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In Vitro Effect of PEG and Proline on Callus Growth and Minerals Values in Basmati Rice (*Oryza sativa*)

S. Adrees[1], M. I. Khokhar[2*], R. N. Fatima[1] and S. Rehman[2]

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ABSTRACT

Purpose: *Drought is a universal issue that disturbs photosynthetic system, adversely affecting quality and crop yield. The study was conducted to determine the response of polyethylene glycol (PEG) as a stressing agent alone or in combination with proline on four fine rice genotypes for callus components as well as biochemical parameters like Chromium (Cr), Magnesium (Mg), Manganese (Mn), Iron (Fe), Phosphorus (P).*

Research Method: *Callus was induced on media having 4.43g/L MS + 4mg/L 2, 4-D (2, 4-dichlorophenoxy acetic acid) + 30g/L sucrose + 1.76g/L phytagel with different levels of PEG "1.25g/L and 2.5g/L" with and without 1ml/L proline.*

Findings: *Callus was used for different analyses after 30 days and results showed that PEG treatments have a negative effect on callus fresh weight, callus dry weight, days to callus induction, callus percentage, and mineral nutrients like K, Cr, Mg, Mn, Fe, P. PEG 2.5g/L showed a greater decrease as compared to PEG 1.25g/L. Proline significantly raises callus growth alone or in combination with PEG but less than control whereas callus dry weight, K, and Ca contents increased more than control when proline applied alone or with PEG combination.*

Originality/value: *Exogenous proline application improves callus growth rate as well as macro-micro elements by mitigating the effect of PEG.*

Keywords: *callus induction, drought stress, macro- micronutrients*

INTRODUCTION

Rice is the second staple food in Pakistan after wheat. Pakistani basmati rice has the most global importance as compared to non-basmati due to its unique aroma character with high-quality characteristics (Rabbani *et al*., 2008). A grain of rice has 3 parts, the germ which includes lots of antioxidants, the endosperm made of carbohydrates and lastly the outermost covering (bran) which fully consists of vitamins and fiber (Shahzadi *et al*., 2018).

It is anticipated from a climatic model that variations in rainfall and average temperature in the coming years will lead to an increase in drought levels (Fischer and Schar, 2010). Abiotic stresses like heavy metals, temperature, radiation, oxidative stresses, and salinity in addition to droughts are worldwide problems that decreased crop productivity (Krishania and Agarwal, 2012). Greenhouse gas levels raised in the atmosphere

[1] *Department of Botany, Government College Women University, Faisalabad*

[2*] *Agricultural Biotechnology Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan.*

ilyasabri@yahoo.com

https://orcid.org/0000-0003-2405-9384

cause rain pattern and temperature fluctuations which has a harmful impact on agricultural crop production in developing countries (Kurukulasuriya *et al*., 2006; Mendelsohn 2014).

Agriculture and weather conditions (high temperature, flooding, rainfall etc.) are directly associated with each other which in turn affect food production. The most imperative issue that limits crop production is the lack of water resources and short rainfall periods. Water shortfall during the rice production stage, particularly in the vegetative or flowering stage causes disturbed floweret prompting period, distracts spikelet infertility and alters terminal plowing duration (Jiang *et al*., 1998; Kamoshita *et al*., 2004). Water stress enhances rates of evaporation and transpiration, reduced water contents and roots spreading in soil (Farooq *et al*., 2009). Certain amino acids play a vital role under drought stress environments as osmoprotectants (choline, proline and glycine betaine) (Kishor *et al*., 2005). Proline which is the most significant osmolyte as well as osmoprotectant accumulates in large quantities under drought environments that interrelate phospholipids, act as a scavenger of reactive oxygen species, protects macromolecules from denaturation, protects cellular organelles in addition to the role as an antioxidant (Kishor and Sreenivasulu, 2014).

