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Accumulation of antioxidants in rice callus (*Oryza sativa* L.) induced by β-glucan and salt stress

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Abstract

Salinity stress causes a considerable reduction in callus growth. Mushroom polysaccharides (β-glucan) are the most promising groups of antioxidant compounds. An *in vitro* experiment was conducted to evaluate the potential function of exogenously applied β-glucan in alleviating the accumulation of antioxidants in rice callus. In this study, morphological, chemical, and biochemical parameters of an embryogenic callus of the rice variety, MR269, were investigated under 200mM NaCl stress conditions after pre-treatment with (0, 0.5, 1 and 1.5 mg/L) β-glucan in culture media. The present study sought to evaluate chemical, biochemical, and callus growth characterization. The results revealed that callus exposed to stressful media exhibited a significant decrease in callus growth and contents of K^+ and Ca^{+2}. In addition, significant accumulation of Na^+ and Na^+/K^+ was found, as well as an enhanced enzymatic antioxidant system and elevated proline activities under NaCl conditions. Furthermore, exogenous addition of β-glucan at 0.5-1.5mg/L under NaCl stress induced a pronounced, significant increase of callus growth, K^+ and Ca^{+2} contents, and enzymatic antioxidant and proline activities. In contrast, a significant decrease was found in the levels of Na^+ and Na^+/K^+, particularly in callus treated with NaCl. β-Glucan ameliorates the adverse effects of NaCl stress, and the extent of amelioration depends on the type of β-glucan agent as well as treatment duration (three months). The changes mentioned are important for determining the morphological, chemical, and biochemical parameters of salinity tolerance in callus. This study demonstrated that exogenous β-glucan exhibits alleviation of the harmful effects of salt stress and increases salinity resistance in callus rice.

Keywords: NaCl stress, polysaccharides (β-glucan), antioxidant activity, proline, callus.
Abbreviation: 2,4-D_2,4-Dichlorophenoxyacetic acid; BA_β-glucan; BAP_6-Benzyl amino purine; CAT_Catalase; POD_Peroxidase; H_2O_2_Hydrogen peroxide; $O_2^{-\bullet}$_Superoxide radicals; $OH^\bullet$_Hydroxyl radicals; PVP_Polyvinylpyrrolidone; RGR_Relative growth rate; ROS_Reactive oxygen species; SD_Standard deviation; SOD_Superoxide dismutase

Introduction

Soil salinization is a primary environmental issue that limits the growth performance and yield of plants. Salt stress is a threatening global issue that causes massive decreases in crop production. More than 20% of all irrigated soil on earth is affected by salinity (Hazman, 2014). Salt stress has significantly damaging effects, specifically osmotic effects that decrease water uptake and ion toxicity stress (mainly Na^+ and Cl^- ions), leading to generative stress. Oxidative stress results in uncontrolled production of reactive ROS, such as $OH^\bullet-$, $O_2^{-\bullet}$, and H_2O_2. These unstable molecules increase toxicity levels and trigger oxidative damage to valuable biochemical molecules, such as RNA, DNA, enzymes, and proteins (Sharma et al., 2012).

Rice (*Oryza sativa* L.) is the world's most significant cereal crop. Rice is a primary source of calories and food for humans (Khush, 2005) and is becoming a staple food for more than half of the world's population. In research, rice is a model species for cereal plants and monocotyledons (Cotsaftis and Guiderdonis, 2005). However, particularly at the seedling stage, rice is extremely sensitive to salt stress, particularly reflected by its height, dry matter reduction, root length, and emergence of new roots (Hazman, 2014).

The use of tissue and plant cell culture focuses on the physiological, chemical, and biochemical developments characteristics of a cell affecting adaptability to salinity stress (Bhat et al., 2013). *In vitro* techniques that utilize cells or tissues are valuable tools for stress studies, facilitating the unravelling of halophyte plants salinity tolerance mechanism at the organized tissue level or unorganized cellular level. Moreover, these investigations can supply information on growth potential as well as physiological and biochemical responses to NaCl stress at various levels of tissue organization (Alhasnawi et al., 2014b).

