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Research article

Regulation of phytosterol biosynthetic pathway during drought stress in rice

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ABSTRACT

Plants respond to drought stress in the form of various physio-biochemical and molecular changes at both cellular and molecular levels. Drought stress causes the destruction of cell membranes by disintegration of membrane lipids. One of the major groups of membrane lipids that plays important role in preserving the integrity of cell membranes is phytosterols. HMG-CoA reductase (HMGR) is the principal enzyme in the biosynthesis of plant sterols, synthesized via mevalonic acid pathway. Phospholipid: sterol acyltransferase (PSAT) is another important enzyme that plays an important role in turnover of phytosterols into steryl esters and helps maintain homeostasis of membrane lipids. In this study, the expression of both *HMGR* and *PSAT* genes in drought sensitive (IR64) and drought tolerant (N22) rice cultivars under applied drought conditions were found to be elevated. The increase in expression of these genes was proportional to the level of severity of applied drought stress. This is substantiated by the negative correlation of *HMGR* and *PSAT* expression to relative water content (RWC) and membrane stability index (MSI). Expression of *PSAT* was also found to be positively correlated to ABA content and HMGR expression.

1. Introduction

Plants, due to their evolutionary particularity are sessile and face greater challenges compared to animals in avoiding environmental hostilities. One of the major manifestations of stress in plants is the disintegration of plasma membrane (PM) which is made up of phospholipid bilayer reinforced by macromolecules such as structural proteins and sterols. Plants are endowed with a vast range of sterols which includes β-sitosterol, stigmasterol and campesterol collectively called as phytosterols, as opposed to animals in which cholesterol is mostly the only sterol performing this function (Piironen et al. 2002).

Phytosterols play an important role in maintaining the integrity and fluidity of membrane lipid bilayers (Demel and De Kruyff, 1976). The rate limiting step in this pathway is the synthesis of mevalonate from HMG-CoA with the help of HMG-CoA reductase (HMGR) enzyme. Apart from phytosterols there are other isoprenoid compounds being synthesized in this pathway having diverse roles in the plant system. Many isoforms of HMGR gene have been detected in a single species and it is hypothesized that these isoforms catalyze separate sub-cellular pathways leading to different isoprenoid compounds (Schaller et al., 1995; Chappell et al., 1995). Hence, it is assumed that at least one isoform of HMGR is involved in biosynthesis of sterols.

Steryl esters forms a storage pool of sterols when they are present in amounts more than that is needed immediately especially during seed maturation and senescence (Dyas and Goad, 1993). Acylation of sterols is considered as an important process in maintaining homeostasis of membrane lipids (Valitova et al. (2016). Phospholipid: sterol acyltransferase (*PSAT*) gene is said to be involved in the turnover of sterols into steryl esters for a steady supply of membrane sterols thereby maintaining the integrity of plasma membrane during stress. Elevation of steryl ester (SE) levels during senescence and aging has been well documented and is known to be a mechanism for reclaiming membrane lipids. Hence it is fair enough to assume that a similar mechanism may be operational under drought stress which is also linked with membrane lipids metabolism. Previously we had estimated the changes occurring to levels of sterols and steryl esters in response to drought stress as well as maturity (Kumar et al., 2015). The HMG-CoA reductase activity was also found to correlate with the levels of sterols. In the present study, the expression of both HMGR and PSAT genes that are directly involved in sterol biosynthesis as well as turnover was investigated under applied drought stress in drought sensitive (IR64) and drought tolerant (N22) cultivars of rice. Expression of these genes were also correlated with the known indices of drought stress such as abscisic acid (ABA) content, relative water content (RWC), and membrane stability index (MSI). Further, a gene encoding PSAT was also isolated from rice using the orthologous gene sequence information

Abbreviations: HMGR, 3-Hydroxy-3-methyl glutaryl CoA reductase; PSAT, Phospholipid: sterol acyltransferase; ABA, Abscisic acid; RWC, Relative water content; MSI, Membrane stability index; SE, Steryl ester; RP-HPLC, Reverse phase- High performance liquid chromatography

