

**LIPID PEROXIDATION AS A BIOCHEMICAL MARKER FOR OXIDATIVE
STRESS DURING DROUGHT. AN EFFECTIVE TOOL FOR PLANT
BREEDING.**

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Abstract

Oxidative stress can be induced by a wide range of environmental factors, including drought. One of the main cellular components susceptible to damage by reactive oxygen species are lipids (by peroxidation of unsaturated fatty acids in biological membranes). The assay of Thiobarbituric Acid Reactive Substances (TBARS) is a well-established method for monitoring lipid peroxidation. This relatively simple analytical protocol facilitates extensive screening research in plant breeding.

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Introduction

Higher plants have developed the ability to adapt to external, and frequently harmful, environmental factors. Drought is considered to be one of the major sources of environmental stress. It seriously affects crop productivity by inhibiting plant growth and development (Anjum *et al.* 2011a) and results in a 50% or more reduction in average yields (Wang *et al.* 2003). Water stress inhibits photosynthesis, induces changes in chlorophyll content and composition, and damages the photosynthetic apparatus (Nayyar & Gupta 2006). Furthermore, dehydration of tissue inhibits photochemical activities and brings about a reduction in the activity of Calvin–Benson–Bassham cycle enzymes (Monakhova & Chernyadev 2002).

It is well established that chloroplast, mitochondria and peroxisomes are a major source of reactive oxygen species (ROS) in plant cells. ROS play a dual role in plant physiology. They are important secondary signaling molecules, but equally, they are toxic products of aerobic metabolism that accumulate within cells during abiotic stress (Huang *et al.* 2012). The equilibrium between the production and the enzymatic and non-enzymatic scavenging of ROS may be disturbed by drought. During water stress, these disturbances in equilibrium result in a sudden increase in cellular redox potential, which can damage many cell components, including proteins, lipids, and DNA (Mittler 2002; Anjum *et al.* 2011b). Many higher plants, however, have evolved efficient enzymatic antioxidant defence mechanisms involving superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, monodehydroascorbate reductase, dehydroascorbate reductase, glutathione peroxidase, guaiacol peroxidase and glutathione–S–transferase (Gill & Tuteja 2010).

The peroxidation of lipids in biological membranes is the most obvious symptom of oxidative stress in plants. The following paper shortly describes the peroxidation of lipids by

drought-induced oxidative stress in crop plants and presents details of the cause and effect relationship that exists between lipid peroxidation and other symptoms that result from drought.

Biochemical mechanism of lipid peroxidation

When ROS levels exceed the capacity of the plant to scavenge, lipid peroxidation (LP) in biological membranes increases, thereby affecting the physiological processes of the cell. Malondialdehyde (MDA) is one of the final products of oxidative modification of lipids, and is responsible for cell membrane damage including changes to the intrinsic properties of the membrane, such as fluidity, ion transport, loss of enzyme activity and protein cross-linking. These changes eventually result in cell death (Sharma *et al.* 2012). The polyunsaturated fatty acid (PUFA) components of membrane phospholipids are especially susceptible to ROS activity. Fundamentally, the process of lipid peroxidation comprises three distinct stages: initiation, propagation, and termination. The initiation phase of LP is the step by which a fatty acid radical is produced. The hydroxyl radicals or superoxides can react with PUFA methylene groups, consequently generates hydroperoxides, lipid peroxy radicals and conjugated dienes (Smirnoff 1995), whereas the peroxy radicals are highly reactive and capable of undergoing a propagation chain reaction (Fig. 1).

The decomposition of lipid hydroperoxides can readily produce aldehydes (malondialdehyde, crotonaldehyde and acrolein), lipid alkoxy radicals alkanes, lipid epoxides and alcohols (Stadtman 1986). The view that lipid peroxidation is solely a destructive process has changed during the past few years. It was shown that lipid hydroperoxides and other products of lipid degradation, as well as LP initiators (*i.e.* ROS), can mediate in the signal transduction cascade (Blokhina *et al.* 2003; Molassiotis & Fotopoulos 2011; Bhattacharjee 2012; Boguszevska & Zagdańska 2012).

