ORIGINAL ARTICLES

Evaluating the Impact of Brassinolide on Ascorbate Peroxidase Activity During Brassica napus Embryo Development in Vitro

Khalil Khamiss, Mahmoud Sabouh and Adnan Kanbar

Department of Field Crops, Faculty of Agriculture, University of Damascus, Syria.

ABSTRACT

The *Brassica napus* embryogenesis has great importance for physiological studies due to the androgenesis system which is released a large number of matched embryos in a short period of time from immature pollens *in vitro*. Thus, the present study aimed to evaluate the effect of different levels of brassinolide (BL) on ascorbate peroxidase (APX) activity during *Brassica napus*, Bactool variety, embryo development. Five different treatments were applied; Control, two levels of BL (10^-6 and 10^-5 M), one level of brassinazole (BrZ), a BL biosynthetic inhibitor, (4x10^-6 M) and BL (10^-6 M) + BrZ (4x10^-6 M). Exogenous applications of brassinolide (BL) increased the activity of ascorbate peroxidase (APX) of microspore-derived embryo which will have a positive profound effects on ascorbate metabolism by altering the amount of it reduced form (ASC) to oxidized form; dehydroascorbate (DHA), ascorbate free radicals (AFRs). At the concentration of BrZ (4x10^-6 M), the activity of ascorbate peroxidase (APX) decreased and that will increase the ascorbate reduced form and therefore increase the amount of a toxic compound, hydrogen peroxide (H2O2).

Key words: *Brassica Napus*, Brassinolide, Brassinazole, Embryogenesis.

Introduction

The capability to regenerate embryos from plant cells *in vitro* is through the use of androgenesis system. Androgenesis is the development of sporophyte from the male gametophyte; immature pollen grains, on artificial medium. Accomplishment of *in vitro* androgenesis is based on adjustment of developmental stage of pollen, minerals of culture medium and growth regulators as well as heat shock or other treatments (Yao et al., 1997). Androgenesis is also called microspore- derived embryogenesis and here pollen directly acts as a zygote and therefore, passes during different embryogenic stages similar to zygotic embryogenesis and it has been applied to a variety of species such as *Brassica napus* (Ferrie et al., 2005 and Stasolla et al., 2008). This system is released a large number of matched embryos in a short period of time and the embryos develop cotyledons, then the plants (Prakash and Giles, 1987), and this allowing to choose any stage of embryonic or plant development to be used for physiological and molecular studies.

Physiological studies have shown that one of the key regulators of embryo development is represented by ascorbate peroxidase (APX) which regulates ascorbate redox status by controlling the balance between the respective reduced ascorbate (ASC) form, oxidized dehydroascorbate (DHA) form and ascorbate free radicals (AFRs) (Belmonte and Stasolla, 2007) and APX activity depends on many different aspects including Steroids (Morkunas I, Gmerek J., 2007).

Steroids are a group of natural products to promoting growth ubiquitous to both plants and animals. Participated in some aspects of development (Bishop and Koncz, 2002). Hundreds of steroids are found in plants, animals, and fungi. Plants are able to produce a variety of sterols and steroids, including brassinosteroids (BRs) (Clouse, 1996). In past few years the detection and characterization of mutants have more established the key function of BRs during plant growth and development as a phytohormones (Clouse and Sasse, 1998 and Arora et al., 2008). One of the most active forms of BRs is Brassinolide (BL) and exogenous applications of BL induced adventitious shoot regeneration from hypocotyl segments of *Brassica oleracea* (Sasaki, 2002). A new study by (Ferrie et al., 2005) discovered that two forms of BRs, 24-epibrassinolide and BL, improved the yield of MDEs from some Brassica species probably by protecting microspores from the heat stress necessary for the initiation of the embryogenic programme. Besides these studies, there is no clear specific information on the requirement for BRs, and BL especially during embryogenesis. This study is to manipulate this phytohormone endogenous level experimentally and here BrZ is used to assess the role played by BL during B. napus microspore-derived embryogenesis and it effects on APX activity.

