Saudi Journal of Biological Sciences (2016) xxx, xxx–xxx

King Saud University

Saudi Journal of Biological Sciences

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ORIGINAL ARTICLE

Triterpenoid modulates the salt tolerance of lanosterol synthase deficient *Saccharomyces cerevisiae*, GIL77

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Received 10 March 2016; revised 5 August 2016; accepted 12 October 2016

KEYWORDS

Salt tolerance;
Triterpenoids;
Oxidosqualene cyclase gene;
Yeast;
GIL77

Abstract This study examined the effect of triterpenoid on the salt tolerance of lanosterol synthase deficient yeast mutant GIL77. The expression of the triterpenoid synthase gene under *GAL1* promoter in GIL77 increased the triterpenoid concentration of both whole cell and plasma membrane fractions. Without the induction of the genes, the growth curve of *BgbAS* or *RsM1* transformant depicted patterns similar to control cells in both the presence and absence of salt with growth inhibition at 500 mM NaCl. The induction of *BgbAS* and *RsM1* gene expression slightly repressed growth compared with control cells in the absence of NaCl. The growth of GIL77 was significantly suppressed by the expression of *BgbAS* or *RsM1* under salinity conditions. Of the triterpenoid synthase genes, *BgbAS* rather than *RsM1* was found to strongly inhibit the growth of GIL77 cells under salt stressed conditions. The expression of the triterpenoid synthase gene in GIL77 also influenced their tolerance to other abiotic stresses. In contrast to the endogenous synthesis, the exogenous supply of triterpenoid in the culture medium appeared to occur in the plasma membrane fraction and enhanced the salt tolerance of GIL77. This study thus discussed the physiological significance of triterpenoid in relation to its possible role in modulating salt tolerance.

1. Introduction

Mangrove plants are unique in that they can grow under a wide range of salinity conditions, ranging from freshwater to a hypersaline environment (Tomlinson, 1986). Several types of salt tolerance mechanisms have been proposed for mangrove plants: (1) adjustment of osmotic pressure by the accumulation of small molecule osmolytes, such as glycinebetaine or sugar alcohol (Popp, 1984; Sakamoto and Murata, 2000); (2) salt extrusion across the plasma membrane using an ion transporter (Allen et al., 1995); (3) compartmentalization of salt in the vacuole

Abbreviations: FID, flame ionization detector; GC, gas chromatography; LS, lanosterol synthase; OSCs, oxidosqualene cyclase; MES, 2-morpholinoethanesulfonic acid; *BgbAS*, β-amyrin synthase; *BgLUS*, lupeol synthase; SC, synthetic complete; S.E.M., standard error of the mean; *RsM1*, multifunctional triterpenoid synthase; TLC, thin layer chromatography.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2016.10.009
1319-562X

Please cite this article in press as: Inafuku, M. et al., Triterpenoid modulates the salt tolerance of lanosterol synthase deficient *Saccharomyces cerevisiae*, GIL77. Saudi Journal of Biological Sciences (2016), http://dx.doi.org/10.1016/j.sjbs.2016.10.009

(Blumwald and Poole, 1987; Mimura et al., 2003); and (4) expression of certain functional genes to neutralize the salt toxicity (Sugihara et al., 2000; Yamada et al., 2002). In addition to these well-documented explanations, we observed increases in triterpenoid concentration and triterpenoid synthase gene expression in mangrove plants with increasing salt concentration (Basyuni et al., 2009; Oku et al., 2003). Surveys of salt tolerance gene expression by our and other studies suggested that triterpenoids are involved in the late stage of the salt tolerance mechanism in mangrove plants, being integrated as a component of long-term adaptation in combination with other short-term regulation mechanisms to confer salinity tolerance (Basyuni et al., 2012a; Ezawa and Tada, 2009; Yamanaka et al., 2009). This salt-dependent change in triterpenoid concentration was reversible upon transfer of the plants to fresh water (Basyuni et al., 2012b). Furthermore, our previous study found that the basal level of triterpenoid concentration in mangrove species correlated well with their habitat zonation along the coast to the inner-island axis: mangrove species growing closer to the sea showed higher triterpenoid concentrations (Basyuni et al., 2012a).

