IN VITRO DEVELOPMENT OF MICROSPORE AND PRODUCTION OF FROM ISOLATED MICROSPORES IN OIL SEED RAPE(Brassica napus L. spp. oleifera)

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ABSTRACT

Isolated microspores from three cultivars of winter type and three cultivars of spring type(Brassica napus L.)were cultured in modified Nitsch and Nitsch(NN) medium supplemented with 13% (W/V) sucrose, 0.05mg/ℓ BA and 1.0mg/ℓ NAA. Incubating, temperature was treated with 32.5℃ for 3days of the initial stage and then maintained at 25℃ in the dark. Embryo yields and frequency of normal embryo production showed remarkable contrast according to genotypes. The highest frequency of embryogenesis was obtained from cultures of buds sampled with sizes of 2.8-3.2mm at approximately 1 week after first flowing and decreased with the aging of the inflorescence. Embryogenic microspores commonly were found in buds with a petal/anther length ratio between 1/3 and 3/4. In cultivars of spring types, the optimum embryo yield was from microspores of buds around the 1/2 ratio with mainly late uninucleate stage microspores. But embryo yield of winter oilseed rape was only obtained from microspores of buds with 1/3 ratio.

INTRODUCTION

Embryogenesis by isolated microspore culture is regarded as ideal method of producing high-quality haploid embryos in many species including Brassica. The main advantage of the technique is that it is possible to produce very large number of embryo in comparison with anther in several crops. It is for this reason that technique has been applied to the isolated microspore culture system of Brassica napus (Lichter, 1982; Chuong and Beversdorf, 1985; Swanson et al., 1987; Polsoni et al., 1988; Pechan and Keller, 1988). In order to realize the greatest efficiency from this system it is important to be able to accurately identify and select buds containing embryogenic microspores and to determine which segement of the microspore population under goes embryogenesis. Moreover, various factors including physiological condition of the donor plant, genotype of donor plant, composition of the culture medium and culture temperature are crucial to the production of Brassica embryos (Licther, 1982; Thurling and Chay, 1984; Chuong and Beversdrof, 1985).

In this paper, we have studied in order to investigate the effect on the recovery of a high yield of microspore-derived embryos of the following as the relationship of bud and anther sizes to the precise stage of microspore development at the start of culture and the difference between donor plant genotypes.

EXPERIMENTAL

Evaluation of genotype and bud size

Table 1 shows the yield of embryogenesis by isolated microspores for bud size among spring genotypes. Gynotypic differences for embryogenic capability according to genotypes were evident. And a range of bud sizes were examined, 2.3-2.7mm, 2.8-3.2mm, 3.3-3.7mm and 3.8-4.2mm. The buds of all range produced embryos but the buds of 2.8-3.2mm range were the most effective for embryogenesis in all spring genotypes. Lisandra was the most embryogenic cultivar of the three cultivars tested

TABLE 1. Camparison of genotype, bud-size and microspore nuclear stage on embryo yield from isolated microspore culture in Springtypes(Brassica napus L.)

Varieties	Bud-size (mm)	Microspore stage(%)					Embryo* number
		EU	MU	LU	VG	VSS	
							*
Lisandra	2.3-2.7	51.6	36.2	12.2			1526b
	2.8-3.2	7.4	35.2	57.4			4737a
	3.3-3.7	5.4	33.2	53.0	8.4		1293b
	3.8-4.2		25.2	47.4	27.4		451c
Lergo	2.3-2.7	44.2	45.0	10.8			577.3b
	2.8-3.2	4.0	39.6	47.0	9.4		1399.7a
	3.3-3.7	2.2	28.2	29.2	40.4		380.0c
	3.8-4.2		3.8	37.2	57.0	2.0	114.6d
ATRrTower	2.3-2.7	36.8	47.8	15.4			41.2c
	2.8-3.2	2.2	52.8	41.2	3.8		321.6a
	3.3-3.7		19.8	37.4	42.8		431.4a
	3.8-4.2			38.6	58.0	3.4	181.9b

* No. of embryos per $1x10^5$ microspore cultured.

The difference of embryo yield among winter genotypes were not evident. All genotypes including Hallayuche, Youngsanyuchae and Naehanyuchae were only obtained embryos from bud sizes with 2.3-2.7mm and 2.8-3.2mm (Table 2). In the efficency of embryogenesis by isolated microspore culture, spring genotypes were higher than winter genotypes. The reason was caused by the difference of developmental stage in microspore according to increase of bud size and winter genotypes were higher speed than spring genotypes in the development of microspore of the same bud size.

Cell divisions were observed in microspore suspensions within 3days of culture and gloubar and heartshaped embryos were visible after 10days.

TABLE 2. Camparison of genotype, bud-size and microspore nuclear stage on embryo yield from isolated microspore culture in Wintertypes(Brassica napus L.)

Varieties	Bud-size (mm)	Microspore stage(%)					Embryo* number
		EU	MU	LU	VG	VSS	
							*
Hallayu-	2.3-2.7	36.0	43.0	21.0			322.2a
chae	2.8-3.2		56.8	32.0	11.2		393.9a
	3.3-3.7			23.0	68.8	8.2	0b
	3.8-4.2			16.6	58.4	25.0	0b
Youngsan-	2.3-2.7	35.0	47.2	17.8			315.6a
yuchae	2.8-3.2		53.6	37.8	8.6		325.9a
	3.3-3.7			25.0	65.0	10.0	0b
	3.8-4.2			14.4	59.2	26.4	0b
Naehanyu-	2.3-2.7	34.8	48.0	17.2			267.7b
chae	2.8-3.2		52.4	40.4	7.2		311.6a
	3.3-3.7			26.0	63.8	10.2	0c
	3.8-4.2			15.4	57.0	27.6	0c

* No. of embryos per $1x10^5$ microspore cultured.

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REFERENCES

Chuong, P.V. and Beversdorf, W.D.(1985). High frequency embryogenesis through isolated microspore culture in Brassica napus L. and B.carinata Braun. Plant Science. 39, 219-226.

Lichter, R.(1982). Induction of haploid plants from isolated pollen of Brassica napus. Z.Pflanzenphysiol. Bd, 105, 427-434.

Pechan, P.M. and Keller, W.A.(1988a). Identification of potentially embryogenic microspores in Brassica napus. Physiologia Plantarum, 74, 377-384.

Polsoni, L., Kott, L.S. and Beversdorf, W.D. (1988). Large-scale microspore culture technique for mutation-selection studies in Brassica napus. Canadian Jurnal of Botany, 66, 1681-1685.

Swanson, E.B., Coumans, M.P., Wu, S.C., Barshy, T.L. and Beversdorf, W. D. (1987). Efficient isolation of microspores and production of microspore-derived embryos from Brassica napus. Plant cell Reports, 6, 94-97.

Thurling, N. and Chay, P.M.(1984). The influence of donor plant genotype and environment on production of multicellular microspores in cultured of Brassica napus spp.oleifera. Annual Botany, 54, 681-693.