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Effects of ROS progenitors on the sporophytic development of maize microspores

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ABSTRACT The sporophytic development of maize microspores was investigated in anther culture under oxidative stress conditions. The oxidative stress was induced using ROS progenitors such as paraquat (Pq) and menadione (Men), methionine combined with riboflavin (Men+Rib) and tert-butylhydroperoxide (t-BHP). All the ROS progenitors reduced the anther response and the number of surviving microspores, which was manifested in a decrease of the number of microspore-derived structures (MDS) in 7- and 30-day-old cultured maize anthers. The ROS progenitors also reduced the regeneration potential of MDS. Numerous abnormal cell divisions and progeny cell degradation could be observed during the development of microspores treated with paraquat, menadione and methionine+riboflavin. Intense nuclear condensation was also found in 7-day-old microspores treated with menadione and paraquat. Moreover, menadione significantly delayed the formation of microspore-derived structures and increased the proportion of embryos. Although a less drastic effect on microspore development was observed in the t-BHP treatment, it increased the ratio of calli in the microspore-derived structures.
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KEY WORDS

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Environmental stresses causing the formation of reactive oxygen species (ROS) may occur during the whole life cycle of plant, from the beginning of germination till the senescence (Scandalios 1993). Some of these (for example high light intensity, UV radiation, drought stress) are mainly experienced during the summer period, and thus influence the development of the male and female gametophytes. However, these processes have rarely been investigated.

Male gametophyte development follows a tightly controlled sequence of events that can be divided into two major processes: microsporogenesis and microgametogenesis. *In planta*, during microgametogenesis the haploid uninucleate microspore divides asymmetrically, resulting in vegetative and generative cells, which develops into a mature pollen grain containing sperm cells. *In vitro* during microsporogenesis, the polarized uninucleate microspores can divide symmetrically due to various stresses (cold, heat, etc.) resulting in binucleate or bicellular microspores (Touraev et al. 1997). It may also happen that the first division during the androgenic development of the microspores is asymmetrical, but usually only the vegetative cell divides further. If the first mitotic division is followed by nuclear fusion, a doubled haploid microspore is formed. Intensive cell division occurs leading to the development of multicellular microspores from the binucleate microspores, over the course of approx. one week. Over the following 2-4 days the outer wall (exine) of the microspores bursts, leading to the formation of microcalli and embryo-like structures (ELS) during a 1- month incubation period. Finally haploid and doubled haploid plants are regenerated from these microspore-derived structures, generally by means of secondary embryogenesis in the case of maize (Sunderland and Evans 1976). These processes can be investigated *in vitro* in anther culture.

In the present work, the sporophytic development of maize microspores was investigated *in vitro* in anther culture under oxidative stress conditions. The oxidative stress was induced using ROS progenitors such as paraquat (Pq) and menadione (Men), which generate superoxide radicals, methionine combined with riboflavin (Met+Rib) under light conditions producing H_2O_2, or tert-butylhydroperoxide (t-BHP), which is a lipid peroxide. The androgenic development of microspores was monitored by determining the viability of the microspores and the rate of calli and embryoid formation and of green plant regeneration.

Materials and Methods

The *in vitro* microspore development of maize hybrid A18 was investigated in anther culture.

The anther culture procedure was described in detail by Barnabas (2003). Briefly, tassels containing uninucleate microspores were pretreated at 7°C for 7 days, then, surface sterilised with 20% bleach and rinsed 5 times with sterile distilled water. The anthers were placed onto modified YP medium containing different ROS progenitors and were incubated at 29°C for 1 month in the dark (cultures treated with methionine + riboflavin were incubated in the light). Calli and embryo-like structures developed from microspores