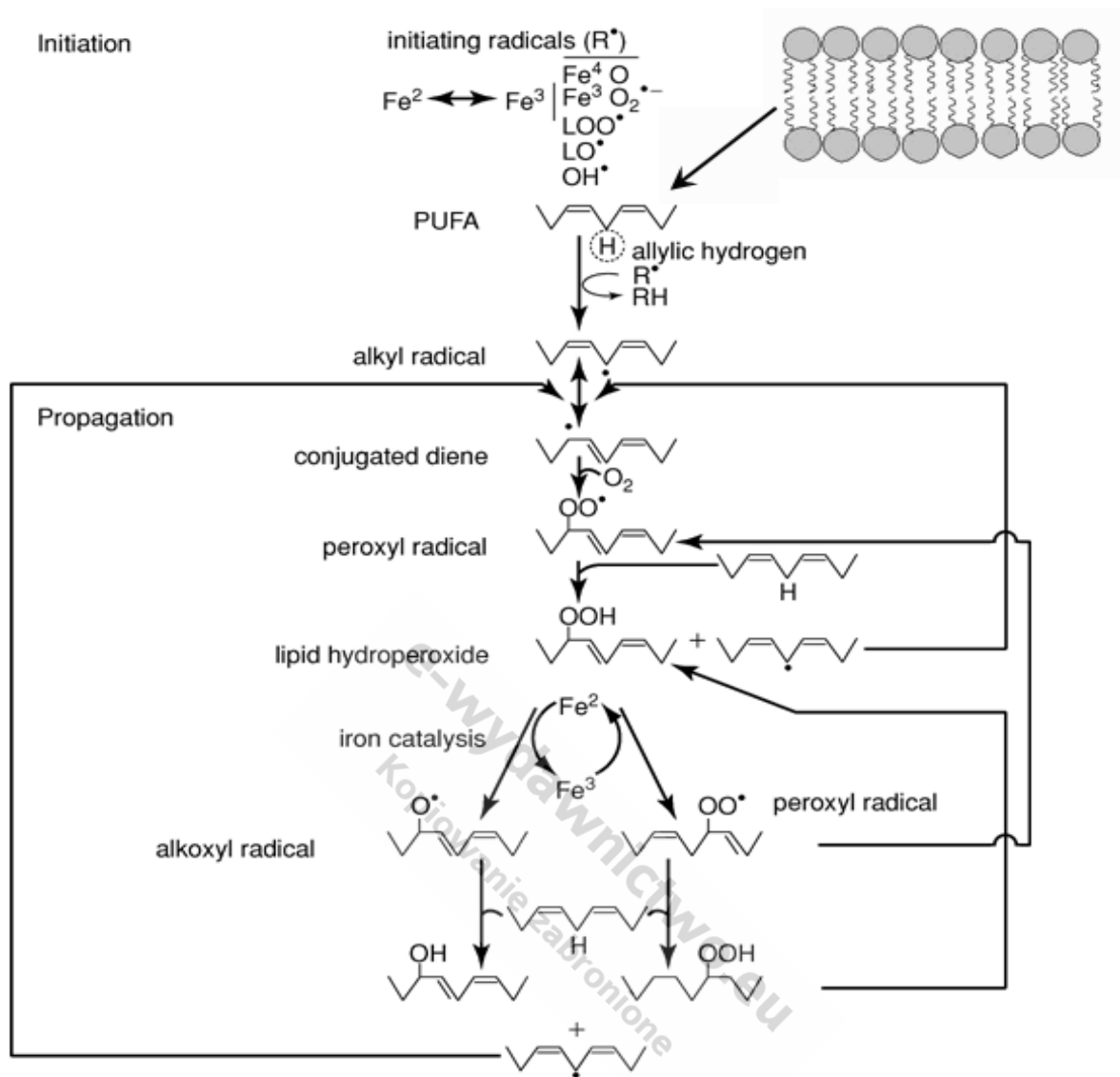


Figure 1. Initiation and propagation phases of lipid peroxidation. According to Hall & Bosken 2009.

Measuring lipid peroxidation

ROS-induced oxidation of PUFAs generates α , β -aldehydes such as MDA and 4-hydroxynonenal (4-HNE). The change in the level of these aldehydic products can be measured, and represents one of the most widely accepted markers of oxidative stress in higher plants (Hulaev & Oliver 2006)

The Thiobarbituric Acid Reactive Substances (TBARS) assay is a well-established method for monitoring lipid peroxidation level. This rapid and easy analytical protocol facilitates extensive research in modern plant breeding e.g. an increased tolerance to environmental stresses, such as drought. MDA forms a 1:2 adduct with thiobarbituric acid (TBA) (Fig. 2), and can be estimated spectrophotometrically A_{532} or fluorometrically (Hodges *et al.* 1999).

