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Short communication

Carbon monoxide sensitivity of cytochrome oxidase in wheat leaves in relation to dwarfing genes (*Rht*) in wheat cultivars

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Summary. Leaves of young seedlings of a number of tall cultivars of wheat, lacking the dwarfing *Rht* genes, readily responded to a brief 2 min exposure to CO, as assessed by in vivo aerobic assay of nitrate reductase. This test depends on the inhibition of cytochrome *c* oxidase by CO, which in turn renders cytosolic NADH available for the reduction of nitrate to nitrite in vivo. Semi-dwarf cultivars of wheat (*Rht* present) did not respond to CO in this way. Since CO forms a complex only with reduced cytochrome a_3, the results indicate differences in the redox state of cytochrome a_3, during in situ respiration of leaves from tall and semi-dwarf plants which are likely to be under genetic control.

Key words: *Rht* genes – Cytochrome oxidase – CO effects – *Triticum aestivum* L.

Introduction

Wheat cultivars containing *Rht* dwarfing genes are known to be insensitive to gibberellins (Gale and Marshall 1972, 1975; Singh and Paleg 1984a, b). During the course of investigations on the role of the cytochrome pathway in regulating NADH supply for nitrate reductase in wheat leaves in vivo (Naik and Nicholas (1986a), we observed differential effects of CO (which inhibits cytochrome *c* oxidase E.C. 1.9.3.1) on in vivo nitrate reduction in tall and dwarf cultivars. The CO effect was monitored by measuring nitrite production in the in vivo nitrate reductase assay under aerobic conditions (Sawhney et al. 1978a, b). In the tall cultivar 'Halberd' (lacking *Rht* genes), the CO treatment resulted in an appreciable production of nitrite from nitrate, while in the semi-dwarf cultivar 'Bindawarra' (*Rht-1* present), this did not occur (Naik and Nicholas 1986b). We now report on our results with 18 tall and semi-dwarf wheat cultivars, which show that cultivars containing the *Rht* genes do not respond to CO treatments. These results may be of interest to plant breeders and geneticists.

Materials and methods

Plant materials

Wheat (*Triticum aestivum* L.) cultivars supplied by the Agronomy Department of this Institute were grown in coarse, sterilized sand in a phytotron (16 h light/8 h dark cycle). The seedlings were supplied with 0.5 strength Hoagland solution containing 10 mM KNO_3. Leaves from ten-day-old seedlings were used. Information regarding the *Rht* genotype of the different cultivars was kindly provided by Dr. K. W. Shepherd of the Agronomy Department.

Experimental

Whole leaves (0.2 g) were placed in open tubes (2.5 × 15 cm) in the absence of aqueous solution and covered with black plastic to eliminate light. High purity CO from a cylinder was forced through the open tubes for 2 min. The tubes, which were open to the air, were then placed in a water bath and covered with a black plastic sheet. Under these conditions the atmosphere was saturated with water vapour so that leaves were not dehydrated during incubation. After incubation in the dark at 30° for 1 h, the reaction was terminated by adding 5 ml of boiling distilled water to the leaves. In order to extract all the nitrite from the leaves, the tubes were then placed in a boiling water bath for 10 min. Nitrite was then determined in suitable aliquots as described previously (Sawhney et al. 1978a). Additional experimental details are given in Table 1.

In vivo nitrate reductase

Leaves (0.2 g) were placed in Thunberg tubes (50 ml). After a rigorous evacuation with a two-stage vacuum pump, to remove the last traces of air, the tubes were closed and incubated for 1 h at 30° in the dark and then the nitrite produced was determined as described previously (Sawhney et al. 1978a).

Table 1. Response of leaves of wheat cultivars to CO treatment. Whole leaves of 10-day-old seedlings of tall and semi-dwarf cultivars were used for the in vivo aerobic assay of nitrate reductase after 2 min CO exposure as described in Methods. In vivo nitrate reductase activity was also measured under anaerobic conditions as described in "Materials and methods". Units, NO_2^- produced (μmol/gf · wt/h)

Tall wheats			Semi-dwarf wheats		
Cultivar	In vivo anearobic	In vivo CO-aerobic	Cultivar (*Rht* gene)	In vivo anaerobic	In vivo CO-aerobic
'Halberd'	3.83	2.64	'Bindawarra' (*Rht-1*)	3.24	Nil
'Olympinc'	3.85	2.66	'Warigal' (*Rht-1*)	3.93	Nil
'VDM'	3.38	0.74	'Egret' (*Rht-1*)	3.64	Nil
'Kolbee'	3.79	1.80	'Sunstar' (*Rht-1*)	2.79	Nil
'Kewell'	3.65	0.60	'Millewa' (*Rht-1*)	4.45	Nil
'Sabre'	3.31	0.58	'Oxley' (*Rht-1*)	3.18	Nil
'PF/41/W4E'	2.87	0.59	'Olsen' (*Rht-1,2*)	2.99	Nil
'60048 Aldirk'	2.63	0.63	'Bayonet' (*Rht-2*)	3.03	Nil
'60019 Festiguay'	4.01	0.48	'Spear '(*Rht-2*)	3.34	Nil

Results and discussion

As seen in Table 1, all cultivars, tall and semi-dwarf, have significant in vivo nitrate reductase activity, assayed under anaerobic conditions, when oxidation of cellular NADH by O_2 is completely inhibited. However, marked differences in response to CO were observed in the two types of cultivars. Thus, all the tall cultivars responded to exposure to CO resulting in nitrite production in vivo under aerobic conditions. The responses varied between 0.48 μmoles NO_2^-/gf·wt/21 h in 'Festiguay' to 2.66 in 'Olympic'. On the other hand, all the semi-dwarf cultivars tested were completely insensitive to CO treatment.

It is known that under aerobic conditions, nitrite production is not detected during in vivo assay of nitrate reductase, unless oxidation of cellular NADH by O_2 via the mitochondrial electron transfer chain is inhibited (Sawhney et al. 1978a, b). Cytochrome *c* oxidase, which reacts with O_2 is inhibited by CO, since a cytochrome a_3-CO adduct is produced (Wikström et al. 1981; Brunori et al. 1985; Denis and Richaud 1985). CO complexes with cytochrome a_3 only when it is in the reduced form. When this occurs cellular NADH becomes readily available for nitrate reduction, even under aerobic conditions. It therefore appears that a lack of response of semi-dwarf cultivars to CO, is a direct consequence of the oxidised state of cytochrome a_3 in leaf mitochondria during steady state respiration. This could be due to a tight coupling between electron transfer and oxidative phosphorylation, so that cytochrome a_3 is always maintained in an oxidised state. We have reported that in the semi-dwarf cultivar 'Bindawarra' (*Rht-1*), pretreatment of the leaves with uncouplers of oxidative phosphorylation such as 2,4-dinitrophenol or carbonyl cyanide m-chlorophenylhydrazone rendered them responsive to CO treatment (Naik and Nicholas 1986b).

The redox state of cytochrome a_3 in various cultivars, would depend on the oxidation reduction state of the mitochondrial electron transfer chain, as well as on the in situ tightness of the coupling to phosphorylation. These parameters involving respiration may well vary between cultivars, since they are genetically determined and this may explain the differential responses to CO in tall and semi-dwarf wheats.

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