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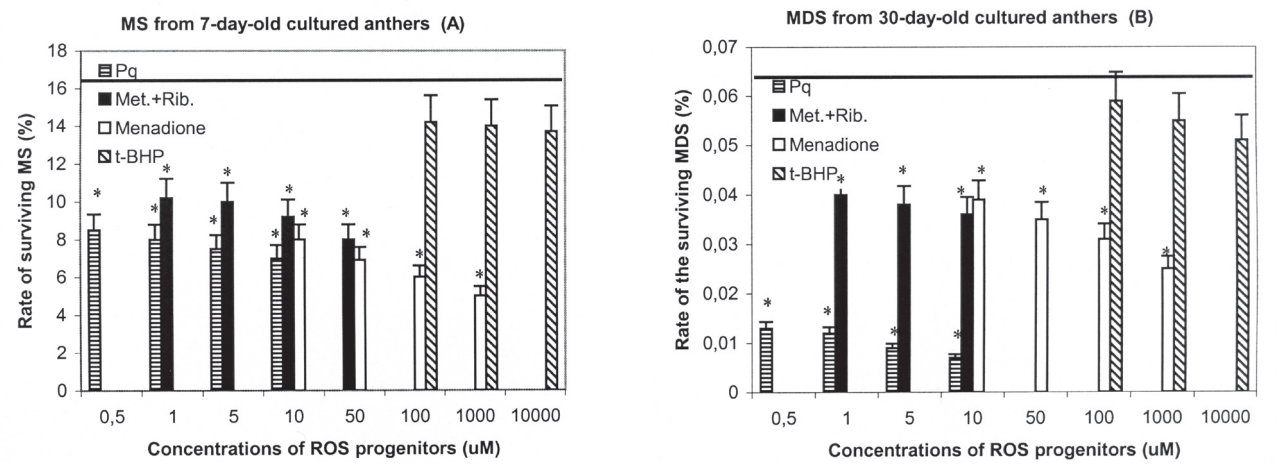

Figure 1. Viability of 7-day-old microspores (MS) (A) and 30-day-old microspore-derived structures (MDS) (B). Horizontal lines indicate control values, without ROS progenitors. *Significantly difference from the control at the p<0.01 level.

were transferred to modified N6 differentiation medium. The regenerated plantlets were placed into larger glass containers containing modified N6 medium without hormones. The healthy green plantlets were planted into soil and were grown till maturity in a Phytotron chamber. The DH lines were self-pollinated.

Oxidative stress was induced by applying ROS progenitors in various concentrations (paraquat at 0.5, 1, 5, 10 μM; menadione at 50, 100, 200 μM; methionine combined with equal amounts of riboflavin at 1, 10, 100 μM; t-BHP at 0.1, 1, 10 mM).

The viability of the 7-day-old microspores and 30-day-old microspore-derived structures (MDS) was determined after FDA staining (Wilholm 1972) using an Olympus BX 51 fluorescence microscope fitted with a Camedia digital camera (Olympus Optical Co. Ltd. Tokyo, Japan).

Results and Discussion

Since no information was available on the effects of ROS progenitors on microspore development, they were applied at several concentrations.

Cytological investigations demonstrated that during the first week most of the nuclei in microspores in anthers became degenerated, resulting in empty pollen grains. As indicated by FDA staining, approximately 16% and 0.062% of the microspores were alive after a week and after 30 days, respectively, in the control cultures. ROS progenitors reduced the number of surviving microspores both in 7- (Fig. 1A) and 30- (Fig.

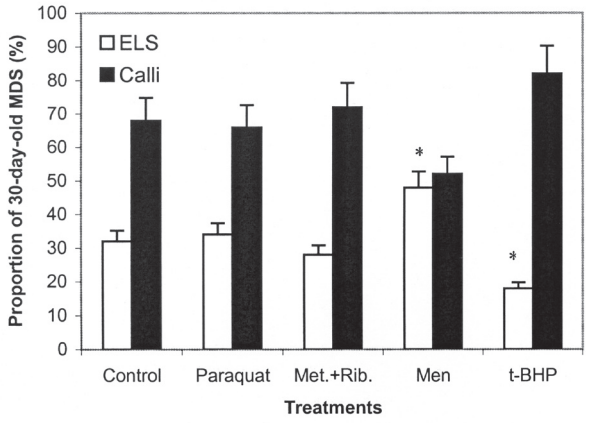

Figure 2. Proportion of embryo-like structures (ELS) and calli in 30-day-old MDS. Pq was applied at 1.0 μM, Met+Rib were applied at 10 μM, Men was applied at 100 μM and t-BHP was applied at 10 mM concentrations.