β-Glucans are polysaccharides found in the cell wall of certain bacteria, fungi, and plants (Gawronski et al., 1999). Mushrooms are one of the best sources for the structure of β (1→3) and β (1→6) glucan (Jantaramanant et al., 2014). Polysaccharides from plants have been shown to be a number of the most promising groups of antioxidant compounds (Ng et al., 2004). However, mushroom β-glucan can influence several cellular functions, including cellular glucose uptake (Jantaramanant et al., 2014). These polysaccharides are employed as natural antioxidants with strong antioxidant activities that protect against oxidative damage (Kong et al., 2010). Salinity stress enhancement alters in the regulation of plant cell polymers, thereby modifying the extractability of cell wall polysaccharides (de Lima et al., 2014). Based on salinity, negatively-charged species are important in cell wall polysaccharides. The increase in negative charge in cell wall polysaccharides is important for coping with salinization. The negative charge facilitates ion transport under high salinity conditions, and the negative charge in polysaccharides serves to delay the entrance of Na^+ (de Lima et al., 2014). Polysaccharides from mushrooms function in the activation of innate defences to cytotoxic macrophages in the cell.

At present, no information is available on the influence of β-glucan on salt stress and the morphological, chemical, and biochemical activities in rice callus. This knowledge can provide information on the possible involvement of antioxidant enzyme activity in the defence against ROS and β-glucan in the salt tolerance response. Therefore, in this investigation, we aimed to study the effect of exogenous β-glucan supply and NaCl stress on the activity of various antioxidant enzymes in callus rice in relation to salt tolerance.

Results

Callus growth

The ANOVA results (Fig 1a) exhibited that there was a marked reduction in fresh weight recorded when rice callus was exposed to 200 mM NaCl-induced salt stress throughout the experimental period of three months. Interaction of NaCl (200mM) and β-glucan (0, 0.5, 1, or 1.5mg/L) was significant for fresh biomass. The exogenous application of β-glucan to culture medium ameliorated the effects on fresh biomass under saline conditions. The protective effect of fresh weight was compared - application of salinity alone to the callus medium diminished callus growth to 47.36%, whereas exogenous application of 1mg/L β-glucan to salinity reduced callus growth to 26.83% in comparison with the control (no NaCl) (Fig 4) .

As well, the ANOVA results showed a significant adverse effect from NaCl presence in callus medium on callus dry weight (Fig 1b). Under stress conditions, additional β-glucan in the medium led to the highest significant increases in dry weight callus versus salt stress (Fig 4).

Further, the ANOVA indicated that a significant variation in NaCl resulted in a rise in RGR (Fig 1c). Data in the same figure also revealed that callus growth under salinity conditions in culture medium containing β-glucan results in a significant increase and reached maximum RGR values in comparison with salinity alone.

Chemical estimation

Salinity stress in callus medium usually retards certain chemicals' flux for the examined callus. The amount of Ca^{+2} decreased significantly with NaCl salinity, whereas the level of Ca^{+2} rose more pronouncedly in the presence of β-glucan that with salt stress alone, as shown in Fig 2a.

Fig 2b depicts salinity stress causing a significant drop in K^+ parameters. Culture medium containing salt-treated callus with β-glucan improves K^+ flux, and 1mg/L β-glucan yielded maximum increments.

The effect of salinity levels on Na^+ parameters are shown in Fig 2c. Na^+ increased significantly under saline conditions. In the present investigation, β-glucan applied to callus at different concentrations diminished the amount of Na^+ in comparison with the control. This finding indicates that β-glucan helped the callus mitigate the adverse effects of salinity stress.

Implementation of NaCl stress resulted in increased Na^+/K^+ contents. In the case of inhibitory effects, all concentrations of the added polysaccharide (β-glucan) affected the accumulation of Na^+/K^+ in salinized callus (Fig 2d).

Biochemical estimation

The effects of POD activity of callus uncovered significant differences between the NaCl-treated samples and the controls. As outlined in Table 1, POD activity in callus was increased. Furthermore, exogenous treatments of culture medium with β-glucan at 1mg/L to NaCl showed the highest levels of POD.

CAT activity was influenced differently. Activity under NaCl stress and exogenous treatment with β-glucan also caused a maximum increase in CAT activity (Table 1).

The data presented in Table 1 shows that, under salinity stress in callus media, SOD activity increased significantly with NaCl-applied salinity in the culture medium. However, salt-induced SOD activity was increased significantly to reach maximum values with the addition to callus medium of β-glucan and NaCl.

