NITROGEN ASSIMILATION IN MICROSPORE-DERIVED EMBRYOS OF *BRASSICA NAPUS* L.

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ABSTRACT

Nitrogen assimilation during androgenetic embryogenesis was investigated in *B. napus* L. Nitrate salts were not used while cultured microspores developed to heart-shaped embryos. Once androgenetic embryogenesis was initiated, microspores developed to torpedo-shaped stage in a medium with ammonium salt and glutamate. Glutamine was an essential component for initiating androgenetic embryogenesis as well as for normal embryo development in *B. napus*.

INTRODUCTION

In *Brassica napus*, isolated microspores(single cells) developed into embryos via globular, heart-shaped and torpedo-shaped stages(Lichter,1982). The microspore-derived embryos contained mRNAs of seed storage protein, napin and cruciferin(Crouch, 1982). Accumulation of the identical fatty acids as in zygotic embryos was also detected in cotyledon of androgenetic embryos(Taylor *et al*, 1990). Therefore, an isolated microspore culture is a good model system to study the early stage of embryogenesis in *B. napus*.

The zygotic embryos receive nutrients from their mother plants via seed coats and embryosac fluid(endosperm) during the early stage of development(Peoples *et al*, 1985). High concentration of glutamine was detected in embryosac fluid of developing *B. napus* seeds(Ohkawa & Maeda, 1992). In the present paper, the effect of glutamine in culture media on androgenetic embryogenesis was investigated. The chemicals included in assimilatory nitrogen metabolism were also examined.

EXPERIMENTAL

TABLE 1. Composition of the media for microspore culture

Component	Concentration(mg/l)									
	A	C	B	J	G	I	O	M	N	D
Nitrate salts	-	+	+	+	+	+	-	-	-	-
Ammonium salt	-	-	-	+	-	+	-	+	-	+
L Glutamate(Glu)	-	-	-	-	+	+	-	-	+	+
L Glutamine(Gln)	+	+	-	-	-	-	-	-	-	-

Other components are the same as MN 13 medium(Keller *et. al.* 1987).

The cell division was observed during the first four days in the media A and C(Table 1) which contain Gln. The androgenetic embryos cultured in the media A or C ceased their development when they were transferred into medium B, a Gln-free medium(conditions 1,

2, 4, 5, 6 and 7 in Table 2). In contrast, the microspores cultured in medium B for the first four days or more did not show any androgenetic embryogenesis(conditions 3, 8, 9, 10 and 11). Under the condition without any ammonium salts(media A, B and C), Gln was required for the first cell division and normal development of the androgenetic embryos.

TABLE 2. Effect of glutamine on embryo formation from microspores

Culturing Days		Culture Condition 1	2	3	4	5	6	7	8	9	10	11
0	4	C[1)]	C	B	A	A	A	A	B	B	B	B
4	6	C	B	C	B	|	|	|	A	|	|	|
6	8	|	|	|	|	B	|	|	|	A	|	|
8		|	|	|	|	|	B	|	|	|	A	|
Size of embryos		No. of Embryos/1 x 10^{-4} microspores										
50	100 μm		52	0	35	41	12		0	0	0	0
100	250		0	0	0	0	34		0	0	0	0
250		100	0	0	0	0	0	78	0	0	0	0

1)The details are in Table 1.

Gln is produced from the nitrate salts by nitrate reductase, nitrite reductase and glutamine synthetase in assimilatory nitrogen metabolism of plant. Very low activity of nitrate reductase was detected in young embryos derived from microspores cultured in medium C on the fourth and eighth day of culture(0.027 and 0.017 μmoles NO_2 synthesis/g f.w./hr., respectively). The activity of the enzyme in larger embryos, heart-shaped or more developed embryos, was about 50 times higher than young embryos(1.457 μmoles NO_2 synthesis/g f.w./hr.). So, the microspore could not initiate androgenetic embryogenesis and the immature androgenetic embryos could not continue their development in medium B including nitrate salts only as nitrogen sources.

Medium D contains ammonium salt and Glu, substrates of glutamine synthetase, but lacks nitrate salts and Gln(Table 1). The microspores cultured in medium D for the first four days or more hardly developed to embryos(Table 3, conditions 17 to 21). Table 3 also shows that the microspores cultured in medium C for the first four days or more developed into normal embryos in the following medium D(conditions 13, 14 and 15). The numbers of embryos developed under these conditions were similar to those continuously cultured in medium C(conditions 12 and 16). Once the androgenetic embryogenesis was initiated, the microspores continued to develop into normal embryos in the medium which contains ammonium salt and Glu as well as in a Gln-containing medium. The results suggest that glutamine synthetase is active after day 4.

TABLE 3. Effect of Glutamate and ammonium salt on embryo formation from microspores

Culturing Days		Culture Condition 12	13	14	15	16	17	18	19	20	21
0	4	C[1)]	C	C	C	C	D	D	D	D	D
4	6	C	D	|	|	|	D	C	|	|	|
6	8	|	|	D	|	|	|	|	C	|	|
8		|	|	|	D	|	|	|	|	C	|
Size of embryos		No. of Embryos/1 x 10^{-4} microspores									
50	100 μm	52	75	36	0	50	0	0	0	0	0
100	250	124	90	128	118	149	1	1	1	1	0
250		103	70	159	230	100	4	11	20	15	5

1)The details are in Table 1.

In assimilatory nitrogen metabolic pathway, two molecules of Glu are produced from a Gln and a α-KG with an enzyme GOGAT. But the microspores cultured in a medium only including Glu as a nitrogen source hardly developed to embryos(data not shown). Gln is considered to be essencial for the initiation of androgenetic embryogenesis.

As shown in Table 4, the microspores, initiated androgenetic embryogenesis in medium C, developed to embryos in media I and D which contained ammonium salt and Glu, but the ones in media J and M, which contained ammonium salt but not Glu, ceased their development as well as the ones in media B and O. On the other hand, many smaller(100 to 250 μm) embryos were developed in media G and N, which contained Glu but not ammonium salt than the ones in media I, D and C. Gln was more suitable than Glu for androgenetic embryo development. From these results, there might be a small size of Glu metabolic pool and Gln could not be used as a substrate of GOGAT for producing Glu. It was suggested that other metabolic pathway of Gln was important for androgenetic embryogenesis, especially for the initiation.

In conclusion, glutamine is an essential component for initiating androgenetic embryogenesis as well as for normal embryo development in *B. napus*.

TABLE 4. Embryo development from microspores in different medium

	No. of Embryos/1 x 10^4 microspores								
	Culture Medium								
Size of embryos	C	B	J	G	I	O	M	N	D
50 - 100 μm	68	611	691	74	52	484	424	96	50
100 - 250	550	11	38	662	430	4	34	423	350
250 -	350	0	0	18	247	0	0	13	202

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