SHORT PAPERS

Photoacoustic Assessment of the *in vivo* Genotypical Response of Corn to Toxic Aluminium

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An open-cell photoacoustic (OCPA) spectroscopic technique is described which can assess genetically rooted differences in the response of live specimens of corn (*Zea Mays* L.) to the toxic action of aluminium. Specimens from a susceptible and a tolerant inbred line were examined. The differences in their OCPA *in vivo* leaf spectra were evident and an interpretation is given based on the genetically controlled response of the inbreds to aluminium toxicity. An earlier discrimination among specimens is obtained with this technique than has been possible so far with previous techniques.

Keywords: *Photoacoustic spectroscopy; open-cell photoacoustic detector; aluminium toxicity in plants; genetically induced tolerance to aluminium toxicity; corn leaf spectrum*

Several reports have appeared on the development of inbred lines of corn (*Zea mays* L.) in which both genetic variability and tolerant genotypes occur in relation to the toxic effects of aluminium.[1–5] Hence a non-destructive analytical method for evaluating the differences in plant response to aluminium toxicity would be of value.

Photoacoustic (PA) spectroscopy has been shown to be an increasingly important tool in plant science related analytical investigations.[6–11] This paper discusses its use in connection with the assessment of the aforementioned differences. Although the interpretation of the data, as far as accounting for a response pattern is concerned, is still preliminary, the demonstration of the usefulness of PA spectroscopy in the present context is conclusive.

Experimental

Specimens

Details of the specimens used have been given elsewhere.[4,5] Two lines were chosen: L1037 (Al^{3+}-susceptible) and L922 (Al^{3+}-tolerant). The aim was to compare the *in vivo* PA leaf spectra while keeping the specimens continuously exposed to different concentrations of aluminium.

Set-up and Procedures

In vivo PA leaf spectra were recorded using the open-cell photoacoustic (OCPA) configuration described by da Silva *et al.*[12] and Bento *et al.*[13] This acted as the detecting element in a laboratory-built PA spectrometer consisting of a 1000-W xenon arc lamp (Oriel), a Jarrell-Ash monochromator, a Princeton Applied Research (PAR) rotating blade light chopper, a PAR lock-in amplifier, a Hewlett-Packard *x* - *y* recorder and a suitably interfaced Commodore micro-computer for on-line data acquisition and processing. Modulation was set to 20 Hz and all spectra were normalised to a carbon-black reference. The aim was to detect significant spectral differences between seedlings from a given inbred line and those from an equivalent line differing from the former only by the fact that they had been grown in a nutrient solution to which 9 p.p.m. of Al^{3+} had been added. Experiments were carried using pairs of seedlings of either the L1037 or L922 line. One member of each pair was grown under a zero concentration of Al^{3+} and served as a control specimen; the other, grown under a concentration of 9 p.p.m. of Al^{3+}, served as the experimental specimen.

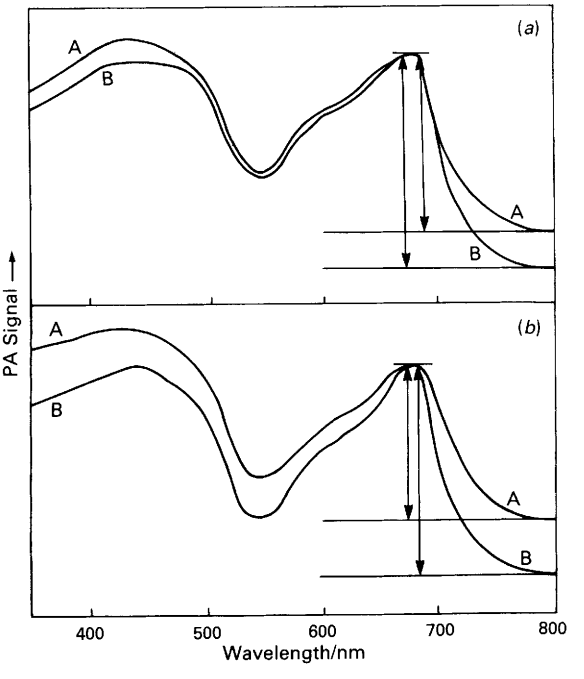

Fig. 1. Photoacoustic spectra for Al^{3+}-susceptible L1037 specimens. A, Specimens grown in a nutrient solution to which 9 p.p.m. of Al^{3+} has been added; and B, specimens grown in Al^{3+}-free nutrient solution; the arrowed lines indicate intensities measured at the chlorophyll band peak with respect to the corresponding spectral base line. (*a*) Spectra of specimens exposed to the corresponding nutrient solution for 33 h; and (*b*) spectra of specimens after 57 h of exposure