Exogenous application of NaCl (200mM) and its interactions resulted in a significant difference in proline activity under salinity conditions (Table 1). Salinity of callus in the absence or presence of β-glucan exhibited a significant increase in proline activity in comparison with control callus. The data in Table 1 also demonstrate that exogenous treatments under both conditions, namely, NaCl and β-glucan, caused a significant elevation in proline activity.

In the present study, 200mM NaCl led to significant degradation in callus growth; these results are consistent with that of (Wani et al., 2010). . Salt stress in culture media can inhibit callus growth by ion imbalance, ion toxicity, water deficit, or a combination of any of these factors (Alhasnawi et al., 2015; Hosseini et al., 2012). In this context, Younis et al., (2010) reported that growth degradation caused by NaCl is because of the reduction of apical growth in cells, as well as the imbalance of endogenous hormonal growth. In both cases, inhibition could have been produced by the adverse water and toxicity effects of both Na^+ and Cl^- ions on metabolism. In addition, a secondary aspect of salinization in plants involves the stress-induced production of ROS (Manchanda and Garg, 2008). Sathish et al., (1997) found that Na^+/K^+ levels in callus cells rise when exposed to salinity, though the mechanism by which such action is accomplished was different. In callus cell growth, cellular K^+ is considerably reduced with only negligible accumulations of Na^+. A high Na^+ concentration has been shown to lower K^+ uptake, mediated by low affinity. Taking into consideration the relationships between ROS metabolism and signalling of plant reactions to NaCl stress, oxidative stress is described by the overproduction of ROS, mainly signified by superoxide anion, singlet oxygen, hydroxyl radical, and

Table 1. The effect of β-glucan under salinity stress on biochemical activities [Peroxidase activity (mg/g), Catalase activity (units/ml), Superoxide dismutase activity (units/mg), and Proline (µmoles/g)] of callus rice var. MR269.

Treatment	Peroxidase activity	Catalase activity	Superoxide dismutase activity	Proline
Control	0.009±0.002^{e}	4.32±0.97^{d}	5.58±1.43^{d}	5.45±0.69^{e}
200mM NaCl	0.020±0.003^{d}	8.50±1.04^{c}	29.06±1.94^{c}	11.73±0.84^{d}
200mM NaCl + 0.5 mg BG	0.038±0.003^{b}	10.32±1.07^{b}	40.32±1.72^{b}	13.67±0.50^{c}
200mM NaCl + 1 mg BG	0.053±0.004^{a}	14.76±1.39^{a}	45.84±1.26^{a}	27.42±0.74^{a}
200mM NaCl + 1.5 mg BG	0.025±0.005^{c}	14.04±1.35^{a}	30.05±1.33^{c}	21.53±0.90^{b}

Values are the mean ± SD. Data of each column bearing different superscript are significantly different at α=0.05 level by Duncan's New Multiple Range Test (DMRT).

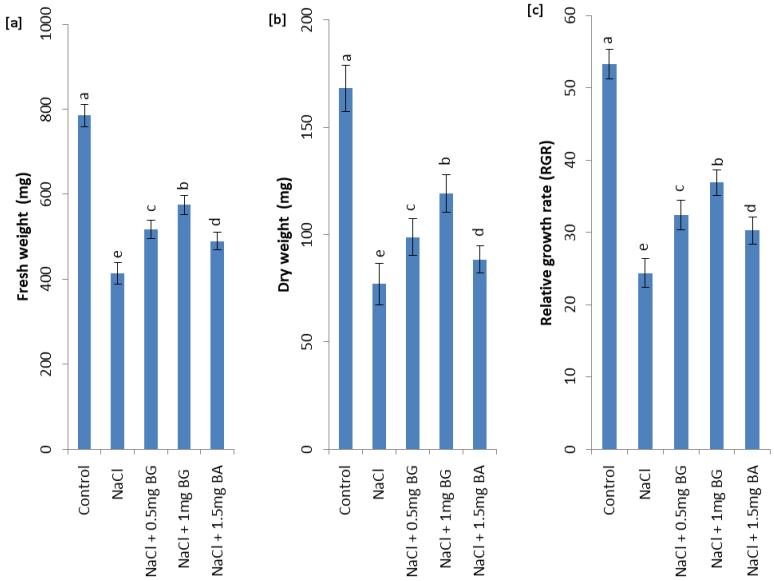

Fig 1. Fresh weight (mg), Dry weight (mg) and Relative growth rate (RGR/week) of callus treated with 200 mM NaCl and (0, 0.5, 1 or 1.5 mg/L) β-glucan. Within each figure, bars with different letters were different at α=0.05 level by Duncan's New Multiple Range Test (DMRT). Vertical bars are mean ± SD.