Plants need 17 basic nutritional elements including some macro-elements like P, K and Mg and some micro-nutrients like Mn and Fe to complete their life cycle (Waraich *et al*., 2011). Many physiological, metabolic and biochemical processes in plants activate with micro and macro-elements to overcome drought stress (Lee *et al*., 2010). The growing population rate leads to a greater demand for rice for food. Therefore, rice production improvement strategies help mitigate hunger, contribute to financial improvements and secure food safety (Wyatt *et al*., 2011). Biological and chemical processes that can tolerate shortages of precipitation rate in highland situations drive support toward better water preservation. Growing resistant genotypes against stress, infection and pathogen attack cause to increase in rice yields.

In tissue culture, a number of stress-stimulating osmotic agents like PEG and mannitol. The highly used osmoticum is PEG due to its high molecular mass, non-ionic, non-poisonous effects and soluble in water (Newton *et al*., 1989). PEG available with various molecular weights (1500, 4000, 6000 and 8000) are not absorbed by plant roots due to its high molecular size and develop stress conditions (khani *et al.,* 2008; Hamayun *et al*., 2010).

Mature seeds are used as explants for callus development and regeneration at various PEG levels under in vitro conditions and explants are selected to have the capacity to survive under water deficit conditions imposed with PEG (Rashid *et al.,* 1996). Resistant cultivars could be selected with two methods; (1) duration methods in which osmotic agent levels increased regularly in culture medium after callus induction also known as the well-stepped method (2) shock treatment technique in which callus is initiated in PEG growth medium, then surviving callus lines are selected (Purohit *et al*., 1998).

Hormonal stability generally influences the regeneration and callus induction from embryos in growth media (Jiang *et al*., 1998). Callus induction capacity improves with auxin (2, 4-dichlorophenoxy acetic acid) or mixing with cytokinins (Castillo *et al*., 1998). Callus formation of rice is stimulated by glutamine as well as proline which is also used as natural nitrogen as well as carbon source in the callus media (Shahsavari, 2011; Szabados and Savoure, 2009). The callus induction capacity of genotypes under stress response is also influenced by genomic factors, due to this effect, explants and their genetic makeup cause to differentiate embryonic callus induction and regeneration (Ganeshan *et al*., 2003). Tissue culture practices help us to recognize stress tolerance mechanism en route for the development of resistant cultivars; it also anticipated that there is a correlation between in vivo and in vitro plants (AL-Taha, 2013). In the tissue culture technique, the growing medium is complemented with selective agents to check the reaction of desired genotypes against drought (Rai *et al*., 2011).

The objective of this study is to detect the effect of PEG on callus growth and essential minerals growth elements of various rice genotypes in the medium. Further, this study used drought-tolerant rice genotypes screening purposes at the cellular level for the breeding program and also able us to develop some clones that perform best under water deficit conditions.

MATERIALS AND METHODS

The experiment was conducted at a cytogenetic laboratory, Agricultural Biotechnology Research Institute (ABRI), Faisalabad. Four rice genotypes named Super Basmati, Basmati-385, Basmati-2000 and Basmati-515 were taken from Rice Research Institute (RRI), Kala Shah Kaku. Seeds were de-husked and surface sterilized with 70% ethanol for 30 seconds then treated with 20% (v/v) sodium hypochlorite (Clorox) for 10-15 minutes at 154 rpm on a shaker, washed 3-4 times with sterilized distilled water for three minutes in a laminar flow cabinet which illuminated with UV-light for 20 min before each operation. Hands and all instruments were regularly disinfected with 70% ethanol. Sterilized seeds were cultured on Murashige and Skoog (1962) (MS) medium (4.43g/L) containing 30g/L sucrose, 1.76g/L phytagel, 4mg/L 2,4-D in 100mL test tubes (Erlen-Meyer, Pyrex) for control as well as at various PEG levels (1.25g/L and 2.5g/L) for callus induction. Callus was also obtained on an alternative medium containing 1mg/L proline at similar levels of PEG (molecular weight 6000) and 2, 4-D (Table 01). For one month tubes were retained in a dark place at 23 ± 2 ^{0}C after culturing. Each treatment was replicated thrice and parameters were studied from one month-old callus.

