South African Journal of Botany 111 (2017) 176–181

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Morphological characterisation of lettuce plasma membrane ultrastructure and vesicle formation caused by nonylphenol: A scanning electron microscopy study

W. de Bruin [a,b], C.F. van der Merwe [a], Q. Kritzinger [a,*], R.M.S. Bornman [b,c], L. Korsten [a,b]

[a] Department of Plant and Soil Sciences, University of Pretoria, Pretoria 0002, South Africa
[b] Institute for Food, Nutrition and Well-being, University of Pretoria, Pretoria 0002, South Africa
[c] School of Health Systems and Public Health, University of Pretoria, Pretoria 0002, South Africa

ARTICLE INFO

Article history:
Received 27 September 2016
Received in revised form 3 March 2017
Accepted 10 March 2017
Available online xxxx

Edited by JS Boatwright

Keywords:
Endocrine disruptor chemical
Nonylphenol
Plasma membrane
Protoplast
Scanning electron microscopy
Surfactant

ABSTRACT

Fresh produce can come into contact with nonylphenol (NP), a widely used surfactant and industrial endocrine disrupting chemical, through various routes of exposure in the agricultural environment. Nonylphenol has been linked to immunological and behavioural abnormalities as well as infertility and cancer in mammals. One of its modes of action is believed to be through damaging the plasma membrane of cells. While it has been demonstrated that NP can be phytotoxic, its effect on plant cell membrane integrity has not yet been examined. In this study, the plasma membranes of lettuce protoplasts were analysed with a high- resolution scanning electron microscope (SEM). The protoplasts were isolated from plants that had been exposed to 800, 1600, 3200, 6400 and 12,800 μg/l NP, respectively, for three weeks. The plasma membrane of the protoplasts that were exposed to 6400 and 12,800 μg/l NP showed vesicle-like protrusions, accompanied by flattening of the membrane in its direct vicinity. This study showed that NP caused changes in the ultrastructure of lettuce plasma membranes, thereby compromising its functionality. To the best of our knowledge, this paper describes for the first time the morphology of the plasma membrane using high-resolution SEM.
© 2017 SAAB. Published by Elsevier B.V. All rights reserved.

1. Introduction

Plants are constantly exposed to detergents as a result of industrial pollution and contact with agrochemicals and contaminated water (Coleman et al., 1997). The quantity and variety of these compounds have increased as a result of intensified industrialisation, especially in countries with economies in transition. One of the surfactants increasingly being used in developing countries is nonylphenol ethoxylate (NPEO) (Bergé et al., 2012). The primary breakdown product of this extensively used class of surfactants is called nonylphenol (NP) (Mann and Boddy, 2000; Belmont and Metcalfe, 2003), which has become an ever-present toxic contaminant in the environment (Pretorius and Bornman, 2005). The majority of NP is primarily released through treated wastewater effluent into aquatic systems. Concentrations previously detected in South African waters range from 0.12 to 6360 μg/l (Barnhoorn et al., 2004; Burger, 2008).

The majority of research on NP has focused on its toxic and endocrine disruptive influence on mammalian cells. Nonylphenol can induce apoptotic and necrotic cell death through breakage in plasma membranes (Pretorius and Bornman, 2005; Pretorius et al., 2006). Reports on the action of NP on higher plants are, however, limited (Ferrara et al., 2006). Studies that have focused on NP in plants monitored its uptake and reported on its phytotoxic effects, but did not adequately describe its mode(s) of action (Bokern and Harms, 1997; Dembitsky et al., 2002).

To study the plasma membrane of plant cells, the cell wall can be a major impediment. Morphological investigations can be done more easily if the walls are first removed enzymatically, leaving exposed protoplasts for visual assessment (Ruesink, 1971). Isolated root and leaf protoplasts have previously been used to study the action of surfactants on plant membrane structure and permeability (Miller and St John, 1974; St John et al., 1974; Watson et al., 1980). The most striking feature of surfactant treated plants is the disruption of the plasma membrane (Baird et al., 1978). At low concentrations it affects the structure and increases permeability of the plasma membrane (Currier and Dybing, 1959), while causing extensive damage at high concentrations (Davis et al., 1982). Increased permeability is manifested by the presence of pores where normal membrane constituents are replaced with surfactants (Currier and Dybing, 1959; Deamer and Crofts, 1967), thereby displacing phospholipids, opening up the membrane (Currier and Dybing, 1959) and facilitating protein interaction (Knoche et al., 1992). In spite of the

* Corresponding author.
E-mail address: quenton.kritzinger@up.ac.za (Q. Kritzinger).

http://dx.doi.org/10.1016/j.sajb.2017.03.027
0254-6299/© 2017 SAAB. Published by Elsevier B.V. All rights reserved.

large number of studies on surfactant-induced membrane permeability, the possible effect of NP on the plant plasma membrane and its mechanism are still not fully understood.

In order to establish whether NP causes membrane damage to plants, it is important to characterise the baseline plant plasma membrane morphology at the ultrastructural level. Our current understanding of the plant plasma membrane is based on the "fluid mosaic" model of Singer and Nicolson (1972). This familiar model states that the membrane consists of a hydrophobic matrix, formed by a double layer of phospholipids in which proteins are embedded. Some of the proteins are confined to one side of the membrane (peripheral proteins), while others extend entirely through the membrane (integral proteins) (Singer and Nicolson, 1972). The membrane proteins, like the phospholipids, are amphipathic and it is this attribute that positions them in the membrane. The lipid bilayer forms a permeability barrier while the proteins perform specific functions (Miller and St John, 1974). Goñi (2014) further states that the bilayer is heterogeneously structured, with diameters ranging between 0.1 and 1.0 μm (Goñi, 2014).