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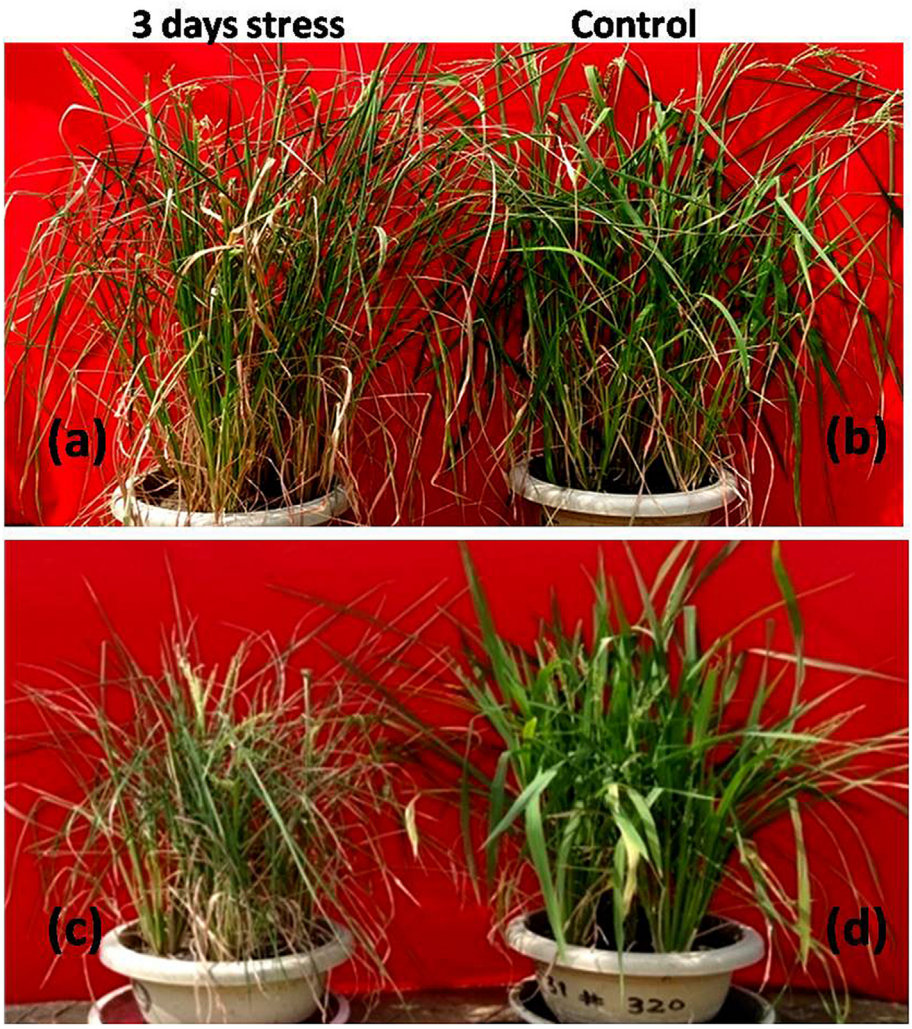

Fig. 1. Rice seedlings given drought treatment in phytotron (a) N22 3 days stress (b) N22 control (c) IR64 3 days stress (d) IR64 control.

from Arabidopsis.

2. Materials and methods

N22 and IR64 cultivars of rice that are drought tolerant and drought susceptible respectively were chosen for the study. 30 days old seedlings were transplanted in pots in the glass house of National Phytotron Facility, IARI, New Delhi (Fig. 1). The seedlings were regularly watered till the soil was saturated and submerged under water. Ideal temperature of 25 °C was maintained inside phytotron till maturity. Drought treatment was imposed on rice seedlings after 45 days of planting by withholding water. Water stress was given for varying time periods in different sets of pots.

2.1. Relative water content (RWC) and membrane stability index (MSI)

Relative water content of leaf was estimated as per method given by Barrs and Weatherley (1968). Leaf blades were collected from mature tillers from the control and drought stressed plants. RWC was measured at 3, 6, 9 and 12 days after withholding water. Membrane stability index was estimated by the method of Premchandra et al. (1990) as modified by Sairam et al. (1997).

2.2. Estimation of ABA by HPLC

ABA content was measured as reported by Zeevaart et al. (1999). Standard ABA solution was prepared by dissolving in 95% methanol. 100 ppm, 200 ppm and 300 ppm solutions were prepared and their corresponding concentrations were measured using RP-HPLC. C18 column was used as the stationary phase. MeOH: H_2O (60:40) was used as mobile phase at a flow rate of 1 ml/min. Run time was set at 6 min.

2.3. Estimation of sterol and steryl ester content

250 mg lyophilized leaf tissue was ground in an extraction mixture of dichloromethane/methanol (2:1) using pestle and mortar. Total lipids were extracted at 70° C. The dried residue was saponified with 5 ml of a solution of 6% (w/v) KOH in methanol at 90° C for 1 h to release the sterol moiety of steryl esters. The total sterols obtained after saponification were extracted with 3 vol of n-hexane. The dried residue was eluted in dichloromethane.

2.4. Separate titration of steryl esters

The dried residue of total lipid extract was chromatographed on silica plates with dichloromethane as the developing solvent. Steryl esters (R_f = 0.9) were then treated separately. They underwent a saponification step as above to obtain free sterols which are eluted in dichloromethane.