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RESPONSE OF RICE POLYPHENOL OXIDASE PROMOTER TO DROUGHT AND SALT STRESS

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Abstract

Polyphenol oxidases (PPO) widely exist in plants that catalyze oxygen dependent oxidation of phenols to quinines and assumed to be involved in plant defense against environmental stresses. In this study transgenic T1 seeds of *Arabidopsis thaliana* containing *Oryza sativa* Polyphenol oxidase (*OsPPO*) gene promoter fused with *GUS* (β-glucuronidase) were analyzed for drought and salt stress. These seeds were already available in our research group. Seeds were germinated on Murashige and Skoog (MS) mediato get T2 plants which were screened in drought stress (5, 10, 15, 20, 25 and 30% PEG-6000) and salt concentrations (50 mM, 100 mM, 150 mM, 200 mM, 250 mM and 300 mM). Experimental data showed that relative *GUS* expression of *OsPPO* gene promoter increased with the increase of drought stress. In case of salt stress, *Os*PPO induction showed similar trend *GUS* expression was increased. The response of *OsPPO* to drought and salt stress suggest the possible participation of *PPO* in plants defense against drought as well as salt stress.

Key words: *Oryza sativa*, *PPO* promoter, *GUS*, *Arabidopsis thaliana*, Salt and drought stress.

Introduction

Plants are unavoidably subjected to various abiotic stressors including drought and salt stresses that limit negatively affect plant growth and limit productivity throughout the world (Shinwari et al. 1998; Zhu, 2001; Aroca *et al*., 2012). Almost 51 % of world wide cultivatable land is subjected to drought (45%) and salt (6%) stresses (Bot *et al*., 2000; Anon., 2005). Rice is more vulnerable to drought than other crops (Kato, 2004; Masood et al. 2005). Plants adopt various genetic and cellular strategies to survive in such adverse conditions (Nakashima et al. 2000; Huang *et al*., 2012; Jirschitzka *et al*., 2013). One of these mechanisms is expression of defense or defense- related genes (Dixon & Harrison 1990; Zhu, 2002; Narusaka et al. 2003).

Polyphenol oxidase (*PPO*) activity in plants is a feature with probable economic, agricultural and environmental impact (Kanade *et al*., 2006) and *PPO*s have extensively been studied by biologists because of their possible involvement in plant defense (Constabel & Ryan, 1998; Constabel & Barbehenn, 2008). *PPO* activity was found to be altered when exposed to drought and saline conditions. Regulation of PPO was found as its activity changed by water stress in coconut (Shivishankar, 1988), in olive (Boughalleb & Mahammadi, 2011), in rice (Emam, 2012) and in wheat (Aldesuquy & Ghanem, 2015). Similarly salt stresses also induced PPO activity in *Trigonella* calli (Niknam *et al*., 2006), in soybean (Weisany *et al*., 2012) and in wheat (Abd Elhamid *et al*., 2014). Kaur *et al*. (2015) showed that *PPO* increased under water and salt stress in drought tolerant wheat (C 306). *PPO* activity was also enhanced in polluted city conditions (Polovnikova & Voskresenskaya, 2008).

Transgenic approach for plants with modified *PPO* expression is a unique tool to evaluate the involvement of *PPO* in plant defense. Transgenic tomato revealed that *PPO* expression was differentially induced and down-regulated to stand against water stress (Li & Steffens, 2002; Kidokoro et al. 2009). In this study rice (*Oryza sativa*) *PPO* promoter was cloned and transformed in *Arabidopsis thaliana* and response of *PPO* promoter was characterized in drought and salt stress by relative *GUS* activity.

Materials and Methods

Seeds collection: T1 transgenic *Arabidopsis thaliana* seeds containing *OsPPO* promoter fused with *GUS* (β-glucuronidase) (*OsPPOGUS*) were used to check the *OsPPO* promoter response towards drought and salt stress.