In as much as the "fluid mosaic" model (Singer and Nicolson, 1972) is a comprehensive one that has explanatory value, a physical description, using high- resolution electron microscopy, has its own indubitable value. At present there is no scanning electron microscope (SEM) imagery available as reference material for a plant plasma membrane, healthy, or damaged. Information on the nature of the plasma membrane surface can therefore assist in the interpretation thereof and on the extent of potential damage that NP causes.

The purpose of this paper is twofold: firstly to characterise the plant plasma membrane morphology using high- resolution electron microscopy and secondly, to demonstrate the effects of NP on the plant membrane. Lettuce (*Lactuca sativa* L.) was used as a model plant, as it is particularly sensitive to water quality (Wiel-Shafran et al., 2006).

2. Materials and methods

2.1. Preparation of nonylphenol stock solution

A stock solution was prepared by dissolving NP (technical grade, mixture of ring and chain isomers, Sigma-Aldrich, Kempton Park, South Africa) in 95% acetone (Merck, Johannesburg, South Africa) to a concentration of 937 mg/l.

2.2. Plant material

Tissue for protoplast isolation consisted of fully expanded leaves and the taproot excised from 10-week old greenhouse-grown cos lettuce (*Lactuca sativa* L. var. *longifolia* Lam.) cv. Triple Play plants. These plants were hydroponically cultivated in glass jars filled with water (Fig. 1), 1/2-strength nutrient solution (Nutrifeed, Starke Ayres, South Africa) and appropriate amounts of a tenth-dilution of the NP stock solution that was added to make up working concentrations of 0 (control), 800, 1600, 3200, 6400 or 12,800 μg/l. A 9.5% acetone solvent control was also prepared.

2.3. Isolation of protoplasts

Representative plants from each treatment (five plants per treatment) were dissected and separated into shoots and roots. Mature leaves were taken from the 3rd rosette, counting from the inside of the plant. Laminar pieces (2.5 × 5.0 mm) were excised from the middle-part which included the mid rib and veins. As for the taproot, a section of 1 mm in thickness was taken from the area of cell maturation. The excised pieces of root and leaf material were cut into 1 mm^3 pieces and placed in a protoplast isolation solution. The isolation solution consisted of 10 g/l cellulose (from *Penicillium funiculosum*, 250,000 U) (Sigma-Aldrich) and 1 g/l pectinase (from *Rhizopus* sp., 1000 U) (Sigma-Aldrich) (digesting enzymes to remove the cell wall) as well

Fig. 1. Cos lettuce (*Lactuca sativa* L. var. longifolia Lam.) cv. Triple Play plants grown hydroponically in water filled glass jars amended with nutrient solution and nonylphenol.

as 0.15 M NaCl (Merck) (osmoticum) that was dissolved in a 0.15 M phosphate buffer (Merck). The pH was adjusted to 5 to ensure an optimal working condition for the enzymes. Protoplasts were isolated by incubating the isolation medium at 25 ± 1 °C for 90 min.

2.4. Sample preparation for scanning electron microscopy

Following the incubation time, the suspension of digested tissue in the isolation medium was centrifuged at 1.2 × 1000 U/min for five min to remove the debris from the protoplasts. The pellet of protoplasts was rinsed three times in 0.075 M phosphate buffer where after it was post-fixed in 1% aqueous osmium tetra-oxide (OsO_4) (SPI-Chem, West Chester, United States of America) for 15 min. Thereafter, the samples were rinsed again three times with 0.075 M phosphate buffer, followed by serial dehydration in 30%, 50%, 70%, 90% and three times with 100% ethanol. The samples were finally dried in a plastic container using Hexamethyldisilazane (HMDS) (reagent grade, ≥99%) (Sigma-Aldrich) and coated with carbon. Samples were viewed with a Zeiss Crossbeam 540 FEG SEM (Germany) and micrographs were taken at 1 kV accelerating voltage. Twenty root cells and 20 leaf cells of each of the five plants per control, solvent control and treatment were analysed.

3. Results

3.1. Morphological characterisation of the plasma membrane

After enzymatic digestion of the cell wall, the appearance of the plasma membrane of the remaining fixed protoplasts was typical of the "fluid mosaic" model of biological membranes. The plasma membrane consisted of a matrix of phospholipids with embedded proteins forming heterogeneous patches. Its thickness varied between 0.012–0.1 μm (Fig. 2a). The cytoplasm formed a continuum with the plasma membrane and was usually not easily discernable from the plasma membrane itself. It varied in thickness from 0.4–3.3 μm. Remnants of cytoplasm from ruptured cells often adhered to surrounding protoplasts or covered the surface of the specimen stub (Fig. 2b).

The membrane surface-texture differed between cells. At times the plasma membrane looked smooth (Fig. 3a & b), while in other instances it appeared rougher (Fig. 3c & d). The size and amount of proteins varied, with individual proteins measuring 40–91 nm (Fig. 3).

3.2. The effect of nonylphenol on the plant membrane of cos lettuce cells

While the plasma membrane structure of all NP treated leaf cells and 800, 1600 and 3200 μg/l NP exposed root cells looked similar to that of the control and solvent control leaf and root tissue, the 6400 and

Download English Version:

https://daneshyari.com/en/article/5762958

Download Persian Version:

https://daneshyari.com/article/5762958

Daneshyari.com