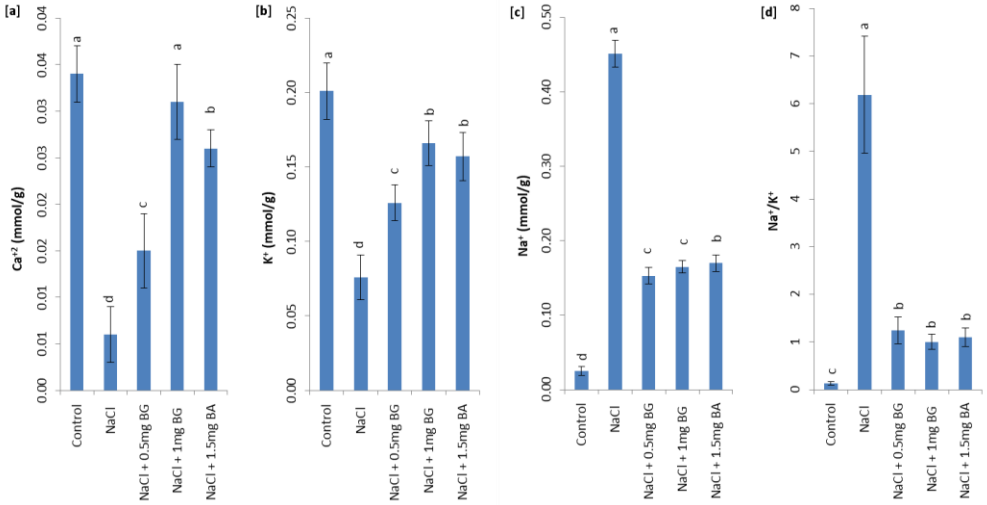

Fig 2. Ca^{+2}, K^{+}, Na^{+} and Na^{+}/K^{+} mmol/g parameters of callus treated with 200mM NaCl and (0, 0.5, 1 or 1.5 mg/L) β-glucan. Within each figure, bars with different letters were different at α=0.05 level by Duncan's New Multiple Range Test (DMRT). Vertical bars are mean ± SD.

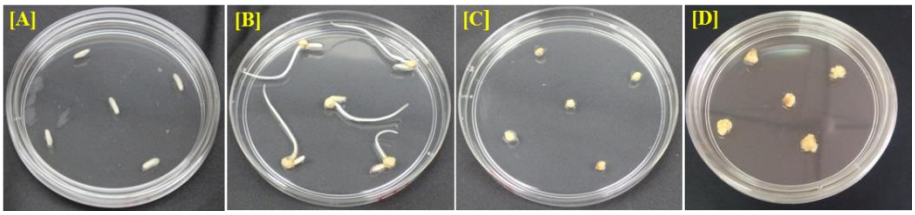

Fig 3. [A] Mature seeds of the MR269 rice variety callus induction medium (MS media added with 3 mg/L of 2,4-D, 0.1 mg/L of BAP, vitamins, 3% sucrose, and 3 g/L of Gelrite) under dark conditions, [B] Callus induction after two weeks, [C] Callus subculture in same callus induction media, and [D] Callus proliferation at approximately 100 mg.

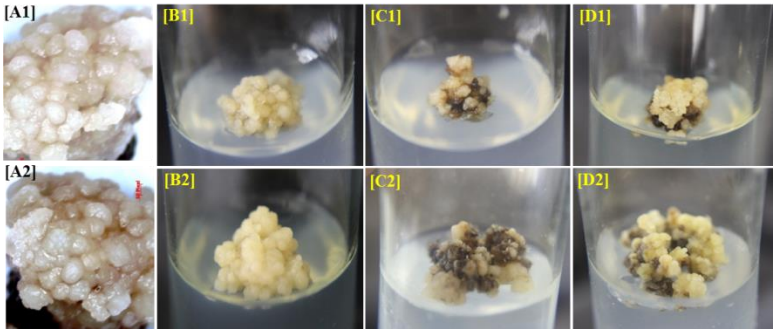

Fig 4. [A1, 2] Photographs were taken under electron microscope for embryogenic callus, [B1, 2] Embryogenic callus morphology at control treatment same callus induction media (without NaCl and BG) for one and three month respectively, [C1, 2]] Embryogenic callus morphology at 200mM NaCl level supplemented in same callus induction media for one and three month respectively , [D1, 2] Embryogenic callus morphology at 200mM NaCl + 1 mg BG levels supplemented in same callus induction media for one and three month respectively.

