The Association of Gibberellin-like Activity
with the Chloroplast Fraction of Leaf Homogenates

J. L. STODDART
Welsh Plant Breeding Station, Aberystwyth

Received March 14, 1968

Summary. Significant gibberellin-like activity has been detected in the 1000 \( \times \) g fraction of leaves of \textit{Brassica oleracea} var. \textit{acephala} (Canson kale) and \textit{Hordeum vulgare} (cv. "Himalaya"). In kale and barley the qualitative pattern of activity found in the chloroplast fraction differs from that normally seen in total-leaf extracts. When expressed on a total chlorophyll or sample fresh-weight basis, approximately 16\% of the gibberellin-like activity found in the leaf can be accounted for in the 1000 \( \times \) g or chloroplast fraction. The physiological implications of this finding are discussed.

Introduction

The effects of photoperiod on the free gibberellin content of leaves have been described by numerous workers (e.g. Harada and Nitsch, 1959; Lang, 1960; Stoddart, 1962). More recent findings have also suggested that the gibberellin biosynthetic pathway in red clover includes at least one light-dependent step (Stoddart and Lang, 1968), and that synthesis can be rapidly induced in etiolated barley or maize leaves by exposure to red light (Reid et al., 1968).

There is, therefore, a clear implication of a photo-receptive system in leaf-gibberellin synthesis which is unlikely to be operative in root or fungal pathways.

In order to investigate this point it is necessary to have knowledge of the sub-cellular location of the sites of gibberellin synthesis within the leaf and their contribution towards the total content.

Of the leaf organelles, the chloroplasts would seem to be the most likely sites for a light-mediated synthetic system. They have been shown to be biosynthetically autonomous in almost all respects, including the formation of phytosterols (Mercer and Treharne, 1966), and thus could be expected to have a gibberellin synthesising capability. Permeability considerations would impose certain limitations on the significance of chloroplast gibberellins but would not detract from their possible importance in the regulation of leaf metabolism.

This study represents an initial attempt to detect gibberellin-like activity in the chloroplast fraction and to assess the importance of any such activity in the context of the whole leaf.
Materials and Methods

Plant Material

Leaf samples were obtained from plants grown in a heated glasshouse (15–18°) under 10–11.5 hours of natural illumination. The following species were used: *Brassica oleracea* var. *acephala* cv. *Cansons*, *Hordeum vulgare* cv. *Himalaya*.

During sampling care was taken to ensure that each sample contained only leaves of comparable age.

Preparation of the Chloroplast Fraction

Half of each sample (ca. 20 g) was homogenised for 1 minute in 200 ml of cold 0.1 M, pH 7.0 phosphate buffer made 0.4 M with respect to sucrose. The homogenate was decanted through a double layer of glass wool and muslin and the resultant suspension centrifuged briefly at 200 × g to remove debris. A further centrifuging at 1000 × g for 10 minutes produced a crude chloroplast preparation. After resuspension in 0.4 M sucrose, the chloroplasts were again centrifuged at 200 × g to remove aggregates and the final pellet obtained after a second treatment at 1000 × g. This was taken as the chloroplast preparation and inspection under the light microscope showed the preparations to be predominantly composed of chloroplast material with very little contamination by non-pigmented debris. There were, however, a number of small particles which exhibited Brownian motion. In the absence of an electron microscope, it was not possible to comment upon the structural integrity of the chloroplasts or the degree of mitochondrial contamination.

Sucrose density gradient studies were carried out with the discontinuous gradient technique (Leech, 1966), using the recentrifuged 1000 × g pellet as the starting material.

Ultrasonic disintegration was achieved, when required, with the M.S.E. model 7120 disintegrator, operating at a frequency of 21 kc/sec and with a peak to peak amplitude of 6–8 μ. The treatment was continued for a total of 1 minute in bursts of 20 seconds each and with the tube embedded in ice to provide adequate cooling. In order to remove chloroplast fragments, sonicates were passed through a Metricel GA-8 membrane filter with a pore size of 0.2 μ.

Estimation of Gibberellin-like Activity

Total-leaf gibberelin content was determined on the remaining half of the sample. The tissue was homogenised in absolute methanol and the extract filtered before reducing to dryness in a rotary-film evaporator. Phosphate buffer (0.5 M, pH 8.3) was added to redissolve the residue and this was repeatedly partitioned against petroleum ether (b.p. 40–60°) to remove pigments.

The aqueous phase was then partitioned 3 times with equal volumes of ethyl acetate, after which the pH was lowered to 2.8. Gibberellin-like activity was extracted at the low pH with redistilled ethyl acetate. The bulked organic phases were reduced to a small volume for thin-layer chromatography.

Chloroplast pellets were extracted in a similar fashion, the initial stage being achieved by the addition of 30 ml of absolute methanol to each centrifuge tube. After resuspension, the chloroplasts were repeatedly agitated during a period of 15 minutes. This extracted all the chlorophyll and was assumed to have a similar efficiency with respect to gibberellins.