HORMONAL REGULATION OF MALE STERILITY IN THE *OGURA* CYTOPLASMIC MALE STERILE LINE OF *BRASSICA NAPUS*

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ABSTRACT

The endogenous levels of plant hormones, viz. cytokinins (CKs) and abscisic acid (ABA) were determined by enzyme-linked immunosorbent assay (ELISA) in floral parts of wild type and the *ogura* cytoplasmic male sterile (*ogu* CMS) line of *Brassica napus*. Stamens of the *ogu* CMS line had 9-fold more ABA in comparison to those of wild type. The mutant stamens contained lower levels of CK bases [zeatin (Z) and dihydrozeatin (DZ)], dihydrozeatin riboside (DZR), and nucleotides (ZNT and DZNT) but had higher levels of CK o-glucosides than those of wild type. The other floral parts, eg. sepals, petals and pistils of wild type and the *ogu* CMS line exhibited similar but smaller differnces in CKs and ABA levels. We suggest that male sterility in the *ogu* CMS line may be associated with altered levels of CKs and ABA in stamens.

INTRODUCTION

Rapeseed (*Brassica napus*) exhibits all three types of male sterility, viz. genic (GMS), cytoplasmic (CMS) and gene-cytoplasmic (G-CMS) (Kaul, 1988). The *ogu* CMS line was developed by the transfer of the rapeseed nucleus into the cytoplasm of radish (*Raphanus sativus*) CMS line (Ogura, 1968). The *ogu* line was backcrossed and maintained in *B. napus* cv. Westar (Polowick and Sawhney, 1987). The development of both stamens and pollens is impaired in the *ogu* CMS line (Polowick and Sawhney, 1987).

Exogenous applications of plant hormones appear to regulate flower development including male sterility in several plant species (Kaul, 1988). However, there are only few reports in the literature dealing with endogenous CKs and ABA in relation to male sterility (Singh and Sawhney, 1992; Shukla and Sawhney, 1992, 1993, 1994). Recently, we reported that the various vegetative parts, mature flowers and inflorescence of the *ogu* CMS line had reduced levels of active CKs, especially DZ (Singh and Sawhney, 1992). In the present study, we characterized various CKs and ABA in floral organs, eg. sepals, petals, stamens and pistils of wild type and the *ogu* CMS line of rapeseed.

EXPERIMENTAL

The seed source of the *ogu* CMS line of rapeseed and wild type (cv. Westar)

was similar to that reported earlier (Polowick and Sawhney, 1987). Plants were grown in a growth chamber maintained at 23°C/18°C (light/dark) and light intensity of 180 $\mu E\ m^{-2}s^{-1}$. The floral parts, eg. sepals, petals, stamens and pistils were excised, frozen in liquid nitrogen and freeze-dried. CKs and ABA were purified by RP-HPLC and quantified by ELISA (Singh and Sawhney, 1992; Shukla and Sawhney, 1994).

RESULTS AND DISCUSSION

Stamens of the *ogu* CMS line had 9-fold higher ABA level than the wild type stamens (Table 1). Similarly, the mutant sepals had 4-fold more ABA in comparison to the wild type sepals. However, petals and pistils of the *ogu* CMS line and wild type contained similar ABA levels (Table 1). Stamens of a GMS line of rapeseed

TABLE 1. ABA levels (nmoles g-1DW) in floral organs of wild type and the *ogu* CMS line of *B. napus*.

Genotype	Sepal	Petal	Stamen	Pistil
Wild type	0.9	2.0	1.0	8.1
ogu CMS line	3.6	2.7	9.2	8.9

(Shukla and Sawhney, 1994) and the stamenless-2 mutant of tomato (Singh and Sawhney, unpublished) also have much higher levels of ABA than their respective wild types. Therefore, male sterility appears to be associated with high ABA content in the above-mentioned species, including the *ogu* CMS line of *B. napus*.

Stamens of both wild type and the *ogu* CMS line had higher levels of DZ-type than the Z-type CKs (Table 2). DZ and its nucleotide (DZNT) were identified as the major CKs in the wild type stamens, whereas OGDZ+OGDZR were the main

TABLE 2. CK levels (pmoles g^{-1}DW) in stamens of wild type and the *ogu* CMS line of *B. napus*.

Genotype	Z	DZ	ZR	DZR	OGZ+ OGZR	OGDZ+ OGDZR	ZNT	DZNT
Wild type	74	352	45	122	43	130	75	280
ogu CMS line	28	161	48	59	81	214	58	156

CKs present in the *ogu* CMS stamens (Table 2). The *ogu* CMS stamens contained reduced levels of CK bases (Z,DZ), ribosides (DZR), and nucleotides (ZNT,DZNT) but higher levels of o-glucosides [(OGZ+OGZR) and (OGDZ+OGDZR)] (Table 2). We observed similar but smaller differences in CK levels in other floral organs of wild type and the *ogu* CMS line (data not shown). CK bases and ribosides are considered as the active forms, nucleotides as their precursors, and the o-glucosides as inactive or storage compounds (Letham and Palni, 1983). In an earlier study, we found reduced levels of active CKs, especially DZ in vegetative tissues and inflorescences of the *ogu* CMS line (Singh and Sawhney, 1992). Similarly, flowers and stamens of a GMS line of rapeseed contained much lower levels of CKs including DZ (Shukla and Sawhney, 1992, 1993). The present findings combined with our previous reports strongly indicate that the *ogu* CMS line is deficient in active CKs. Hence, we propose that reduced levels of active CKs and higher levels of CK o-glucosides and ABA in stamens may have some role in the expression of male sterility in the *ogu* CMS line of *B. napus.*

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