hydrogen peroxide. Plant cells execute self-protective mechanisms and develop several biochemical approaches to evade ROS damage (Alhasnawi et al., 2014c).The enhanced production of ROS during NaCl exposure leads to progressive oxidative damage and subsequent cell death before growth suppression (Ruiz-Lozano et al., 2012). This damage can harm the main cellular components, including nucleic acids, proteins, and lipids (Gill and Tuteja, 2010). In a study by Apel and Hirt, (2004), plant cells were observed to enact a security mechanism that can decrease oxidative damage caused by ROS. With this, the introduction of ROS scavenging enzymes, such as CAT, POD, and SOD, is the most ordinary mechanism for detoxification of ROS manufactured throughout stress reaction. The stable-condition concentration of ROS in plant cells are concluded by the equilibrium between the effectiveness of ROS generation and scavenging enzymes. Abogadallah and Quick, (2009) noted that antioxidant protection systems were an essential function in salinity tolerance in different plant types. Proline accumulation is a common physiological response in numerous plants to various collections of abiotic and biotic stresses (Alhasnawi et al., 2014a). Therefore, the present results reflective of proline accumulation, in line with those of Gandonou et al., (2006); Patade and Suprasanna, (2009), suggests a higher accumulation of proline in callus exposed to salinity stress medium.

Under salinity conditions, growth performance and salt tolerance of common rice callus with regards to fresh weight, dry weight, and RGR were effectively improved with β-glucan supplementation. This treatment can attenuate the harmful effects of NaCl. Throughout the plant cell life cycle, carbohydrates act critically in several physiological functions. Sugars provide energy sources, crucial carbon, and signalling molecules. In addition, sugars have regulatory functions during the early phases of plant development, including controlling metabolism, development, growth, and stress resistance (Alayon-Luaces et al., 2010). de Lima et al., (2014) put forth that salinization enhancement alters the reorganization of cell polymers, thereby modifying the extractability of cell wall polysaccharides. A study by (Jantaramanant et al., 2014), also showed that mushroom β-glucan can affect several cellular functions, such as cellular glucose uptake. In addition, Li et al. (2011), reported that glucose is essential for the activation of plant resistance to abiotic stress and that ion homeostasis is the physiological foundation for living cells. Gunter and Ovodov, (2002) observed that polysaccharides in *Silene vulgaris* callus is the primary metabolite (polysaccharide) cell wall component permanently biosynthesized through cells. Therefore, the results presented here are consistent with those of Geshi et al., (2000); Goubet and Morvan, (1994). These studies reported that polysaccharides have important roles in the expansion phase and cell division. Therefore, β-glucan can exert a protective function by regulating callus growth under NaCl stress.

Experiments that evaluate the effects of exogenous β-glucan application during salinity stress involve increases in Ca^{+2} and K^{+} content and decreases in Na^{+} and Na^{+}/K^{+} content. Polysaccharides exemplify a structurally varied type of macromolecule that prevalently occurs in nature because of their large potential for structural variability, ultimately offering a high capacity for carrying biological information (Carbonero et al., 2006). Ooi and Liu, (2000) also noted that the monosaccharide units in polysaccharides and oligosaccharides can connect at numerous points to form various collections of linear or branched structures. This tremendous potential variance fosters the essential flexibility required for precise regulation mechanisms of different interactions in higher organisms (Carbonero et al., 2006).

In halophytic species, the prominence of negative charge of cell wall polysaccharides is exhibited in salinity related with sulphated polysaccharides (Aquino et al., 2011).The concentration of polysaccharides and their degree of sulphation has been exhibited to be positively correlated with

salt stress. Rabéchault et al., (1974) noticed that glucose levels appear to provide adequate osmotic pressure, which would permit the absorption of mineral nutrients present in media that are fundamental for cell growth. This indicates the enhancement of Ca^{+2} and K^+ content under salt stress.

