culturing of detached ears

Ears at mid-milky stage, i.e. 12 days post anthesis (DPA) were cut under water below penultimate node and cultured according to the method of DONOVAN & LEE 1977 as modified by SINGH & JENNER 1983, keeping eight replications for each treatment. The concentrations in the culture medium of sucrose and L-glutamine were 117 and 17 mM, respectively. Phytohormones namely GA_3, ABA and Kinetin were added at 100 µM each in the culture media. Culture solution devoid of hormone was taken as the control. After adjusting the pH of the culture solution to 5.5, the medium was ultrafiltered through 0.22 µM Millipore membrane. Before culturing, the flag leaf and its sheath were removed and stems were surface sterilized with 40% EtOH followed by quick washing with distilled water. Ear-heads carrying 20 cm peduncle length from the cut end were placed (one ear-head per tube) in culture tube containing 35 ml cold-sterilized liquid medium. These cultured ear-heads were then transferred to an illuminated water bath maintained at 2–4° C (SINGH & al. 1991). After required culturing for 3 days, the grains were separated, freeze-dried and stored desiccated below 0° C till used for analysis. For enzyme studies, fresh grain samples were used.

Determination of free sugars and starch

Free sugars and starch were extracted and estimated from the freeze-dried grain samples as described previously (SINGH & ASTHIR 1988, ASTHIR & SINGH 1995a).

^{14}C-incorporation studies

Ears at mid-milky stage were detached under water. In each detached ear, stalk length was kept as 2 cm. The ears were then transferred to 14.6 mM [$U^{14}C$]-sucrose (one spikelet/0.4 ml plus 148 KBq respective radiolabelled sugar) containing 100 µM phytohormones. Sugar solution without hormone was kept as control. The preparations were aseptically incubated at 28° C for 6 h. After required culturing grains were separated, plunged immediately into hot 80% EtOH and stored below 0° C till used for analysis.

Invertase extraction

Invertase was extracted from the fresh grain samples essentially by the method decribed by ASTHIR & SINGH 1995a. The grain tissue was homogenized in extraction buffer containing 50 mM Hepes-NaOH (pH 7.0), 5 mM $MgCl_2$, 1 mM sodium EDTA, 2.5 mM DTT, 0.5 mg ml^{-1} BSA and 0.05% (v/v) Triton X-100, and centrifuged at 10,000 g, extracted again and the pooled supernatants were passed through Sephadex G-25 column equilibrated with the above buffer without EDTA and Triton X-100.

Invertase assay

Activities of soluble acid (pH 4.8) and soluble neutral invertase (pH 7.5) were assayed as per ASTHIR & SINGH 1995b.

The data were statistically analysed by analysis of variance using Duncan's multiple range test.

Abbreviations

ABA – abscisic acid; GA_3 – gibberellic acid; DPA – days post anthesis; DTT – dithiothreitol; EDTA – ethylene diamine tetracetic acid; Hepes-(n-2-Hydroxyethyl piperazine-N'–2-ethane sulphonic acid); BSA – bovine serum albumin.

Results and Discussion

Culturing of detached ears for 3 days in the presence of hormones showed variable effects on free sugar accumulation in grains of three cultivars of wheat (Table 1). Addition of 100 μM each of GA_3 and kinetin increased the content of free sugars in all the cultivars studied while ABA showed inhibitory effect. WAGNER 1974 also reported that the assimilate transport into the ears after treatment with phytohormones was promoted by GA_3 and markedly inhibited in the presence of ABA as compared to control. In short-term culturing of detached ears for 6h, the uptake of ^{14}C-sucrose was clearly stimulated by GA_3 and kinetin, but only in Kalyansona (Table 2). In both long- and short culturing experiments, GA_3 stimulated both sugar uptake as well as its conversion to starch in all the three varieties while kinetin showed this effect only in Kalyansona. The hormonal stimulation of grain sink activity in this way may thus stimulate grain-filling metabolism leading to increased dry matter accumulation, as also reflected in starch content (Tables 1 & 2). This is also indicative of the differential hormone responsiveness of wheat cultivars. Variation in gibberellin (McMASTER 1976), cytokinin (BANOWETZ 1997) and ABA responsiveness (BASRA & al. 1993) of wheat genotypes has been reported.