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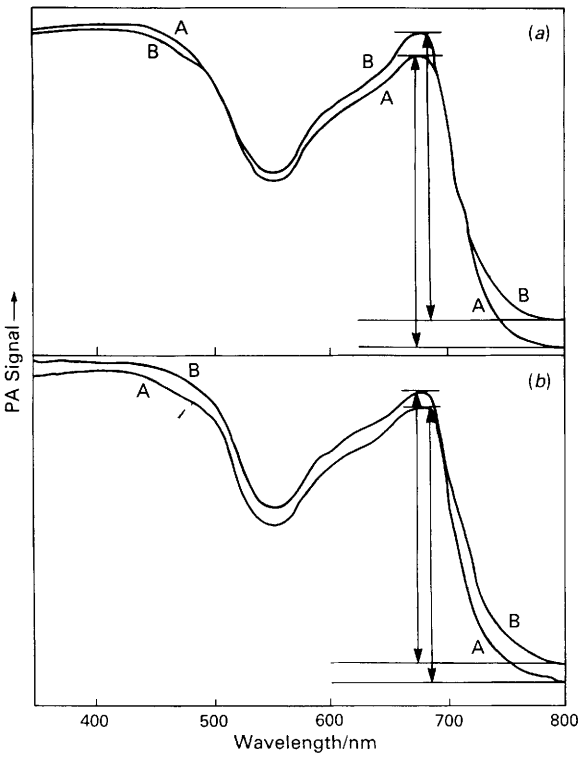

Fig. 2. Photoacoustic spectra for Al^{3+}-tolerant L922 specimens (symbols as in Fig. 1)

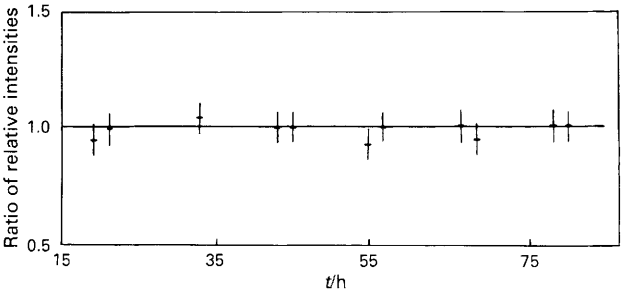

Fig. 3. Plot of ratio of relative intensities *versus* time for Al^{3+}-tolerant L922 specimens (for details see text)

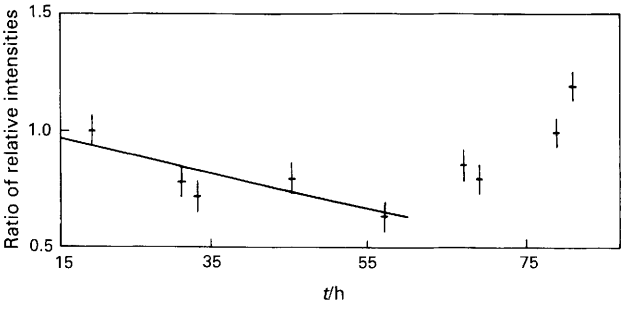

Fig. 4. Plot of ratio of relative intensities *versus* time for Al^{3+}-susceptible L1037 specimens (for details see text)

