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cys1組み替え植物におけるイオウ代謝の制御と大気汚染抵抗性

Molecular regulation of sulphur metabolism and resistance of cys1 transgenic plants to gaseous pollutants.

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Abstract

Transgenic tobacco plants expressing the wheat cys1 gene, encoding O-acetylserine (thiol) lyase which catalyses the conversion of sulphide to cysteine, were previously shown to be resistant to H_2S gas. In the present study, the transgenic plants, unlike control plants, were also found to be resistant to toxic levels of both SO_2 and paraquat, which induce free oxygen radicals, suggesting that the higher cysteine levels in the transgenic plants act to protect the plants from oxidative damage. In addition, cys1 transcript levels were found to be regulated by nitrates and dark conditions, providing a mechanism for co-regulation of the plant sulphur-nitrogen status.

小麦のcys1遺伝子は、O-ｱｾﾁﾙｾﾘﾝ（ﾁｵ-ﾙ）ﾘｱ-ｾﾞをｺ-ﾄﾞしており、この酵素は硫化水素をｼｽﾃｲﾝに変える働きをしている。cys1遺伝子を発現しているﾄﾗﾝｽｼﾞｪﾆｯｸﾀﾊﾞｺは硫化水素ガスに抵抗性であることを以前に示した。本研究では同ﾀﾊﾞｺ二酸化ｲｵｳやﾊﾟﾗｺｯﾄ（これらは酸素ﾗｼﾞｶﾙを生成する）にも抵抗性があることを明らかにした。恐らく量的に増えたｼｽﾃｲﾝやｸﾞﾙﾀﾁｵﾝが酸素による傷害を軽減したものと思われる。さらに、cys1の転写産物は窒素や暗所処理によって制御されていることがわかった。これは窒素とｲｵｳの動態が同時に制御されていることを示唆している。このような制御に関与するﾌﾟﾛﾓ-ﾀ-部位を解析中である。

Purpose

Many sulphur compounds (such as SO_x, H_2S) are highly toxic and have a significant impact on the global environment, health, and animal and plant life. In plants, it has now become essential to understand the molecular mechanisms that regulate the sulphur status and to identify methods by which this status can be altered to suite particular environmental conditions. Previously, we isolated and characterized the gene, cys1, encoding O-acetylserine (thiol) lyase, or cysteine synthase, which catalyses the conversion of sulphide into cysteine. This enzyme has been implicated not only in the control of the sulphur status of plants, but also in mechanisms of detoxification of sulphur and other toxic compounds, such as SO_2, H_2S and HCN. We also showed that transgenic tobacco plants, expressing the wheat cys1 gene, had several-fold higher activities of O-acetylserine (thiol) lyase and were resistant to toxic levels of H_2S gas (Youssefian et al 1993). In addition, more recent results indicated that the level of cys1 transcripts was regulated by both nitrate and dark conditions, providing some insight into the co-regulation of the sulphur and nitrogen status of plants.

The purpose of the current research was therefore to examine the mechanism of resistance of the cys1 transgenic plants to various sulphur compounds, especially SO_2, and to obtain a greater understanding of the molecular mechanisms that regulate expression of the cys1 gene and which thus regulate the plant sulphur-nitrogen status.

Progress

Mechanism of Resistance to SO_2 gaseous pollutants

The possible resistance of the transgenic plants to SO_2 gas was analyzed by exposing control and cys1 transgenic plants to various SO_2 levels. Whereas control plants showed high levels of chlorosis, transgenic plants showed no or very little signs of damage. This differential response of the plants was not due to variation in stomatal conductance, which was equally reduced in all plants by fumigation.

As SO_2 gives rise to free oxygen radicals, which affect photosynthesis notably by inactivating PSII, differences in chlorophyll fluorescence was used as an indicator of differential responses to fumigation. While control plants showed a dramatic reduction in fluorescence, there was only a limited reduction in the fluorescence of transgenic plants in response to fumigation. Similarly, oxygen evolution, as an indicator of photosynthetic activity, demonstrated that fumigation inhibited photosynthesis in the control but not in the transgenic plants. These results clearly demonstrated that the cys1 transgenic plants were more resistant to SO_2 than control plants and that this was due to internal metabolic processes.

In order to determine whether this resistance was directed solely to sulphurous compounds or to the free oxygen radicals produced by SO_2, leaf discs were exposed to various concentrations of paraquat (methyl viologen (MV)) which induce free radicals. Again, while control plants showed high levels of chlorosis at concentrations of MV greater than 0.4uM, transgenic plants did not show chlorosis until subjected to concentrations greater than 2.0uM. A time-course of electrolyte leakage from leaf discs exposed to 20mM MV showed a greater and faster leakage from control plants than transgenics. Finally, the levels of non-protein SH groups, including cysteine and other sulphydryl compounds were found to be consistently higher in the transgenic than in control plants.

Regulation of cys1 expression by nitrate and dark treatment

The cys1 transcript levels, as determined by Northern blot analysis, were found to be suppressed in the absence of nitrates (N) and in the light. In the presence of N, there was a dramatic increase in transcript levels in the dark but only a slight increase in the light. Kinetic analysis showed that transcripts were induced by N or by dark between 4 and 12 hours after the start of treatment. Neither sulphates nor ammonium affected cys1 transcript levels. The OASTL enzyme activity in wheat plants was also found to be significantly increased by N and growth in the dark. In order to clarify more precisely the cys1 transcriptional regulation, a cys1 clone was isolated from a wheat genomic library. A 2.7 kb fragment of the 5' upstream region was subcloned into pBluescript vector, and the nucleotide sequence of the fragment was partially determined. Promoter deletion fragments-GUS reporter gene fusions are to be used to transform tobacco plants, and the inducibility of the GUS gene by nitrates, dark and other factors will be determined.

Accomplishments

The clear resistance of the cys1 transgenic plants to both SO_2 and paraquat provides a basic mechanism by which this resistance is achieved. Overall, the results suggest that the higher cysteine synthase activity of the transgenic plants provides the plants with a higher capacity to convert excess sulphur to cysteine which in turn acts, as does glutathione, as a potent reducing compound which can detoxify free oxygen radicals.

The identification of factors regulating cys1 transcript levels provides a clear understanding of how sulphur metabolism is co-regulated by the plants' nitrogen status and light/dark signals.

Future Prospects

Such cys1 transgenic plants will certainly be of direct applicable importance, providing a genetic source of resistance not only to the increasing levels of global pollutants but also to other environmental stresses, such as cold, drought, UV, ozone and pathogen attack, which also appear to function through the production of oxygen radicals. These studies may constitute the first step in the production of transgenic crop and forest plants resistant to a variety of environmental pollutants.

The study of the regulation of cys1 expression is one of basic importance. It will first provide a more general understanding of how cys1 expression, and indeed the expression of other genes, may be regulated by nitrogen and darkness. More importantly, however, specific motifs involved in the induction of cys1 will be identified and will lead the way for identification of cis-acting transcriptional factors that regulate expression. This will have great potential to explain how the sulphur status of the plant is regulated, and how this regulation may be modified.

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