At the time of culturing, i.e. mid-milky stage, an appreciable activity of the invertases (acid, neutral) was recorded in developing grains and invariably, the activity of acid invertase was higher than that of neutral invertase indicating the predominant role of acid invertase. GA_3 and kinetin application increased the activity of invertase, especially neutral invertase (Table 3) while ABA reduced its activity. Although it has proven difficult to attribute different roles to invertase activities (SCHAFFER 1986, MASUDA & al. 1988), it is quite probable that the ability of wheat grain to express two types of invertases may permit them to utilize assimilates differently. While the soluble neutral invertase (probably cytoplasmic) could regulate the hexoses for grain growth, the acid (vacuolar) invertase could control the assimilate influx by increasing the sucrose gradient (MORRIS 1982,

Table 1.

Effect of phytohormones on the dry matter (DM), free sugars (FS) and starch content (mg g^{-1} FW) in three wheat cultivars.

	KALYANSONA			PBW 343			HD 2329		
Hormones	DM	FS	Starch	DM	FS	Starch	DM	FS	Starch
Control	4060.7^{c}	14.40.7^{b}	50411^{c}	42911^{b}	12.60.4^{b}	56014^{b}	42211^{b}	11.30.4^{b}	56014^{b}
GA_3	43213^{b}	16.10.9^{a}	67413^{a}	45213^{a}	14.20.6^{a}	72012^{a}	50010^{a}	12.80.3^{a}	76012^{a}
ABA	38212^{d}	13.50.8^{b}	41012^{d}	35513^{d}	10.00.6^{c}	2709^{d}	36912^{d}	9.30.3^{c}	40011^{c}
Kinetin	48313^{a}	17.20.8^{a}	60014^{b}	40016^{c}	15.60.7^{a}	42010^{c}	38014^{c}	12.50.4^{a}	42010^{c}

Table 2.

Effect of phytohormones on ^{14}C incorporation (cpm ml^{-1} g^{-1}) from [U-^{14}C] – sucrose into free sugars and starch in three wheat cultivars

	KALYANSONA		PBW 343		HD 2329	
Hormones	Free sugars	Starch	Free sugars	Starch	Free sugars	Starch
Control	2707113^{c}	10412^{c}	111422^{b}	1119^{c}	212294^{b}	10610^{c}
GA_3	5598359^{a}	94423^{a}	201324^{a}	79117^{a}	2959120^{a}	59014^{a}
ABA	84064^{d}	464^{d}	54416^{d}	354^{d}	64529^{d}	796^{d}
Kinetin	4199226^{b}	24319^{b}	82714^{c}	17211^{b}	73328^{c}	21111^{b}

Table 3.

Effect of phytohormones on the activities of acid and neutral invertases (n moles g^{-1} FW) in three wheat cultivars

	KALYANSONA		PBW 343		HD 2329	
Hormones	Acid	Neutral	Acid	Neutral	Acid	Neutral
Control	67424^{b}	32113^{b}	62213^{c}	3509^{c}	61014^{c}	33412^{c}
GA_3	68719^{a}	35412^{a}	64316^{b}	40510^{b}	65512^{b}	3809^{b}
ABA	66117^{b}	3039^{b}	62019^{c}	30511^{d}	61010^{c}	3029^{d}
Kinetin	62913^{a}	3679^{a}	67821^{a}	4829^{a}	68013^{a}	43011^{a}

ESTRUCH & BELTRAN 1991). Such hormonal stimulation results in an increase in sink activity which may then trigger grain growth.

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Recensio

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