Fig. 1 depicts the biosynthetic pathway of triterpenoids and sterols. Triterpenoids and sterols are biosynthesized from a common precursor (2,3-oxidosqualene) by the enzyme oxidosqualene cyclase (OSC). Phytosterol, but not triterpenoid, has been accepted as a sterol component of plasma membrane in the plant kingdom. Several studies have demonstrated the biological activities of triterpenoid and their derivatives (Liu, 2005; Montilla et al., 2003; Safayhi and Sailer, 1997), and these biological activities have been explained by the disruption of membrane integrity caused by insertion of foreign molecules such as triterpenic acid into membrane phospholipid bilayers (Prades et al., 2011). It is therefore possible that the plasma membrane can accommodate a wide array of lipid molecules to some extent, depending on their physicochemical properties (Nes et al., 1993). Thus, it appears plausible that triterpenoid could replace phytosterol in the plasma membrane of mangrove plants.

On the basis of the series of our and other studies, we hypothesized that triterpenoids incorporate into the plasma lipid membrane, thereby changing salt stress tolerance. The scenario we proposed as mentioned above requires further testing, particularly in terms of physiological functions of terpenoids in the salinity stress using a model microorganism or plant. To introduce the triterpenoid synthase gene and analyze the function of their OSCs, lanosterol synthase (LS) deficient *Saccharomyces cerevisiae* GIL77 was used as the host organism (Basyuni et al., 2006; Kushiro et al., 1998). This host organism cannot synthesize ergosterol, a component of their cell membranes in yeast, and accumulates 2,3-oxidosqualene inside cells (Fig. 1). Therefore, transformation of GIL77 with other OSC genes and its expression results in the conversion of the substrate 2,3-oxidosqualene to the corresponding reaction products, depending on the activities of the OSCs. Thus, the present study investigated the effect of triterpenoid in GIL77 on its tolerance to abiotic stress, including salinity.

2. Materials and methods

2.1. Chemicals

β-amyrin and β-sitosterol were purchased from Extrasynthese (Rhône, France). All lipids were dissolved in hexane, and stored at −30 °C until use.

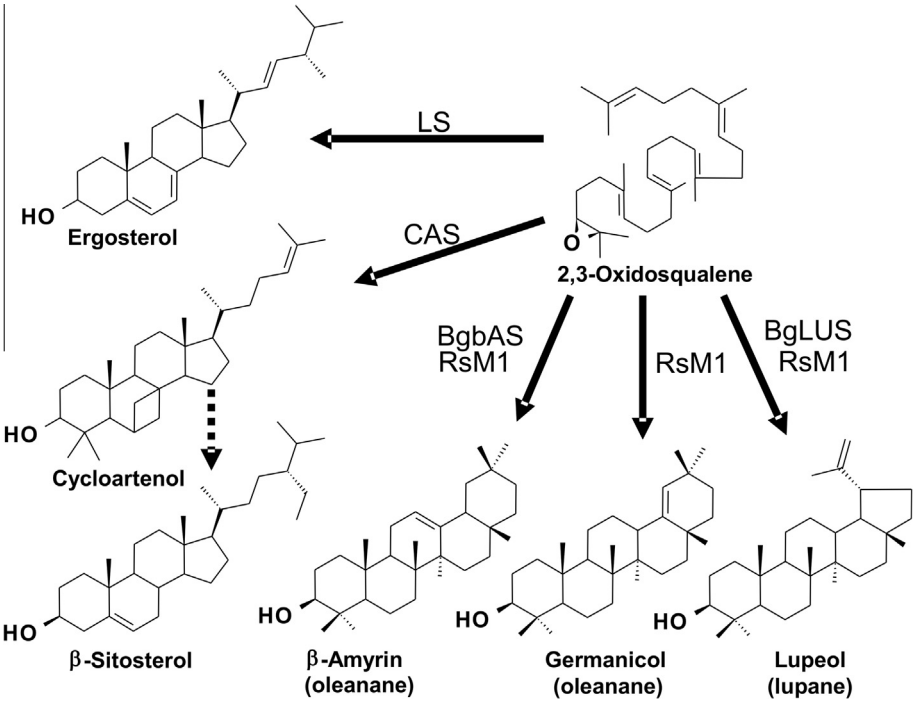

Figure 1 Cyclization of 2,3-oxidosqualene to terpenoids and phytosterol in mangrove trees. Terpenoids and phytosterol are biosynthesized from a common precursor by the enzyme oxidosqualene cyclases. LS, lanosterol synthase; CAS, cycloartenol synthase; *BgbAS*, *Bruguiera gymnorrhiza* β-amyrin synthase; *BgLUS*, *Bruguiera gymnorrhiza* lupeol synthase; *RsM1*, *Rhizophora stylosa* multifunctional triterpene synthase.