Studied Parameters

Callus fresh weight (CFW) (g): After 1-2 minutes washing of the callus with deionized double distilled water with constant shaking it was dried in between tissue papers. Fresh callus weight was noted in grams.

Callus dry weight (CDW) (g): After fresh weight readings, the callus was put in the oven at 65^{o}C for a day and dry weight was calculated in grams with analytical balance then mean values of all readings were calculated.

Days to callus induction (DCI): The callus was checked after 4-8 days and noted days to callus initiation.

Callus percentage (callus %): Callus percentage was calculated after one month of culturing by using this formula

$$\text{Callus percentage} = \frac{\text{Tubes in which callus developed}}{\text{Total cultured tubes}} \times 100$$

Mineral elements: 0.5g callus was dried and perchloric acid (5mL) was added and placed overnight, then digested at 100 ^{o}C, raised temperature at 150 ^{o}C in digestion tubes till 2mL volume remained in tubes. Then volume was made of up to 50mL in a volumetric flask, filtered with filter papers then used for mineral nutrients concentration. Flame photometer (Model: PFPI-7, Jenway, UK) is used for phosphorus (P) determination and atomic absorption spectrophotometer is used for magnesium (Mg), calcium (Ca), manganese (Mn) and iron (Fe) determination.

Statistical Analysis

The experiment was repeated three times, and results were averaged for investigation of variance, then obtained data were subjected to ANOVA (Steel *et al*., 1997). By using appropriate computer software the data was studied statistically with a completely randomized design and factorial arrangement. Mean values were compared with the least significant differences test (LSD) followed by Snedecor and Cochran (1980).

Table 01: Treatments used in this study

Treatments	Medium composition
T0	Control (0g/L) polyethylene glycol (PEG)
T1	PEG 1.25g/L
T2	PEG 2.5g/L
T3	PEG 0g/L + 1ml/L proline
T4	PEG 1.25g/L + 1ml/L Proline
T5	PEG 2.5g/L + 1ml/L Proline

Table 02: Mean square analysis of variance showing the effect of Polyethylene glycol (PEG), Proline and their interaction on callus fresh weight (CFW), Callus dry weight (CDW), Days to callus induction (DCI), Callus %, Calcium (Ca), Magnesium (Mg), Manganese (Mn), Iron (Fe) and Phosphorus (P)

SOV	DF	CFW (g)	CDW (g)	DCI	Callus %	Ca (mg/g)	Mg (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	P (mg/kg)
PEG	2	0.05***	0.01***	17.51***	451 .43***	40.12***	12565.04***	312.83***	5405.18***	53.59***
Proline (Pro)	1	0.02***	0.01***	21.13***	660.06***	41.38***	3669.39***	843.725***	1643.56***	24.67***
Genotype (G)	3	0.05***	0.01***	19.68***	1091.28***	41.81***	33329.96***	2251.74***	2392.26***	53.22***
PEG x Pro	2	0.01*	0.01*	0.13 ns	14.26ns	0.72**	16.01 ns	6.42 ns	17.51 ns	0.29**
PEG x G	6	0.01***	0.01*	0.29 ns	2.37 ns	0.93***	695.28***	8.98 ns	55.66**	4.66***
Pro X G	3	1.99 ns	0.02***	0.27 ns	20.79*	0.51*	38.02 ns	15.59*	11.07 ns	0.54***
PEG X Pro x G	6	4.02 ns	0.01	0.05 ns	3.2 ns	0.37 *	82.03 ns	7.28 ns	14.92 ns	0.11*
Error	48	3.55	0.01	0.33	5.56	0.13	74.64	4.97	13.13	0.04

