ROLE OF ROOTS, HORMONES AND LIGHT IN THE SYNTHESIS OF NITRATE REDUCTASE AND NITRITE REDUCTASE IN RICE SEEDLINGS

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1. Introduction

Light plays an important role in the synthesis of nitrate reductase in higher plants [1, 2]. It was suggested that redox changes associated with the Hill reaction might be involved in the induction of both nitrate reductase and nitrite reductase in plants [3, 4]. In the case of tobacco leaves, however, it was reported that requirement for light could be replaced by providing appropriate concentrations of gibberellic acid and kinetin in the dark, suggesting thereby that light per se is not required but acts by supplying the hormones [5, 6]. It is believed that roots are important centres for the synthesis of the hormones, which are transported to the shoots [7, 8]. We now report that excision of roots depresses the synthesis of nitrate reductase and nitrite reductase in the leaves of rice seedlings and application of gibberellic acid (GA_3) to excised seedlings restores the level of enzyme synthesis. However, even in the presence of GA_3 and kinetin, light is still required for the induction of the enzymes.

2. Materials and methods

Since the removal of roots is likely to affect the uptake of nitrate anions, it is necessary to ensure that the concentration of the inducer in the shoot portion is not a limiting factor in the synthesis of the enzymes. In order to achieve this, rice seedlings (variety Improved Sabarmati), grown in distilled water and normal light, were initially transferred to dark for about two days, so as to eliminate the observed residual effect of light on the induction of the enzymes [4]. At this stage complete Hoagland's solution containing 15 mM KNO_3 was supplied in the dark for 12 hr. During this period the plants absorbed sufficient nitrate but since they were kept in the dark, significant synthesis of the two enzymes did not occur. These plants containing adequate nitrate concentration and with developed chloroplasts were then used to study the effect of excision of roots on the enzyme synthesis in the shoots. After removal of the roots with a sharp knife, the shoots were exposed to normal light [4], for different time intervals as indicated in the tables. Nitrate reductase and nitrite reductase were extracted and assayed by established methods using NADH and reduced methyl viologen as reductants respectively [4]. Where indicated gibberellic acid (GA_3) and kinetin (6-furfuryl aminopurine) were applied as foliar sprays with 0.1% Tween 20, given at every 4 hr interval, with the help of an atomiser. The hormones were also added to the Hoagland's solution. Both GA_3 and kinetin, when added to the assay mixture, did not have any effect on enzyme activities in vitro. Nitrate reductase and nitrite reductase activities were expressed as μmoles of NO_2^- formed or reduced/g tissue/hr respectively.

3. Results

When roots were removed, synthesis of nitrate reductase and nitrite reductase in the shoots of green seedlings exposed to light declined after 8 hr as compared to intact seedlings. After 24 hr the decrease in excised seedlings was more than 60% (table 1).

It is noteworthy that when GA_3 at 4 μg/ml was supplied to excised green seedlings, the activities of

Table 1
Effect of excision of roots on the synthesis of nitrate reductase and nitrite reductase in shoots

Time after exposure to light (hr)	Nitrate reductase μmoles NO_2^- formed/ g tissue/hr		Nitrite reductase μmoles NO_2^- reduced/ g tissue/hr	
	Intact	Excised	Intact	Excised
4	1.12	1.12	10.1	6.0
8	1.57	0.70	22.4	14.1
24	2.53	0.93	20.3	8.40

Twenty days old seedlings grown in light and provided with nitrate in the dark were used and roots were excised. The excised seedlings were then exposed to light. Enzyme activities were assayed at 4, 8 and 24 hr time intervals and compared with those found in intact seedlings receiving identical treatment.

Table 2
Effect of GA_3 on enzyme synthesis in excised seedlings

Treatment	GA_3 added μg/ml	Nitrate reductase	Nitrite reductase
Intact	Nil	3.9	16.7
Excised	Nil	0.7	9.1
,,	4	4.0	19.5
,,	8	14.5	34.7
,,	20	8.8	21.3
,,	40	4.1	16.7
,,	80	2.3	9.3
,,	120	2.3	11.1

Excised seedlings were treated with GA_3 and after 12 hr light exposure, enzyme activities were assayed as described in Materials and methods.

