NUMBER OF MICROSPORES IN IMMATURE AND MATURE FLOWER BUDS IN *BRASSICA* SPECIES

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INTRODUCTION

Hinata and Konno (1975) have reported on the number of pollen grains of *Brassica* and allied genera and determined that flowers developing at the base of the inflorescence produced a greater number of pollen grains than flowers developing on the terminal portion of the inflorescence. This difference may have been the result of microspore abortion. We have investigated the level of microspore abortion in the six *Brassica* species of the triangle of U (1935) by comparing the number of microspores in immature flower buds and the number of pollen grains in buds at one day before anthesis.

MATERIAL AND METHODS

The number of microspores was determined in 21 cultivars/ strains of six *Brassica* species. Ten plants for each cultivar/ strain of *B. rapa*, *B. juncea*, *B. napus*, and *B. nigra*, and 2 plants for *B. carinata* and *B. oleracea* were grown in a soilless potting mix in the greenhouse, one plant per 12.5 cm diameter pot. From the three-leaf stage onwards, the plants were fertilized weekly with a dilute solution of 20-20-20.

The length of flower buds containing microspores at the uninucleate stage was determined for each genotype at the onset of flowering. Anthers were excised and fixed in Carnoy's fluid I (95% ethanol:glacial acetic acid 3:1) for 30 min, rinsed with distilled water, and crushed on a glass slide in a drop of the fluorochrome DAPI (Coleman and Goff 1985). The developmental stage of the microspores was examined using fluorescence microscopy. Flower buds containing microspores at the uninucleate stage, referred to as immature buds, and flower buds at one day before anthesis, referred to as mature buds, were harvested separately from the main raceme of each plant.

Each sample consisted of three flower buds from each of the two developmental stages. The buds were homogenized in 1 ml of distilled water and the homogenate was mixed with a Vortex mixer and filtered through a nylon screen (100 µm pore size) into a centrifuge tube. After centrifugation (3000 rpm, 3 min) and removal of the supernatant, the microspores were suspended in 1 mL of a 50% aqueous solution of glycerine. Microspore density was determined using a haemocytometer. Six sub-samples were counted for each preparation and the average number of microspores per bud was calculated. A number of smaller microspores were observed in all mature buds and they were not included in the count if their diameter was less than half that of the other microspores.

RESULTS AND DISCUSSION

Mature buds of B. oleracea, B. rapa, B. napus, B. nigra, and B. juncea contained significantly less microspores than immature buds (Table 1). The decrease in the number of microspores during development ranged from 48.3% for broccoli to 12.7% for the mustard cultivar Scimitar. In B. rapa ssp. yellow sarson (Y.S.) and B. carinata, the number of microspores in immature and mature buds was not different.

Table 1. Number of microspores ($x10^3$) of immature and mature flower buds in Brassica species and average number of buds on the main inflorescence

Species	Cultivar/ strain	No. of microspores in Immature buds	No. of microspores in Mature buds	Decrease (%)	No. of buds on the main raceme
B. oleracea	Kohlrabi	421.7	254.4	39.7**	33.0
	Broccoli	190.0	98.3	48.3**	30.0
	Mean	**305.9**	**176.4**	**42.3****	**31.5**
B. rapa	Tobin	167.9	122.6	27.0**	29.1
	Echo	166.8	113.4	32.0**	34.2
	Torch	166.2	110.0	33.8**	33.4
	Mean	**167.0**	**115.3**	**31.0****	**32.2**
B. napus	Golden	156.8	118.7	24.3**	31.0
	Topas	131.6	95.6	27.4**	29.1
	Midas	127.9	97.3	23.9**	26.2
	Westar	127.8	90.7	29.0**	29.4
	Mean	**136.0**	**100.6**	**26.0****	**28.9**
B. nigra	R4134	76.5	59.4	22.4**	19.6
	R4126	71.5	60.3	15.7**	19.5
	R4152	68.0	51.9	23.7**	17.2
	R4125	63.3	44.5	29.7**	22.8
	Mean	**69.8**	**54.0**	**22.6****	**19.8**
B. juncea	Scimitar	97.5	85.1	12.7*	22.0
	Donskaja	90.8	67.5	25.7**	20.4
	ZEM 87-1	90.6	72.6	19.9**	29.4
	Cutlass	71.9	57.3	20.3**	18.1
	Mean	**87.7**	**70.6**	**19.5****	**22.5**
B. rapa Y.S.	R500	110.6	109.3	1.2ns	17.9
B. carinata	Dodola	103.3	108.3	-4.8ns	9.0
	R4194	93.3	83.3	10.7ns	10.0
	R4177	71.7	75.0	-4.6ns	16.0
	Mean	**89.4**	**88.9**	**0.6ns**	**11.7**