= significant at 0.05 confidence level, **= highly significant at0 .01 confidence level, *= extremely significant at 0.001 confidence levels; ns=non-significant*

RESULTS AND DISCUSSION

Assessment of Callus Growth Parameters Under PEG-Induced Drought Stress and Proline Combination

Callus fresh weight: Table 02 depicts mean square value of callus fresh weight. PEG, proline treatments, and genotypes exhibited extremely significant differences in the fresh weight of the callus. PEG x Genotypes (G) and PEG x Proline (Prol) interactions displayed highly significant variance while non-significant variance exhibited by Prol x G as well as the PEG x Prol x G combination. Under all PEG levels callus fresh weight decreased however more reduction was noted at 2.5g/L PEG. Callus fresh weight of all genotypes consistently decreased under all PEG levels, on the other hand under all PEG treatments Super basmati as well as Bas-2000 executed better than Bas-515 and Bas-385; however, Bas-385 showed more sensitivity than other studied genotypes (Fig. 01). Under proline application alone or in combination with all PEG levels, callus fresh weight for all genotypes was enhanced. Maximum callus fresh weight (0.387g) was noted in Super basmati while minimum (0.199g) in Basmati-385 at T0. With increasing levels of

PEG, there was a reduction in the fresh mass of callus in all genotypes. When proline (1ml/L) was added in T3, T4 and T5, the high response was found in Super basmati (0.402, 0.375 and 0.301g) followed by Basmati-2000 (0.335, 0.312 and 0.281g) while low (0.229, 0.201 and 0.198g) in Basmati-385, detailed in figure 01.

Callus dry weight Table 02 presented that highly significant interactions were observed between PEG x Prol, PEG x G, Prol x G and PEG x Prol x G. Callus dry weight was observed high at all PEG levels in media but high increment noted at 2.5g/L PEG. While in dry weight steady increment was noted of all genotypes under all PEG treatments, but the performance of Super basmati, as well as Bas-2000, was better as compared to Bas-515 and Bas-385 under all PEG treatments. From all genotypes Bas-385 was observed more sensitive. All genotypes' callus dry weight was improved by proline application alone or in combination with PEG. Minimum callus dry weight was noted in all rice genotypes at T0 as compared to other treatments (Fig. 02). It was increased in the PEG medium for all genotypes however Super basmati most response at all PEG treatments. On proline addition in medium, the callus dry weight of Basmati-385 slightly recovered as compared to others as at T1 and T2. When proline added with PEG medium it has better effects on recovery of callus dry weight in Super basmati at T3 (0.0316g), T4 (0.037g) and T5 (0.0435g). This study predicted that proline has an effect on callus dry weight and depended on rice genotypes.

Days to callus induction: Non-significant difference was observed between PEG x Pro, w PEG x G, Pro x G and PEG x Pro x G in Table 02. Days to callus induction increased at all PEG levels, but more days were observed at 2.5g/L PEG. With the increment of PEG concentration in the medium, all genotypes exhibited a continual rise in days to callus induction; in contrast, Super basmati and Bas-2000 executed better in all PEG levels than other studied genotypes while from all observed genotypes Bas-385 was more sensitive. Proline application alone or with a PEG combination improved all genotypes' days to callus induction ability. Minimum days to callus induction were found in Super basmati (4.333), while maximum (7.333) in Basmati-385 at T0. Callus induction days increased in all genotypes with increased PEG levels. With the proline addition, the highest response was noted in Super basmati at T4 (4.333) and T5 (5.333), while the lowest in Basmati-385 at T4 (6.666) and T5 (7.666) as shown in Figure 03. It was noted days to callus induction were dependent on rice genotypes however its number of days was enhanced with proline application under PEG treatments as shown in Figure 03.