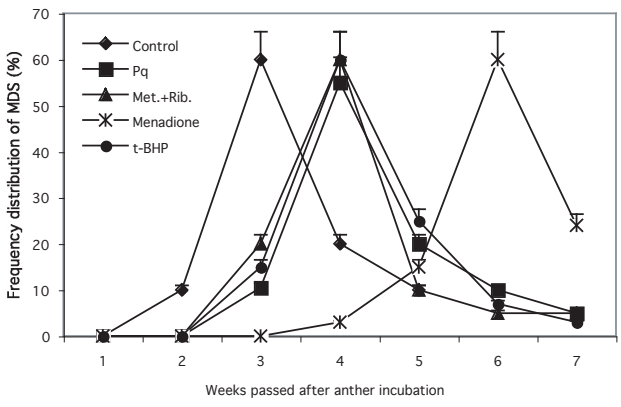

Figure 3. Time dependence of the appearance of calli and embryo-like structures (MDS) on the culture media.

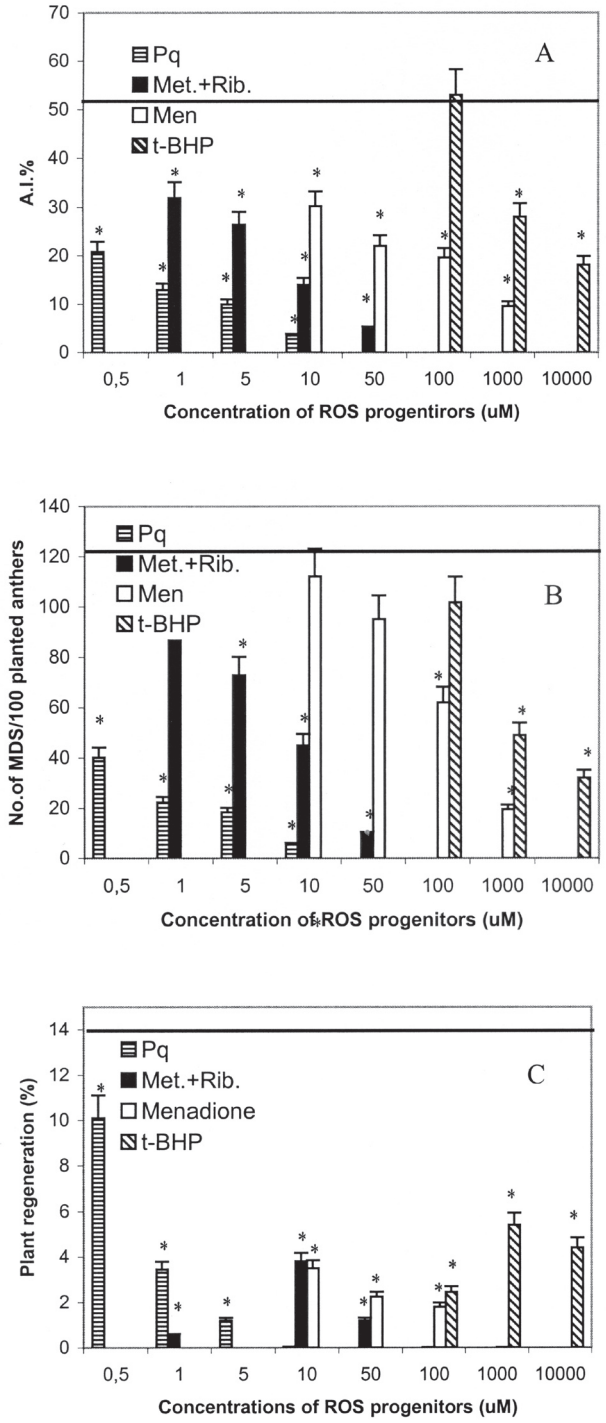

Figure 4. Dose response to ROS progenitors given by anther induction (A.I.%) (A), the formation of microspore-derived structures (indicated as number of MDS/100 anthers) during the 30-day incubation period (B) and the regeneration potential (%) of MDS (C). Horizontal lines indicate the control values, without ROS progenitors. *Significantly different from the control at the p<0.01 level.