*:p<0.05; **:p<0.01; ns: non significant

Kohlrabi buds (B. oleracea var. gongylodes) contained the largest number of microspores in both immature and mature buds (Table 1). This is the largest number of microspores reported for Brassica species. Brassica nigra strains had the smallest number of microspores in both immature and mature buds. There did not seem to be a relationship between the number of microspores and ploidy level. The diploid species had either high or low number of microspores, and the same was true for the allotetraploid species.

It was possible that the lower number of microspores of the mature buds was the result of microspores becoming trapped in the floral tissues during sample preparation. Using B. napus Topas, we determined that there was no difference in microspore counts when crushing excised anthers or whole buds, suggesting that the number of microspores trapped by the floral tissues was negligible.

Microspores in immature buds were uniform in size, but microspore size in mature buds varied greatly. In most genotypes, 20-30% of the microspores were smaller and darker than the rest. Even when including these microspores in the counts, the mature buds of certain genotypes contained fewer microspores than the immature buds.

The number of microspores present at four bud developmental stages was determined in four plants of B. napus Topas and three plants of B. nigra R4126 (Fig. 1). For each developmental stage, three consecutive flower buds were sampled on the main inflorescence. To account for differences in bud size between the species, bud length was translated into a scale of 1-10, 1 corresponding to buds containing uninucleate microspores, and 10, to buds 1 day before anthesis. The decrease in the number of microspores from immature to mature buds appeared to occur mainly at the binucleate stage in Topas, while the number of microspores declined gradually until the trinucleate stage in R4126.

The reasons for microspore degeneration are unclear. The aborting microspores may be genetically deficient and hence unable to mature normally. Microspore degeneration may also be caused by environmental factors. We have observed that B. rapa yellow sarson R500 and B. carinata, which do not show a decrease in the number of microspores from immature to mature buds, developed fewer buds on the main inflorescence (Table 1). A greater number of buds on the main inflorescence may result in stronger competition for nutrients by each individual bud, increasing the rate of microspore degeneration.

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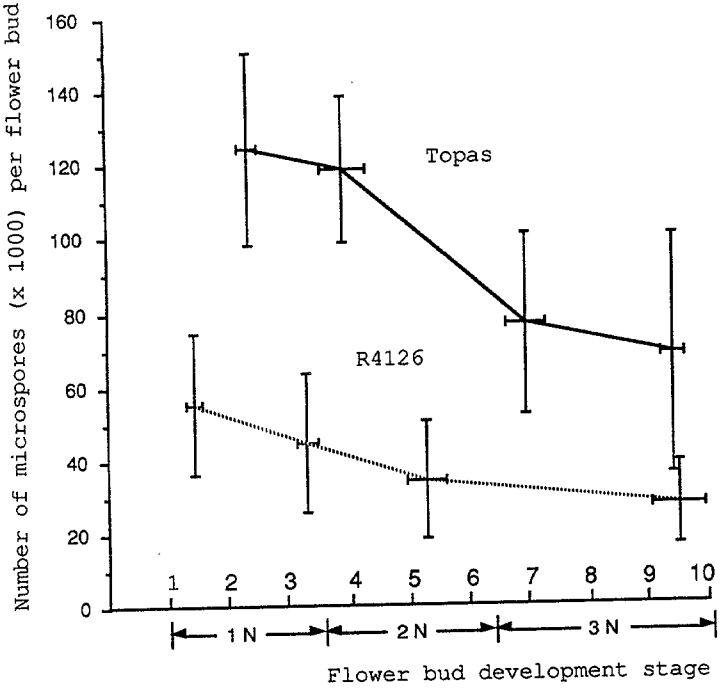

Fig. 1. Number of microspores in uninucleate (1N), binucleate (2N), and trinucleate (3N) bud developmental stages of B. napus Topas and B. nigra R4126 when grown in the greenhouse. Bars represent standard deviations.