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ARTICLE

The effect of n-butanol and 2-aminoethanol on the *in vitro* androgenesis of maize

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ABSTRACT Contrary to wheat, in our experiment *in vitro* androgenic induction of maize microspores was not substantially influenced by *n*-butanol and 2-aminoethanol treatments. Embryo production of the treated live microspores was increased by max. 7-8% after 6-hour treatment with 0.4% *n*-butanol and 4 mM 2-aminoethanol. The plant regeneration frequency of the long-term treated (18 hs. with 2mM 2-aminoethanol and 18 hs. with 0.2% *n*-butanol) microspores was almost 5 times higher that of control. Unlike behaviour of the microspores of this two cereal species could have derived from their altered cytoskeletal systems
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KEY WORDS

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Androgenesis (microspore embryogenesis or callogenesis) is an important tool in plant breeding to produce homozygous plants rapidly and in developmental biology to discover the process of *in vitro* embryogenesis. Plant regeneration from isolated maize microspore cultures or dehisced microspores from floating anthers in liquid medium has been reported in a limited number of genotypes (Pesticelli et al. 1989). One of the main factors that hinder the application of microspore culture techniques is the low rate of embryogenesis. Cytoskeletal changes are required at the first steps of microspore (MS) induction towards the sporophytic pathway. Two biogenic alcohols, *n*-butanol (Soriano et al. 2008) and 2-aminoethanol (Rajaeijan et al. 2011) have been reported as a trigger for microspore division in anther culture and embryogenesis due to their effects on microtubules and as activators of Phospholipase D (PLD). The present study was focused on the efficiency of DH maize production *via* microspore cultures.

Materials and Methods

Microspore donor plants (A18 hybrid) were grown in phytotron chamber. Tassels were harvested when most of the microspores were in the late uninuclear stage of development and stored at 7ºC for 10 days. After the cold pre-treatment anthers were placed in modified YP media (Genovesi at al. 1982) in the presence or absence of *n*-butanol (0.2-0.8%) or 2-aminoethanol (2-8mM) (200 anthers/treatment, 5 repetitions) for 6 or 18 hours. Then microspores dehisced from the anthers (approx. 2000 microspores from one anther) were cultured in liquid modified YP medium at 29ºC. Number of the viable microspores was detected before treatments and on the 4th day of culture by means of fluorescent microscope after FDA test. The 3-4 week-old microspore-derived structures (embryos and calli) were transferred individually onto a regeneration medium (N_6O_1). Viable doubled haploid (DH) plantlets were potted to soil and grown up to maturity in the phytotron.

Results and discussion

Viability of the microspores before treatments varied between 60-68%. It decreased to 10-15% after the 4th day of culturing. The longest exposure (18 hours) to the highest concentration of *n*-butanol (0.8%) and 2-aminoethanol (8 mM) resulted in a drastic decrease of microspore viability (Table 1). Concerning

Table 1. Androgenic response of cultured maize microspores. [1] Percentage of viable MS in the 4th day of culturing. [2] Percentage of microspore-derived structures (MDS: embryos+calli) obtained from live microspores. [3] Percentage of viable plants differentiated from MDS.

		Viable MS %[1]	MDS %[2]	Embryo %	Plant reg.%[3]
6-hour treatment	Control	9.12	0.2741	25.62	0.83
	0.2% but.	11.245	0.1990	20.09	1.98
	0.4% but.	9.995	0.1304	32.18	2.30
	0.8% but.	19.295	0.2798	27.58	3.79
	2mM 2-AE	5.94	0.1339	27.27	2.00
	4mM 2-AE	13.465	0.4787	33.61	2.25
	8mM 2-AE	20.905	0.3324	20.12	2.52
18-hour treatment	0.2% but.	15.085	0.3017	21.00	1.50
	0.4% but.	15.73	0.0826	26.66	4.76
	0.8% but.	6.125	0.0208	30.88	1.47
	2mM 2-AE	17.235	0.3990	24.62	4.10
	4mM 2-AE	14.445	0.3135	23.96	1.15
	8mM 2-AE	5.04	0.0832	21.51	2.12

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the efficiency of our methodology, maintenance of microspore viability during the treatments and the whole procedure seems to be a very important problem.

The results (Table 1.) show that the embryo yield obtained from the viable microspores was slightly increased in most treatments with *n*-butanol and 2-aminoethanol as compared to the control. The same tendency was observed by Soriano et al. (2008) using wheat anther cultures, but in that case the increase in the production of embryos was significant. Controversial behaviour of maize microspores to antimitotic agent colchicine was also observed earlier (Barnabás et al. 1999) which indicates that differences can be exist in the cytoskeleton of the two cereal species.

N-butanol and 2-aminoethanol treatments had a more significant influence on the green-plant regeneration from the microspore-derived structures. Six-hour treatment with 0.8% *n*-butanol and 8 mM 2-aminoethanol, furthermore 18-hour treatment with 0.4% *n*-butanol and 2 mM 2-aminoethanol caused a significant increase in plant regeneration. This observation is similar to that of wheat (Soriano et al. 2008).

In summary we found that *n*-butanol and 2-aminoethanol had no positive effect on the induction of maize microspores. At the same time the embryo yield was slightly elevated and the plant regeneration was significantly increased. However, further biochemical and structural investigations are necessary to discover the mechanism of the effects of the two compounds on maize androgenesis.

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