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# Synergistic enhancement of tolerance mechanisms in response to photoactivation of cationic tetra (N-methylpyridyl) porphyrins in tomato plantlets

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ABSTRACT

Antimicrobial photodynamic treatment (APDT) is largely used in medical domain and could be envisaged as a farming practice against crop pathogens such as bacteria and fungi that generate drops in agricultural yields. Thus, as a prerequisite for this potential application, we studied the effect of water-soluble anionic (TPPS and Zn-TPPS) and cationic (TMPyP and Zn-TMPyP) porphyrins tested on tomato (*Solanum lycopersicum*) plantlets grown in vitro under a 16 h photoperiod. First of all, under dark conditions, none of the four porphyrins inhibited germination and induced cytotoxic effects on tomato plantlets as etiolated development was not altered. The consequences of porphyrin long-term photoactivation (14 days) were thus studied on in vitro-grown tomato plantlets at phenotypic and molecular levels. Cationic porphyrins especially Zn-TMPyP were the most efficient photosensitizers and dramatically altered growth without killing plantlets. Indeed, tomato plantlets were rescued after cationic porphyrins treatment. To gain insight, the different molecular ways implied in the plantlet tolerance to photoactivated Zn-TMPyP, lipid peroxidation, antioxidative molecules (total thiols, proline, ascorbate), and ROS detoxification enzymes were evaluated. In parallel to an increase in lipid peroxidation and hydrogen peroxide production, antioxidative molecules and enzymes (guaiacol peroxidase, catalase, and superoxide dismutase) were up-regulated in root apparatus in response to photoactivated Zn-TMPyP. This study showed that tomato plantlets could overcome the pressure triggered by photoactivated cationic porphyrin by activating antioxidative molecule and enzyme arsenal and confining Zn-TMPyP into cell wall and/or apoplasm, suggesting that APDT directed against tomato pathogens could be envisaged in the future.



## 1. Introduction

Molecules such as porphyrins, naturally present in all living kingdom, are photoexcitable by sunlight and able to produce reactive oxygen species (ROS) such as superoxide anion radical, hydrogen peroxide, hydroxyl radical, and singlet oxygen which, in turn, lead to cell damage and ultimately cell death [1,2]. Under dark, porphyrins do not exhibit significant cyto- or genotoxicity [1,4]. Two studies performed on root meristematic cells of *Allium cepa* and tobacco Bright Yellow cell suspensions showed opposite results with photoactivated porphyrins [4,5]. Study performed on *Allium cepa* root apex demonstrated that cationic porphyrins, either free or Zn-metallated, induce DNA photodamage whereas in tobacco cell suspension, anionic and cationic porphyrins were not cytotoxic and anionic porphyrin was the most efficient death inducer compared to cationic one upon illumination. According to literature, this last result was quite surprising, taking into account that cationic or neutral porphyrins are always the most efficient to induce cell death from bacteria to mammal cells and/or tissues. Previous work showed that derivatives of cationic porphyrin were more efficient than neutral and anionic porphyrins on Gram-negative bacteria (*Escherichia coli*) and that these effects were less pronounced on Gram-positive bacteria such as *Enterococcus hirae* or *Staphylococcus aureus* [2,6–10]. Likewise, exogenous supply of cationic porphyrin in cultures of cyanobacteria and green microalgae showed that the latter were more photosensitive than the former [11]. Upon illumination, protoporphyrin IX and other derivatives were proven to be strongly cytotoxic to the yeasts *Saccharomyces cerevisiae* and *Candida albicans* [1,12–14]. Furthermore, tested on mosquito or fly larvae, photoactivated cationic porphyrins also possess an insecticidal power [15–17]. Finally, strategies known as photodynamic therapy including antimicrobial photodynamic treatment are based on the use of photoactivated porphyrins in a large variety of medical domains such as cancerology, dermatology, ophtamology, or odontology [1–3,18]. This strategy is also envisaged to struggle plant pathogens such as *Colletrichum acutatum* and *Aspergillus nidulans* fungi [19].

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All these studies were focused on an optimization of a fatal issue such as death linked to photoactivation of porphyrins. Furthermore, they were essentially performed on isolated cells or tissues from prokayotic to eukaryotic cells, except on mosquito larvae. Nevertheless, it should be of interest to understand the consequence of photoactivation of exogenous porphyrins in whole organisms especially in plants. Indeed, as antimicrobial photodynamic treatment (APDT) could be considered as a potential agronomic strategy against plant pathogens (fungi and bacteria) [19], it would be of interest to study the response of plants to photoactivated porphyrins. In that respect, our aim was to study the effect of two categories of water-soluble synthetic porphyrins: cationic and anionic porphyrins, either free base (TMPyP and TPPS, respectively) or zinc-metallated (Zn-TMPyP and Zn-TPPS) on tomato plantlets grown in vitro. Previous studies on plants were performed to set up modifications of endogenous porphyrins biosynthesis by transgenesis [20–24]. Authors showed that an accumulation of protoporphyrin IX, common precursor of heme and chlorophylls, triggered lethal phenotypic alterations (growth inhibition, browning leaves, desiccation) after illumination [22,23].