Increasing negative charge in cell wall polysaccharides represents the importance of confrontation salinity by aiding ion transfer under high salt conditions. On the other hand, it can be seen that negatively-charged polysaccharides act by delaying the entry of ions, such as Na^+ (de Lima et al. 2014). Carillo et al. (2011) reported that the proliferation of negatively-charged polysaccharides can perform an important task by slowing the movement of Na^+ ions in cells. Na^+ concentrations are described to reach toxicity before Cl^-. Thus, this work's β-glucan data indicates the enhanced decrease of Na^+ and Na^+/K^+ content.

Exogenous β-glucan significantly increased POD, CAT, SOD activities, along with that of proline, thereby demonstrating the protective influences of exogenous β-glucan associated with antioxidant activities. The osmotic potential rise in response to water concentration drops is most likely explained through the enhancement of polysaccharide hydrolyzing enzyme activities in responses to stress. Hence, enzymes such as pectinesterase and polygalacturonase may exhibit increased activity (Inari et al., 2002). Burton et al., (2010) observed an alteration in the rate of polysaccharide polysubstitution that affected cross-linking by hemicelluloses and celluloses. Wall expansion is limited through covalent bonds in matrix polymers, which demand enzymatic cleavage for wall reduction to take place.

β-Glucan is a natural component of grain that possesses important antioxidant activity (Kofuji et al., 2012).Polysaccharides from plants have been demonstrated to be one of most promising groups of antioxidant compounds (Ng et al., 2004). Polygalacturonase extracted from barley exerts a noteworthy antioxidant effect as well as various biological activities (Kofuji et al., 2012).

Polysaccharides are natural antioxidants with strong activity utilized in protecting against oxidative damage (Kong et al. 2010). Faure et al. (2012) observed this when studying the enzymatic protection ability of polysaccharide solutions. In addition, different compounds have been found to be capable of decreasing or altogether stopping the formation of the hydroxyl radical ($^{\bullet}OH$), which is responsible for β-glucan degradation. $^{\bullet}OH$ formed during Fenton reactions is an ROS known as an extremely powerful oxidant (Faure et al., 2012). Von Sonntag (1980) found that free radicals are invasive toward biomolecules and are highly reactive. These free radicals are shown to attack the polysaccharide chain by abstracting the carbon-bound hydrogen. Obviously, $^{\bullet}OH$ has a high degree of reactivity along with the ROS species, $O_2^{\bullet-}$ and H_2O_2 (Faure et al., 2013). Duan and Kasper, (2011) determined that $^{\bullet}OH$ was the most powerful ROS, implicating it in the oxidative harm of a large group of biological molecules that included carbohydrates. Evidently, H_2O_2 contributes to $^{\bullet}OH$ production, to which polysaccharide decrease is attributed; autooxidation and accumulation of $^{\bullet}OH$ also results in further reductions in β-glucan (Faure et al., 2013).

Liu et al., (2005) obtained data that confirmed peroxidase activity in the presence of polysaccharides. The rise in this sort of activity may be caused by the conformational alteration of enzyme molecules. This matter proposed the significance of the degree of polysaccharide acetylation for binding with peroxidases (Maksimov et al., 2012).

Showalter, (1993) found that several proteins and enzymes are involved in the metabolic regulation and structural regulation of various cell-wall polymers. These enzymes include plant glycoside hydrolases, which perform numerous important functions in cell wall metabolism, mobilization of storage reserves, plant defence, signalling, and reorganization of glycans (Minic, 2008).

To investigate whether antioxidant enzymes perform an important function in the defence against salt-induced oxidative harm, the activities of antioxidant enzymes were assayed. These results demonstrate a marked increase in the activities of CAT, POD, and SOD in callus treated with different polysaccharide (β-glucan) concentrations in combination with NaCl. These elevated activities improved the survival rate of rice callus under salt stress. Aquino et al., (2011) established that polysaccharide levels in rice plants in the absence or presence of 200 mM NaCl were ameliorated by more than three-fold in plants with NaCl. These authors' main suggestion was that negatively-charged cell wall polysaccharides, including pectin, perform important roles in coping with salt stress. In this work, the finding agrees with that of Qiu et al., (2014), an external supply of exogenous sucrose could enhance the tolerance of *Arabidopsis* seedlings to salt stress through elevation of CAT, POD, and SOD activities.