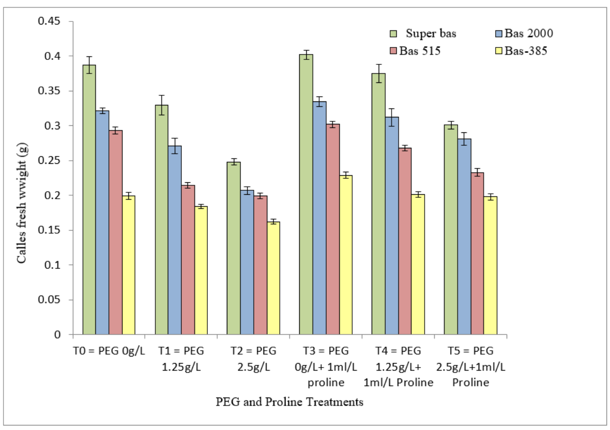

Figure 01: **The main effects of PEG, Proline treatments and their interaction on callus fresh weight (Y- axis), Error bars used in bar chart is standard error.**

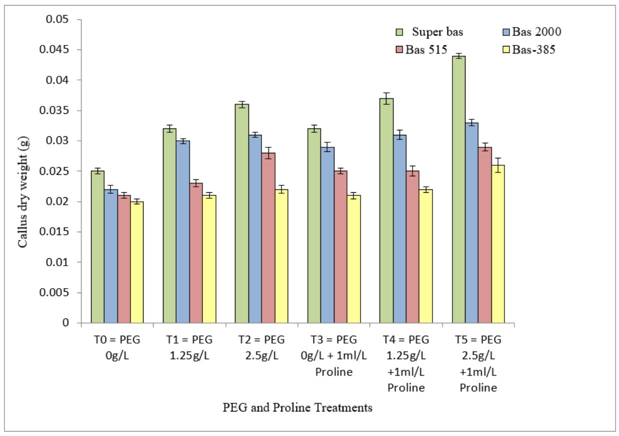

Figure 02: **The main effects of PEG, Proline treatments and their interaction on callus dry weight (Y-axis), The Error bars used in the bar chart are standard error.**

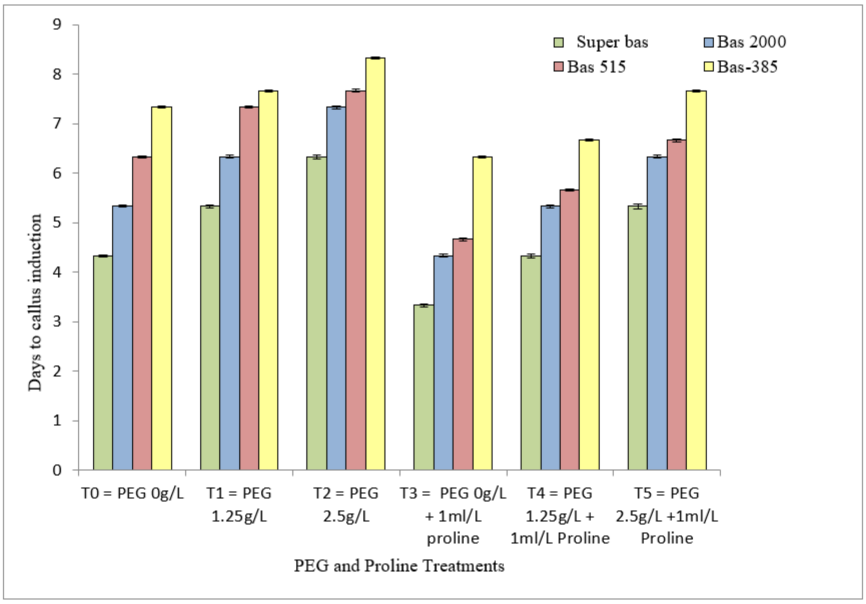

Figure 03: The main effects of PEG, Proline treatments and their interaction on days to callus induction (Y-axis), Error bars used in the bar chart are standard error.