Results and Discussion

Figs. 1(*a*) and 2(*a*) and 1(*b*) and 2(*b*) show representative PA leaf spectra for some selected specimens taken after continuous exposure to the corresponding nutrient solution for *ca.* 33 and 57 h, respectively. The control and experimental specimens show essentially no spectral differences for the L922 seedlings, in contrast to the differences observed for those from the L1037 line, even after exposure for only 30 h. It is apparent that for the latter the presence of Al^{3+} significantly altered the *in vivo* optical response of the seedling leaves. This correlates well with a previous verification of phenotypical susceptibility involving radicular growth inhibition.[5] Most of the spectral differences lie in the region dominated by the chlorophyll band, with a maximum at *ca.* 680 nm. In fact, a 30% decrease in peak intensity was observed after exposure for 57 h. Again, no significant decrease was observed for the L922 line. The exposure time (t) behaviour of the ratio $r(t) = S_{\text{experimental}}/S_{\text{control}}$, S being the appropriately normalised PA signal for each genotype, as measured at that peak, was also studied. The results are shown in Figs. 3 and 4. The data in Fig. 3 are consistent with a constant experimental value of 1.0 for $r(t)$, even for long exposure times; this is consistent with the fact that L922 inbreds are known to show a high tolerance to Al^{3+}. In contrast, in Fig. 4, $r(t)$ exhibits a steady decline at an approximately linear rate of 0.5 h^{-1}, at least for values of t down to 60 h. For longer exposure times, the apparent recovery of $S_{\text{experimental}}$ has still to be explained.

In an attempt to interpret these data it should be remembered that under the experimental conditions used here the OCPA signal can be represented by $S = A\beta(\lambda)\,\alpha/k$, where $\beta(\lambda)$ is the light absorption coefficient at wavelength λ, α the thermal diffusivity, k the thermal conductivity of the sample and A a constant. Now, taking $\alpha = (k/\rho c)$, which gives α in terms of the mass density, ρ, and the specific heat, c, of the sample, and considering measurements at a fixed value of λ, say at the chlorophyll band peak, so that $\beta(\lambda)$ remains constant, we obtain $S \sim (\rho c)^{-1}$, an equation that expresses the dependence of the OCPA signal on the parameters of the sample that are relevant in this instance.

On the basis of previous findings of genetic character,[5] we can assume that for the L1037 seedlings exposed to Al^{3+} the volume of symplastic (intracellular) water decreases with time. As a result, the average heat capacity per unit volume at the plant leaf site where the *in vivo* OCPA measurements were made would increase. Such variations are not observed for the L922 specimens. By combining this information with the equation $S \sim (\rho c)^{-1}$, it is possible to explain not only the t-invariance of S_{control} but also the observed decrease in the values of $S_{\text{experimental}}$ for the L1037 specimens. However, in our view, a complete explanation of the behaviour of $r(t)$ is still required. To this end the assumption could be made that such a decrease is somehow enhanced by the fact that the change in plant physiology results in a lower rate of chlorophyll production[14] and thereby lowers its concentration in the plant leaves.

For medium values of t (say, >60 h) the data for the L1037 specimens appear to be consistent with the hypothesis that for any given concentration the impairing effects of Al^{3+} will eventually show a tendency to subside or stabilise. Photoacoustically, the proof of this would be $S_{\text{experimental}}$ tending to a constant value. The observed trend in $r(t)$ for this range of t values is consistent with this, particularly in view of the fact that, under such long and continued biochemical stress, fluctuations in chlorophyll content and morphological changes that can produce scattered results in the OCPA measurements are to be expected. However, at present we cannot fully explain the increase in $r(t)$ (shown in Fig. 4) for t values outside the ranges considered so far. One possible explanation would be an apparent adaptation response that appears to set in around and above *ca.* 60 h of exposure of susceptible specimens.[5] The associated modifications in the plantules radicular system may enhance a recovery mechanism capable of offsetting, at least in part, the impairing effects of aluminium toxicity. The data presented here are still preliminary, hence further studies are underway to ascertain whether

this correlates with the apparent recovery of the PA response of the specimens.

Conclusions

It has been demonstrated that OCPA spectroscopy is a valuable analytical tool for the *in vivo* assessment of the genotypical response of corn plants to aluminium toxicity. The differences in the visible light OCPA spectra of the experimental and control specimens of either susceptible or tolerant inbred lines are very conspicuous for the former but hardly discernible for the latter, over the entire range of the visible spectrum.

To the best of our knowledge this is the first report describing the use of an optically triggered thermoacoustic open-cell analytical technique for assessing differences that are deeply rooted in genetically controlled plant behaviour. To date the discrimination has relied on measurements associated with Al^{3+}-induced differences in radicular growth. This requires fairly long observation times leading to unwanted effects that make it difficult to interpret the results. In comparison, the technique described here can produce an accurate discrimination of responses at a far earlier stage among plant specimens that still appear normal.

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