Sterilization of seeds: Seeds were sterilized with 70% ethanol and washed three times with autoclaved distilled water. Then seeds were immersed in 0.1% agar and placed on (Murashige and Skoog (MS) media (1962). Plates were kept in growth chamber for getting T2 transgenic plants to conduct the designed study.

Drought and salt stress treatment: One week old transgenic T2 plants were grown on MS media. For drought stress treatment polyethylene glycol gel (PEG-6000) was used. In total 35 ml of MS media in each plate was solidified with 0.7% agar and over laid by 35 ml of liquid MS media containing the 5, 10, 15, 20, 25, and 30% of PEG-6000. The dissolved PEG-6000 yielded -0.3, -0.6, -0.9, -1.2, -1.5 and -1.8 mega pascal (MPa) water potential. T2 plants were grown on these drought stress medium for 24 hour. On the other hand salt stress treatment was given on one week old T2 plants on MS media supplemented with 100, 200, 300, 400, 500 and 600 mM. Plants were grown on salt stressed media for 24 hours. For both stresses control samples were also used under similar incubation conditions. For drought control same T2 transgenic plants were placed on MS media over laid by liquid MS without PEG-6000 and for salt stress T2 transgenic plants were placed on MS without salt. Control samples were also remained on simple MS media for 24 hours like treated samples.

GUS staining: GUS buffer was prepared by 50 mM sodium dihydrogen phosphate, 10 mM disodium ethyle dimethyl tetra acetic acid and 0.01% Triton X 100 and PH was maintained up to 7.0. X-Gluc solution (0.1M) was prepared by 10 mg X-Gluc dissolved in 0.1 ml DMSO. For GUS staining experiments 5 µl of 0.1 M X-Gluc solution was added in 1 ml of GUS buffer. After stress treatments (drought and salt), plants were immersed in GUS solution and incubated on 37°C for overnight. After that plants were de-stained with methanol to remove chlorophyll contents and relative GUS expression was checked for each stress treatments form intensity of GUS staining.

Results and Discussion

Transgenic T2 plants containing *Os*PPOGUS were tested for the evaluation of *Os*PPO promoter in drought stress and saline treatment.

***GUS* expression of *OsPPO* in response drought stress:** One week old T2 plants were treated with drought stress along with control T2 plants on simple MS media. Relative *GUS* expression was noted from stained pictures. No *GUS* activity was noted on control plants. *GUS* activity of *OsPPO* promoter started appearing on -0.3Mpa water potential and continued to increase with more water stress. Upon - 0.9 Mpa it becomes higher and becomes more intense and constant from - 1.2 Mpa up to - 1.8 Mpa water potential (Fig. 1). *OsPPOGUS* activity showed increasing trend with higher PEG-6000 concentrations and achieved constant intensity level at 20%. So *Os*PPO responded more at high drought levels.

Drought responsiveness of *OsPPO* promoter is comparable with *GUS* expression of potato *PPO* in transgenic tomato in response to drought with highest magnitude in adult leaves and abscission regions of plant to cope with drought (Thipyapong *et al*., 2004). Activity of *PPO* induced by water stress has already been previously demonstrated in tomato (Loeb *et al*., 1997). In another report, it was found that *Ramonda serbica* desiccated leaves activated several fold higher *PPO* induction when plants were subjected near to complete drying condition (Veljovic-Jovanovica *et al*., 2008). Moreover, *PPO* induction was found to be elevated by water stress in coconut (Shivishankar, 1988) and wheat (Kaur *et al*., 2015).

***GUS* expression of *OsPPO* in response salt stress:** *OsPPO* showed almost similar pattern of induction in response to salt stress mentioned above for drought stress. Salt stress elevated the *GUS* expression and intensity with higher salt concentration and then level of expression intensity seemed to be uniform. From the (Fig. 2), it was observed that there was no *GUS* activity in plants cultured on unstressed MS media (Control). It was seen that starting from 50 mM salt the *GUS* expression increased up to 200 mM and becomes constant up to 300 mM. Therefore, it can be concluded that *OsPPO* promoter is responsive towards increasing salt concentration.