Corresponding Author: Adnan Kanber, Department of field Crops, Faculty of Agriculture, University of Damascus Syria.
E-mail: adnan_mha@yahoo.com
Materials and methods

Brassica Napus Embryo Development:

*Brassica napus* Bactool variety plants were grown into a growth chamber under 16 hours photoperiod at 25°C day and 16°C night for around 35 days until emergence first buds of flower. Plants were moved to cold environment 12°C day and 7°C night to grow more buds for collecting (Ferrie and Keller, 1995). 2-3 mm length microspores were isolated for microspore-derived embryo (MDE) development and that were carried out exactly as described by Belmonte *et al.*, (2006). Different levels of the two compounds [BL, 10^{-6}-10^{-5} M , BrZ, 4×10^{-6} M and BL (10^{-6}M) + BrZ (4x10^{-6}M)] were applied for BL and BrZ treatments in the culture medium at day zero and preserved in culture throughout the stage of maturity as explained by Ferrie *et al*., (2005). For measurements, control and treated embryos were harvested at days 14, 21, 28, and 35 to assess the optimal stage for conversion.

Metabolic Study:

Measurements of endogenous enzymatic reaction of ascorbate peroxidase (APX) were carried out as indicated by Stasolla and Yeung (2001).

Statistical Analysis:

Tukey’s post-hoc test for multiple variances (Zar, 1999) was used to compare differences between treatments and control.

Results and discussions

Ascorbate production in plant cells depends on the activity of essential enzymes; ascorbate peroxidase (APX) and ascorbate free radical reductase (AFRR) (Fig. 1).

![Fig. 1: Simplified diagram showing the ascorbate system. ASC, reduced ascorbate; AFR, ascorbate free radicals; DHA, dehydroascorbate; H2O, water; H2O2, hydrogen peroxide; AFRR, ascorbate free radical reductase; APX, ascorbate peroxidase.](image)

Table 1: Ascorbate peroxidase (APX) activity (nmole substrate metabolized / mg protein/min) in embryos produced from MDEs and cultured in the presence of brassinolide (BL), brassinazole (BrZ) and BL+BrZ. Embryos were collected at different days during the growth stages.

<table>
<thead>
<tr>
<th>Treatment (M)</th>
<th>Days in Culture</th>
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<tbody>
<tr>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Control</td>
<td>48.2</td>
</tr>
<tr>
<td>BL (10^{-6})</td>
<td>110.2</td>
</tr>
<tr>
<td>BL (10^{-5})</td>
<td>75.1</td>
</tr>
<tr>
<td>BrZ (4x10^{-6})</td>
<td>12.1</td>
</tr>
<tr>
<td>BL(10^{-6}) + BrZ(4x10^{-6})</td>
<td>37.5</td>
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</tbody>
</table>
Fig. 2: Activity of ascorbate peroxidase (APX) (nmole substrate metabolized / mg protein/min) involved in ascorbate metabolism. Embryos were treated with brassinolide (BL) and/or brassinazole (BrZ) and collected at different days during development for enzymatic activity.

Desiccation:

Application of BL in the culture medium increased the activity of ascorbate peroxidase (APX) (Andrzej Bajguz, 2010) which leads to increasing the production of DHA and AFR, thereby switching the redox state towards an oxidized environment (Fig. 1). The activity of APX, the recycling enzyme converting ASC to AFR (Fig. 1) was high in BL-treated embryos and low in the other treatments, especially in the presence of BrZ (Table 1 and Fig. 2). Higher APX activity is required for detoxification by decreasing the level of H$_2$O$_2$ generated during active cellular proliferation and in the regulation of cell wall plasticity by reducing the endogenous H$_2$O$_2$ utilized by peroxidases in cross-linking cell wall polymers (De Gara et al., 1996).

References