1B) day-old cultured anthers especially in the case of paraquat, Met+Rib and menadione treatments (Fig. 1 AB).

During the first week of the incubation period, microspores developing sporophytically reached the multicellular stage. In the control samples, the normal cell division pattern was observed. In many cases, the paraquat or menadione treatments resulted in nuclear condensation and cell degradation. The Met+Rib treatment in light also caused progeny cell degradation (Ambrus et al. 2005). When incubation was continued for a month, the exine of the microspores burst and calli or embryo-like structures were formed. In the control samples, the calli were white or light yellow and friable, while the embryos were mostly compact and regularly shaped (data not shown, Ambrus et al. 2005). The ROS progenitors resulted mainly in irregular embryo structures and friable calli probably due to cell degradation occurring during the early stages of microspore development. In the control the proportions of calli and ELS were 68% and 32%, respectively. Menadione induced the formation of embryo-like structures (48%), while t-BHP induced the formation of friable calli (82%; Fig. 2). The other ROS progenitors did not alter the type of microspore division. The majority of MDS (calli and embryoids) appeared in the third week of the incubation period in the control medium, while they appeared a week later on culture media containing Pq, t-BHP and Met+Rib. Menadione caused a 3-week delay in the development of calli and ELS (Fig. 3). This effect was already observed in 7-day-old multicellular microspores, since they contained fewer cell nuclei than in the other treatments.

In maize anther culture, the microspores in approximately 50% of the inoculated anthers (A.I.%) were able to develop into calli or embryoid structures during the 1-month incubation period without ROS progenitors, resulting in 124 MDS per 100 plated anthers. All the ROS progenitors inhibited the androgenic development of the microspores, as indicated by the decrease in anther induction (A.I.%; Fig. 4A) and in the number of MDS (Fig. 4B).

However, there were differences in the efficiency of various ROS progenitors (Fig. 4AB).

Surprisingly, Pq, which is generally used as a photosynthetic herbicide, dramatically reduced both the anther response (A.I.%) and the frequency of formation of MDS even at the extremely low concentration (0.5 μM; Fig. 4AB). Met+Rib and Men only influenced microspore development when applied at concentrations of higher than 50 μM or 100 μM, respectively. It appears that lipid peroxide (t-BHP) affected *in vitro* microspore development only at extremely high concentrations (10mM), since it only reduced the anther response, the formation of MDS or the regeneration potential at high concentrations (Fig. 4AB).

All the ROS progenitors also reduced the regeneration potential of calli and ELS (R%), which was 14% in the control (Fig. 4C). The least drastic effect could be detected in the

t-BHP treatment. The plant regeneration % was also greatly reduced by the fact that in many cases plantlets with only roots or only shoots could be obtained from media containing ROS progenitors. However, fertile DH plants were obtained in limited numbers from all the ROS progenitor treatments (Ambrus et al. 2005).

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References

Ambrus H, Darko E, Szabo L, Bakos F, Király Z, Barnabas B (2005) In vitro microspore selection in maize anther culture using oxidative stress stimulators. Protoplasma, in press.

Barnabás B (2003) Anther culture of maize (*Zea mays* L). In Malusinszky M, Kasha KJ, Forster BP, Szarejko I eds., Doubled haploid production in crop plants. Kluwer Academic Press, Dordrecht, pp. 103-108.

Scandalios JG (1993) Oxygen stress and superoxide dismutases. Plant Physiol 101:7-12.

Sunderland N, Evans LJ (1980) Multicellular pollen formation in cultured barley anthers. II. The A, B, and C pathways. J Exp Bot 31:501-514.

TouraevA, Vicente O, Heberle-Bors E (1997) Initiation of microspore embryogenesis by stress. Trends Plant Sci 8:297-302.

Widholm JM (1972) The use of fluoresceine diacetate and phenosaphranine for determining viability of cultured plant cells. Stain Technol 47:189-194.