PPO activity was previously noted in salt stress conditions. *Trigonella foenum graecum* callus growing on media supplemented with salt (NaCl) showed PPO activity (Niknam *et al*., 2006). Ali and Abbas (2003) also reported PPO activity by treatment with NaCl. Recently over expression of *Fragaria ananassa PPO* was found to be regulated by NaCl along with some biotic stresses in transgenic strawberry which showed delayed fungal infection (Jia *et al*., 2015). In brief *OsPPO* promoter responded well under water stress and salt stressed indicating its positive correlation with increased abiotic stresses as drought and salt. This drought and salt responsiveness of *OsPPO* demonstrated that rice *PPO* may be involved in defense mechanism against environmental stresses such as water shortage and saline conditions.

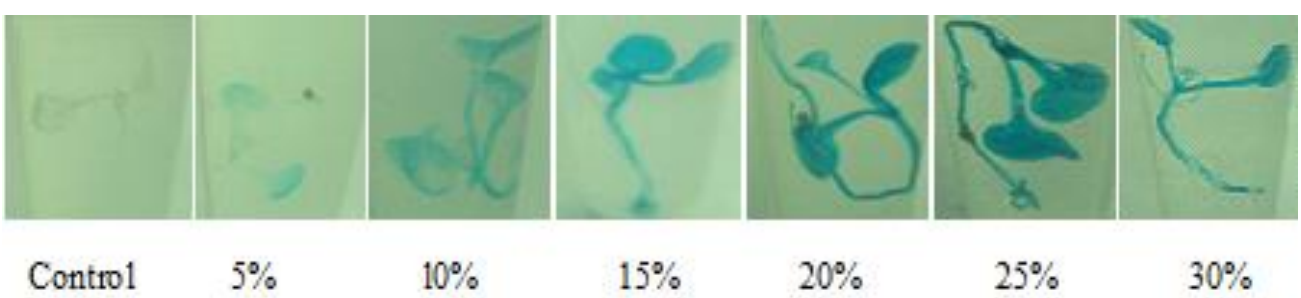

Fig. 1. *OsPPO* promoter induced *GUS* expression in transgenic T2 *Arabidopsis thaliana*plants in response to different PEG-6000 concentration on MS for 24 hours.

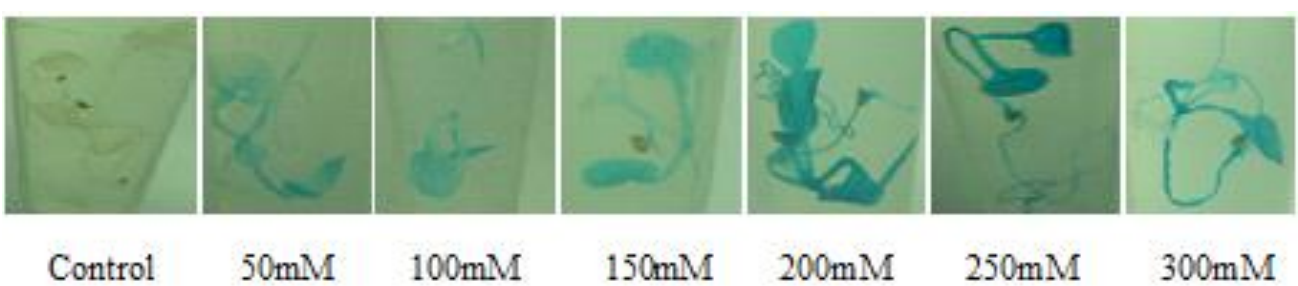

Fig. 2. *OsPPO* promoter induced *GUS* expression in transgenic T2 *Arabidopsis thaliana* plants in response to different NaCl concentrations on MS for 24 hours.

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