Calluspercentage(%): Non-significant difference was noted between PEG x Prol, PEG x G and PEG x Prol x G interactions on the other hand Prol x G showed a significant difference in Table 02. Callus percentage reduced at all PEG levels in media, but the greatest reduction was at 2.5g/L PEG. With the increment of PEG concentration in the medium, all genotypes exhibited a continual decrease in callus percentage. On the other hand, Super basmati and Bas-2000 executed better under all PEG treatments than studied genotypes, while from all observed genotypes, Bas-385 was more sensitive. Under proline application alone or in combination with all PEG treatments enhanced the callus percentage of all genotypes. Callus % depicted in Figure 04, the maximum value observed in Super basmati (45mg/Kg), while the minimum in Basmati-385 (27mg/Kg) at T0. Callus % consistently decreased in all four genotypes in PEG treatments, having the lowest values in Basmati-385 (20 mg/Kg) and Super basmati (36.667mg/Kg) at T2. Moreover, the Callus % was raised when proline was added in treatments 3, 4 and 5. The variety Super basmati showed maximum value (51mg/Kg) at treatment 3 and minimum (44 and 41mg/Kg) at treatment 4 and 5. This study revealed that adding a water-reducing agent (PEG) reduced callus % for all genotypes and showed dependent behavior. It observed an effect of proline on callus % in the PEG medium.

Worldwide the foremost crop yield limiting factor is drought which also immensely defects the rice crop (Yang *et al*., 2008). The addition of stress-inducing osmotic agent polyethylene glycol (PEG) in the callus growth medium stunned the potential of cells grown medium (Gulati and Jaiwal, 1994). Many metabolic processes like nutrients and water uptake, reactive oxygen species production decline of chlorophyll level in addition to developmental practices interrupted by water shortage environments in plants (Tsago *et al*., 2013). As wheat, rice and sunflower cells face a gradient that is constricted by PEG in growth medium that favors the water movement inward however cells leave the water when this gradient is inverted at a high PEG level therefore, this study proposed that growth in addition to the development of callus reduces at high PEG level (Wani *et al*., 2010; Mahmood *et al*., 2012). The plant tissue's water potential alternatively decreases when soil moisture declines under water shortage environments (Mohamed *et al*. 2000). This study revealed that fresh callus weight decreased while dry callus weight consistently increased as PEG concentration increased in the medium, but all genotypes responded differently.

Moreover, some genotypes were noted as more sensitive, revealing a larger decrease in fresh weight and an increase in dry weight compared to others. The most tolerant response to PEG concentration was Bas-2000, in addition to, Super bas meanwhile Bas-385 was noted as more sensitive. Different studies also verified that the callus of tolerant plants reduced osmotic potential in culture media under water shortage conditions which caused to sustained growth rate by additional water uptake (Ahmad *et al*., 2007). In osmotic stress, fresh callus weight decreased while dry weight increased, as proline in addition to soluble sugars accumulated under high drought stress which increases dry callus weight on the other hand photosynthetic activity in addition to growth rate reduced (Shibli *et al*., 1992). The growth rate decreased in cultivars of rice when the PEG level increased in the callus medium (Suriyan *et al*., 2010). Moreover, in osmotic stress, fresh callus weight decreases because of the reduction of the turgor pressure of cells due to the deterioration of the water level (Al-Bahrany, 2002). Qasim *et al*., (2007) studied that proline application showed positive effects on callus fresh and dry weight under all PEG treatments and callus growth rate improved with exogenously proline application.