Under salinity, exogenous β-glucan significantly enhances proline production. This is based on the accumulation effect on proline, which engages in a protective purpose as a scavenger of ROS. This results in enhanced growth and adaptation abilities of callus under salt stress. Proline accumulation is an important factor that aids plant cell systems in adapting to salinity stress (Garcia et al., 1997). Accumulation of proline can improve salinity tolerance by stimulating the accumulation of stress-protective proteins and protein turnover machinery, along with stabilizing the detoxification of protein enzymes (Khedr et al., 2003). In addition, proline also serves as a hydroxyl radical scavenger (Hoque et al., 2007). High proline under salinity stress is related to osmotic adjustment, which can improve salt tolerance in plant cells (Vinocur and Altman, 2005).

Materials and Methods

Explant source and sterilization

Mature seeds of the MR269 rice variety were sterilized using the method described by Zinnah et al. (2013). Seeds were dehulled and sterilized by washing with sterilized distilled water three times prior to immersion for 2 min to 3 min in 70% ethanol. Next, mature seeds were treated with 0.1% $HgCl_2$, which is supplemented with a few drops of Tween-20, for 4 min to 6 min and finally rinsed several times in sterile distilled water.

Callus induction media

Mature seeds were plated on corresponding callus induction media on Murashige and Skoog, (1962) (MS) media, of which was added 3mg/L of 2,4-D, 0.1mg/L of BAP, vitamins, 3% sucrose, and 3g/L of Gelrite. The goal of supplementation was to prepare a semi-solid media in the dark at 25±2°C. Subculturing was carried out on subculture medium for two weeks under the same conditions for further proliferation and to obtain the desired amount of callus (Fig 3A;B;C).

Embryogenic callus under NaCl and β-glucan

Selected high-quality embryogenic callus was divided into 100mg portions (Fig 3D), subcultured in test tubes containing the same induction medium supplemented with NaCl (200mM) and mushroom polysaccharides (β-glucan) (0, 0.5, 1, or 1.5mg/L), and then subcultured every two weeks for a period of three months onto fresh media containing NaCl and β-glucan. At the end of three months under salinity stress and β-glucan conditions, the callus was harvested for morphological, chemical, and biochemical analysis. At the end of the culture period, the fresh and dry weights of embryogenic callus were recorded. Relative growth rate (RGR), which is calculated on the basis of the initial and final fresh weight of callus, is reported (Al-Bahrany, 2002) as follows:

RGR = [In (final weight) − In (initial weight)]/weeks

Chemical analysis

Na^+, Ca^{+2}, and K^+ were analysed by atomic absorption spectrometry (Model No. 3110) (USA).

Biochemical analysis

For antioxidant enzyme activity determinations, 2g of fresh callus was homogenized with 4mL of 0.1 M ice-cold phosphate buffer (pH 7.2) supplemented with 0.1g of PVP. The homogenate was centrifuged and used in assaying enzyme activity for analysis.

Estimation of peroxidase (POD) activity

For the determination of POD activity according to the used method, H_2O_2 and guaiacol solutions for establishing absorbance with a spectrophotometer (model U-1900, Hitachi, Japan) calibrated at 470nm were made. The followed procedure was previously put forth by Racusen and Foote, (1965) with certain modifications.

Estimation of catalase (CAT) activity

CAT activity was determined according to one unit used to decompose 1μM of H_2O_2 enzyme activity with a spectrophotometer at 240nm as described by Beers and Sizer, (1952) and assayed with a slight modification.

Estimation of superoxide dismutase (SOD) activity

SOD activity was determined by a spectrophotometer at 560nm. This method was reported by Maral et al., (1977), with certain modifications.

Estimation of the proline activity

Proline activity was measured by using 0.5 g of freshly ground callus in liquid nitrogen proline and was measured at 520 nm using a spectrophotometer (Bates et al., 1973).

Statistical analysis

The experiment was conducted according to a completely randomized design (CRD) with 10 replications. Analysis of variance method (ANOVA) was performed with SAS software ( ), and a significant difference was determined by Duncan's multiple-range tests at a α=0.05 level.

Conclusion

B-glucan is effective in ameliorating the harmful effect of oxidative agents on callus cells. Accumulation of enzymatic antioxidants and proline was found to be responsible for the regulation of ROS, thereby reducing the damaging effects of ROS and further improving the salt tolerance of rice callus. This study demonstrated that exogenous β-glucan exhibits enhancement functions by alleviating the harmful effects of salt stress, hence elevating the salinity resistance of rice callus. In addition, this system can be used to investigate the function of β-glucan in plant tolerance.

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