ABSTRACT

A series of experiments was conducted to devise a standard procedure for comparing *in vivo* nitrate reductase activities of different cocoa clones. This procedure involved subjecting cultured leaf discs to different treatments in order to first inactivate the enzyme down to a uniform basal level, and then reactivate it.

Dark incubation in a nitrate-free incubating medium gave satisfactory reductions in enzyme activity. Exclusion of light or of nitrate alone was not sufficient to get appreciable and consistent reductions. There was, however, a positive linear relationship between initial enzyme activity and the time to reach 50 per cent activity (half-life) in the absence of nitrate suggesting a regulatory role of nitrate *in vivo*. Exclusion of air hastened the rate of inactivation in nitrate-rich cultured discs in the dark. Discs immersed in the incubating medium lost activity at a faster rate than discs that were afloat. It was suggested that this was due to tissue breakdown and possible microbial contamination.

Reactivation of the enzyme was achieved by light incubation in a nitrate-rich medium. Appreciable levels of enzyme reactivation occurred with all three clonal types investigated irrespective of the method of inactivation. The large variations in the rate of, and extent of reactivations, however, made it impossible to draw any conclusions on the nitrate reducing potential of the different cocoa types. These variations are attributed partly to differences in the physiological condition of the discs, and partly to the experimental procedure.

Suggestions are made for modifications in the experimental technique to reduce variations.