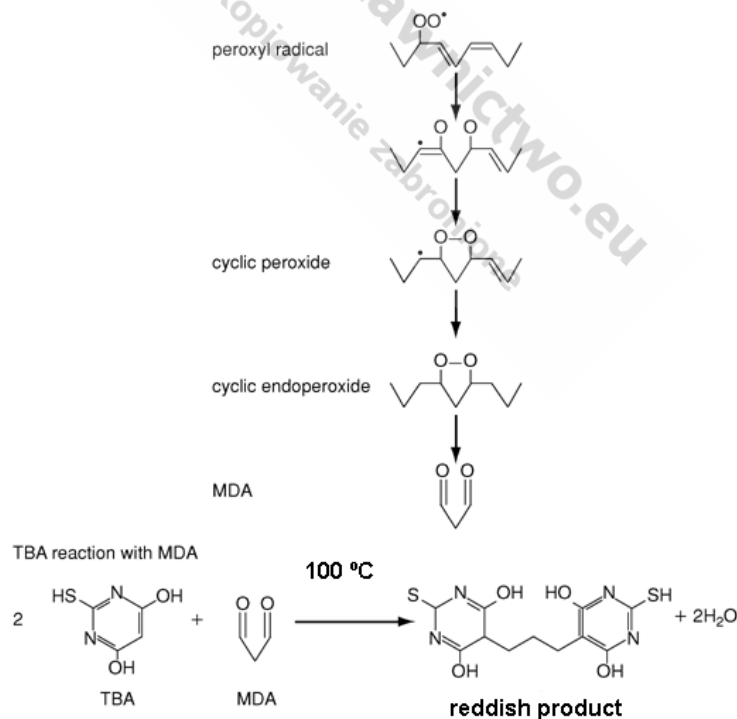


Figure 2. Formation of malondialdehyde (MDA) and measurement by thiobarbituric acid (TBA) reaction. According to Hall & Bosken 2009.

Lipid peroxidation correlates with other biochemical and physiological symptoms

Measurement of TBARS during water stress treatments has generally been accepted as a means of assessing the degree of oxidative stress during drought. This is supported by data collected over a number of years for a range of crop species (Tab. 1).

Table 1. The lipid peroxidation induced by drought in various crop plants.

Species	References
Oats (<i>Avena spp.</i> L.)	Harish <i>et al.</i> 2010
Jutes (<i>Corchorus spp.</i> L.)	Chowdhury and Choudhuri 1985
Apple trees (<i>Malus spp.</i> Mill.)	Wang <i>et al.</i> 2012
Maize (<i>Zea mays</i> L.)	Bai <i>et al.</i> 2006; Ali & Ashraf 2011; Anjum <i>et al.</i> 2011a; 2011c; 2012
Soya bean (<i>Glycine max</i> (L.) Merr.)	Anjum <i>et al.</i> 2011b
Rapeseed (<i>Brassica napus</i> L.)	Liu <i>et al.</i> 2011
Upland cotton (<i>Gossypium hirsutum</i> L.)	Yildiz–Aktas <i>et al.</i> 2009
Wheat (<i>Triticum aestivum</i> L.)	Sairam <i>et al.</i> 1998; Singh <i>et al.</i> 2012
Common bean (<i>Phaseolus vulgaris</i> L.)	Zlatev <i>et al.</i> 2006
Sesame (<i>Sesamum indicum</i> L.)	Fazeli <i>et al.</i> 2007
Chickpea (<i>Cicer arietinum</i> L.)	Mohammadi <i>et al.</i> 2011

It is possible that the detrimental effect of water deficit in soil and genotypic variation in drought tolerance is associated with levels of LP in tissues. Singh *et al.* (2012) showed that instability of biological membranes, as reflected by LP, was greater in drought-sensitive than in drought-tolerant wheat (*Triticum aestivum* L.) genotypes. Furthermore, these authors concluded that the progressive increase in TBARS during plant development may have resulted from greater levels of hydrogen peroxide. Moreover, a very strong and positive correlation was reported to exist between levels of hydrogen peroxide and TBARS in the leaves of wheat plants grown under irrigated and rain-fed conditions. Similar results were obtained in other studies, that demonstrated a negative correlation between the fresh weight of wheat seedlings and their TBARS content. Conversely, the correlation between H₂O₂ and TBARS was positive (Tian & Lei 2007). Sairam *et al.* (1997/98) reported an increase in LP and a decrease in the level of total chlorophyll and carotenoids. Increased TBARS accumulation has been correlated with a reduction in the relative water content (RWC) and photosynthetic pigment content of leaves subjected to prolonged water deficit (Jiang & Huang 2001).

Concluding remarks and future challenges

Water is a renewable resource. However, with the population growth, economic development and improved living standards, the world's supply of fresh water is steadily decreasing and as a result water resources for agricultural production are limited and diminishing. Therefore the breeding of drought-tolerant and water-use-efficient crop varieties should be of global concern. It would appear that breeding crop plants for increased tolerance to drought is an economically justified approach to the improvement of agricultural efficiency, whilst simultaneously reducing the use of fresh water in agriculture. Consequently,

an understanding of the mechanisms of drought tolerance in higher plants should be the priority of all plant researchers and breeders.

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