Table 3
Effect of GA_3 on the synthesis of enzymes in green intact seedlings exposed to light or dark

GA_3 added μg/ml	Light treatment		Dark treatment	
	Nitrate reductase	Nitrite reductase	Nitrate reductase	Nitrite reductase
Nil	13.9	45.4	0.8	8.8
4	15.1	30.3	0.6	7.2
8	12.8	27.7	0.7	7.8
20	13.3	27.7	0.7	7.1
40	13.9	31.7	0.7	7.0
80	13.9	31.7	0.7	6.5
120	13.9	30.5	0.7	6.4

Intact seedlings grown in light were transferred to dark after providing nitrate. One set of seedlings was continued in light. Different concentrations of GA_3 were supplied. After a 24 hr induction period enzyme activities were assayed.

Table 4
Effect of GA_3 and kinetin on the synthesis of enzymes in the dark

Hormone added	Etiolated seedlings		Green seedlings kept in the dark	
	Nitrate reductase	Nitrite reductase	Nitrate reductase	Nitrite reductase
Nil	0.09	1.02	0.27	12.05
GA_3 + kinetin	0.07	1.00	0.54	14.05

GA_3 at 8 μg/ml and kinetin at 20 μg/ml were supplied in combination to completely etiolated seedlings or to green seedlings kept in the dark during induction. After a hr induction period enzymes were extracted and assayed.

both the enzymes were restored to the levels found in intact seedlings (table 2). Maximum increase in enzyme activity was found at 8 μg/ml GA_3 but at higher concentrations upto 120 μg/ml a steady decline occurred. Results in table 3 show that GA_3 had no effect when added to intact seedlings exposed to light and at higher concentrations it did not depress the enzyme synthesis, as was observed in excised seedlings. Thus intact seedlings probably have a mechanism to counter the effect of excess GA_3, which is lost when the roots are removed. Results in table 3 also show that in the dark GA_3 at different concentrations could not stimulate the synthesis of the enzymes in intact green seedlings. Thus GA_3 could not replace the requirement for light.

Similar experiments were conducted with different concentrations of kinetin, ranging from 4 to 80 μg/ml. Kinetin was not very effective in restoring the synthesis of enzymes in excised seedlings. At 20 μg/ml it enhanced the activities of nitrate reductase and nitrite reductase by 25 and 10% respectively in rootless green seedlings exposed to light. When GA_3 and kinetin were supplied together the enhancement in enzyme synthesis in excised seedlings was the same

as was observed with GA_3 alone. GA_3 and kinetin in combination at their optimum concentrations, however, failed to stimulate the synthesis of the enzymes in completely etiolated intact seedlings and in normal green seedlings kept in the dark (table 4). In the latter type of seedlings different concentrations of GA_3 and kinetin ranging from 5 to 100 μg/ml were also ineffective in stimulating the synthesis of the enzymes in darkness.

4. Discussion

Removal of roots depressed the synthesis of nitrate reductase and nitrite reductase in the apical portions and addition of GA_3 to such seedlings stimulated enzyme synthesis. The site of synthesis of gibberellins in plants is not definitely established, but our results indicate that in rice seedlings, the roots must be contributing substantially for maintaining appropriate concentration in the shoots. It is noteworthy that in the absence of light, GA_3 alone or in combination with kinetin could not restore enzyme synthesis in intact green or etiolated seedlings. Thus, the strict requirements for light could not be replaced by hormones supplied externally. This conclusion is contrary to the observations in the case of tobacco leaves, where appropriate concentrations of GA_3 and kinetin were reported to induce the synthesis of nitrate reductase in the dark [5, 6]. Since the latter experiments were conducted with young leaves of tobacco plants which were grown for two months in light, the observed synthesis could be a manifestation of residual effect of light. Even in complete darkness, the activitity of nitrate reductase in control leaves was about 25% of that found in leaves exposed to light [5, 6]. This is quite high as compared to the activities usually observed in completely etiolated seedlings or in green seedlings which have been given dark treatment for a sufficiently long period so as to eliminate the residual effect of light. It has previously been observed that the residual effect of light in rice seedlings persists for about 12 hr even after transfer to the dark [4]. Stimulation of nitrate reductase synthesis by GA_3 has been confirmed by our results also, particularly when the supply of GA_3 is a limiting factor after the roots are excised. However, that does not mean that gibberellins can induce the synthesis of the enzymes in complete darkness. For this light reaction of photosynthesis seems to be essential.

References

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