Based on these studies and because porphyrins could be used as antimicrobial agents in agronomic practices, we studied the consequences of sunlight photoactivation of exogenous porphyrins on tomato plantlets at phenotypic and molecular levels. We supposed that defense mechanisms such as antioxidative machinery would be activated to allow plantlet tolerance to the presence of exogenous photosensitizer. We firstly showed that none of these porphyrins were able to inhibit germination and were cytotoxic under dark conditions. Thus, we compared the consequences of their photoactivation on phenotype of 14-day-old in vitro plantlets. Cationic porphyrins, especially Zn-TMPyP, induced the strongest altered phenotype. Nevertheless, tomato plantlets could resist to high amounts of cationic porphyrins and also be completely rescued afterwards. Thus, taking into account these first results, we focused our study on the cellular and molecular mechanisms triggered in particularly by Zn-TMPyP in plantlets roots, trying to understand how tomato plantlets were able to resist to photoactivated Zn-TMPyP. Indeed, endogenous levels of total thiol, proline, and ascorbate, considered as antioxidant molecules, and enzyme activities such as peroxidases and superoxide dismutase were significantly up-regulated in tomato roots. In conclusion, we suggested that, in response to Zn-TMPyP photoactivation, tomato plantlets were able to produce appropriate amounts of total thiol, proline, and ascorbate as well as to sufficiently activate ROS detoxification enzymes to resist to photoactivated Zn-TMPyP.

## 2. Materials and Methods

### 2.1. Porphyrins

All porphyrins are soluble in water and their chemical structures have been reported in Fig 1. Anionic porphyrin corresponds to TPPS and was purchased from Sigma–Aldrich (St Louis, MO, USA). Zn-TPPS was obtained by metallation of TPPS with zinc acetate [25]. Cationic porphyrins: Tetra (N-methylpyridyl) porphyrin tetrachloride or TMPyP and Zn-TMPyP were purchased from Frontier Scientific (Carnforth, UK).

To evaluate porphyrin photostability in the plant culture medium, they were added to the medium at the wanted concentration. For all experiments, plates were maintained for 15 days in a controlled growth chamber at 22 °C under a 16 h photoperiod and a photon flux density of 250 μmol.$m^{-2}$.$s^{-1}$. Photostability was evaluated by Uv–Vis absorption method, monitoring decrease of the Soret Band at 437 and 414 for Zn-TMPyP and TPPS, respectively. For each measurement, a square (1 $cm^2$) of medium with porphyrin was taken, suspended in 10 mL water under vigorous agitation. Then absorption spectra were recorded. Initial value of Soret band absorbance was used to define 100% level.

### 2.2. Plant Material

Seeds of tomato var Bali F1 hybrid purchased from Tézier (Portes-lès-Valence, France), were germinated in vitro on a Gamborg B5 culture medium (Duchefa Biochemie, Haarlem, Netherlands) at pH 5.8 supplemented with 2% (*w*/*v*) sucrose and 0.8% (*w*/*v*) agar (Difco, Dallas, USA) [26]. Porphyrins were added to the medium after autoclaving. For all experiments, plants were grown on 25 mL of B5 alone or supplemented with porphyrin in a controlled growth chamber at 22 °C under a 16 h photoperiod and a photon flux density of 250 μmol.$m^{-2}$. $s^{-1}$. Cool daylight lamps (OSRAM Lumilux 24W) were used in the growth chamber. After 14 days of growth, tomato plantlets were harvested and used for phenotypic and biochemical analyses. Germination ratio was evaluated after 4 days of culture using a binocular microscope. Under these conditions, 8 tomato seeds were sown on the same plate. The experiments were performed 3 times independently. Pictures from the last experiment were taken with a digital camera and/or under a Leica binocular microscope.

For cytotoxicity assessment, plates containing 8 seeds and surrounding by aluminum foil in order to avoid any light source were maintained vertically for 4 days in growth chamber. The experiments were performed 3 times independently.

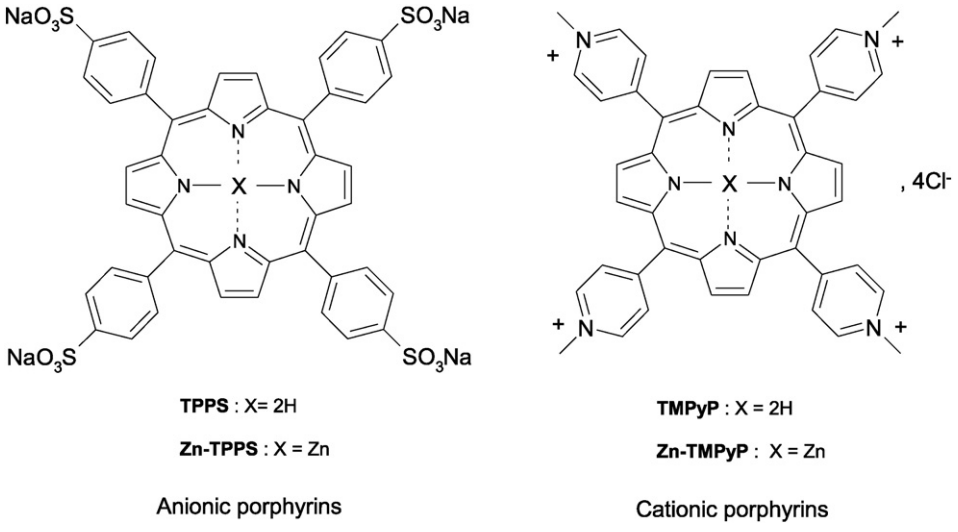


**Fig. 1.** Structures of different porphyrins tested in this study.