ENDOGENOUS CYTOKININS IN NORMAL AND MALE STERILE LINES OF BRASSICA NAPUS

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INTRODUCTION

All three types of male sterility, viz., genic (GMS), gene-cytoplasmic (G-CMS) and cytoplasmic (CMS) exist in rapeseed (Brassica napus L.) (Kaul 1988). The various factors known to influence male sterility in rapeseed are; environmental, especially the temperature (e.g. Fan and Stefansson 1986; Polowick and Sawhney 1987), biochemical (Ogura 1968; Polowick 1989) and molecular (Vedel et al. 1982).

The role of plant growth substances, both applied and endogenous, has been implicated in the regulation of male sterility in several plant species (Frankel and Galun 1977; Kaul 1988). However, to our knowledge there are no reports on the hormonal (endogenous) regulation of male sterility in rapeseed. In barley, the much higher biological activity of cytokinins in root exudates of the normal and restored male fertile lines than the CMS lines (Ahokas 1982) provide a strong evidence for the involvement of cytokinins in fertility restoration. Based on this study, an investigation into the analysis of cytokinins in the vegetative parts, i.e., roots, stems and leaves, and inflorescences of the normal (B. napus cv. Westar), an ogu CMS line (Ogura 1968) and a GMS line of B. napus was conducted.

MATERIALS AND METHODS

Plant Culture

Seeds of the CMS and GMS lines of B. napus and the normal (B. napus cv. Westar) were germinated in a mixture of loam, sand and peat moss (1:1:1) in 13 cm diameter plastic pots in the greenhouse. The seedlings were transferred into a growth chamber maintained at intermediate temperature (ITR, 23°C/18°C, light/dark) when the first leaf was about 1 cm wide. Light was supplied by high intensity fluorescent tubes at an intensity of 180 $\mu E\ m^{-2}s^{-1}$ for 16h per day. All plants were fertilized twice every week with a commercial fertilizer, 20:20:20 (Plant Products Co. Ltd., Bramlea, Ontario).

Purification, Biological activity, and Quantification of Cytokinins in the normal, CMS and GMS rapeseed plants

Roots, leaves, stems and inflorescences of the normal, CMS and GMS rapeseed plants were excised and immediately stored in plastic containers containing ice. Roots were washed repeatedly in tap water to remove soil and rinsed with distilled water, and blotted on layers of filter papers. The individual tissues were frozen in liquid nitrogen, and freeze-dried. The freeze-dried tissues were weighed, followed by extraction in a solvent (methanol-water-formic acid, 15:4:1). The extracts were centrifuged and the solvent evaporated in a rotary flash evaporator at reduced pressure

at 40°C. The resulting extracts were purified on phosphocellulose columns (Badenoch-Jones et al. 1984) yielding two fractions; an acidic fraction (containing cytokinin nucleotides) and a more active basic fraction (containing cytokinin bases, ribosides and glucosides). The basic fraction was subjected to preprative 1-dimensional thin layer chromatography (TLC), using dye markers and cytokinin standards (Badenoch-Jones et al. 1987). Five TLC zones corresponding to R_f values of cytokinins i.e. isopentenyladenine (iP), zeatin (Z) + dihydrozeatin (DZ), isopentenyladenosine (iPA), zeatin riboside (ZR) + dihydrozeatin riboside (DZR), and cytokinin glucosides were eluted with ethanol-water-acetic acid (25:25:2).

The bioactivity of Z+DZ and ZR+DZR fractions was tested in Amaranthus betacyanin bioassay (Biddington and Thomas 1973). Further purification of Z, DZ, ZR and DZR involved the use of reversed phase Baker columns (Baker 10 SPE C_{18}, 6ml, J.T. Baker Chem. Co. NJ, USA) (Jameson et al. 1987). The cytokinin fractions of some samples were analyzed by reversed phase high performance liquid chromatography (HPLC). The individual cytokinins, Z, DZ, ZR and DZR were quantified by specific enzyme-linked immunosorbent assay (ELISA) (Idetek, inc. CA, USA).

RESULTS

In general, the ogu CMS line had a lower cytokinin activity (determined by Amaranthus betacyanin bioassay) in both vegetative parts and inflorescences compared to the normal rapeseed plants. Similarly, the GMS mutant exhibited a lower cytokinin activity especially in stems and inflorescences in comparison to the normal plants. In total, four cytokinins, viz., Z, DZ, ZR and DZR were quantified by ELISA in roots, stems, leaves and inflorescences of the normal than the ogu CMS line. Cytokinin bases (Z and DZ) were present in higher concentrations than the corresponding ribosides (ZR and DZR) in both vegetative parts and inflorescences of the normal and the CMS line. The normal rapeseed plants had higher levels of cytokinin bases (Z, DZ) and ribosides (ZR, DZR) in all organs especially roots and inflorescences compared to the CMS line.

DISCUSSION

There is little information on the endogenous cytokinins of B. napus (de Bouille et al. 1989), and apparently none in relation to male sterility. de Bouille et al. (1989) reported much lower levels of ZR than Z in both flower buds and open flowers of oilseed rape (B. napus var. oleifera). Similarly, we observed reduced levels of cytokinin ribosides (ZR, DZR) than the corresponding bases (Z, DZ) in both the normal and ogu CMS plants.

The results presented here also showed a reduction in biological activity and levels of cytokinins in both the ogu CMS and GMS lines than the normal rapeseed plants. Earlier, Ahokas (1982) reported lower cytokinin activity (by bioassay) in root exudates of the CMS lines than the normal and restored male fertile lines of barley. Using ELISA, we have found reduced cytokinin levels in the roots and also inflorescences of the ogu CMS line than the normal rapeseed plants. It is suggested that the lower cytokinin levels in

the CMS inflorescences may be due to a general decline in biosynthesis of cytokinins in roots and a lower cytokinin uptake from roots to the inflorescences. Because of similar cytokinin bioactivity in the normal and GMS roots, the lower cytokinin activity in GMS inflorescences may be due to a decline in cytokinin transport and/or retardation of cytokinin biosynthesis and a rapid metabolism of cytokinins in the floral buds and open flowers.

In conclusion, male sterility in both ogu CMS and GMS lines of B. napus appears to be associated with a deficiency in endogenous cytokinin content.

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