Assessment of Mineral Elements under PEG Induced Drought Stress and Proline Combination

Calcium (Ca): Table 02 presented a significant difference between PEG x Prol, while PEG x G Interaction depicts a highly significant difference. On the other hand, PEG x Prol x G as well as Prol x G interactions displayed the least significant difference. The highest Ca value was observed at 2.5g/L PEG. Super basmati in addition to Bas-2000 executed better under all-over PEG treatments than other studied genotypes while from all observed genotypes Bas-385 was more sensitive. Under proline application alone or in combination with all PEG treatments, the Ca content is enhanced in the callus of all genotypes. The highest Ca was observed under PEG treatment at T2. Super basmati and Bas-2000 performed better whereas Bas-385 was found most sensitive at all PEG treatments. Ca level of all genotypes improved with the application of proline in PEG treatments, resulting in the best response in Super basmati (13.157mg/g) followed by Basmati-2000 (11.333mg/g) at T5 (Fig. 05). It is concluded that Ca content is also genotypes dependent under all treatments.

Magnesium (Mg) In Table 02, a highly significant difference was observed in PEG x G whereas PEG x Prol, Prol x G and PEG x Prol x G interactions revealed a non-significant difference. The highest Mg content in the callus of all genotypes decreased at 2.5g/L PEG level. Super basmati in addition to Bas-2000 executed better while Bas-385 noted the most sensitive rice genotypes at all PEG treatments (Fig. 06). Mg content in callus of all rice genotypes improved with proline application also in PEG treatments. For Magnesium (Mg) in callus, Super basmati performed at the highest concentrations, obtaining Maximum Mg value ranging from T0 to T5 (872 to 865mg/Kg). Otherwise, Basmati-385 demonstrated the minimum range values (784.667 to 755.333mg/Kg) for all treatments. All genotypes showed a consistent decrease at all PEG levels, but Super basmati had better concentrations under all PEG levels with a maximum Mg value (858mg/Kg) found at treatment-2. Basmati-385 was the most sensitive genotype noted (741.333mg/Kg) at treatment-2. The best response of the proline application was in Super basmati, followed by Basmati-2000. It is concluded Mg content improved with proline application and seemed to decrease under PEG treatments and is also dependent on rice genotypes.

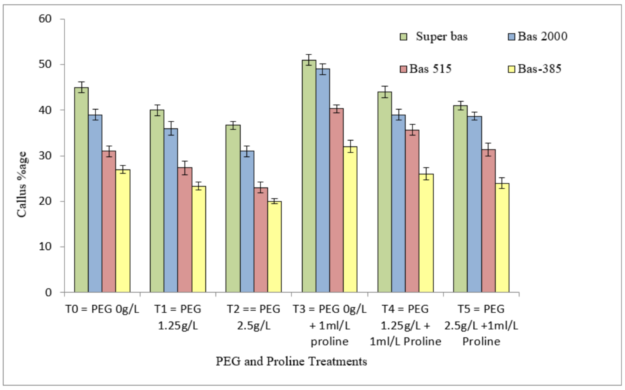

Figure 04: **Main effects of PEG, Proline treatments and their interaction on callus percentage (Y-axis), Error bars used in bar chart is standard error.**

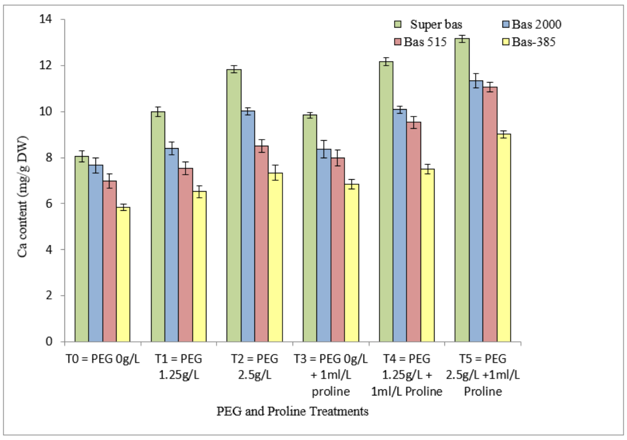

Figure 05: **The main effects of PEG, Proline treatments and their interaction on callus Calcium (Ca) content (Y-axis), The Error bars used in the bar chart is standard error.**

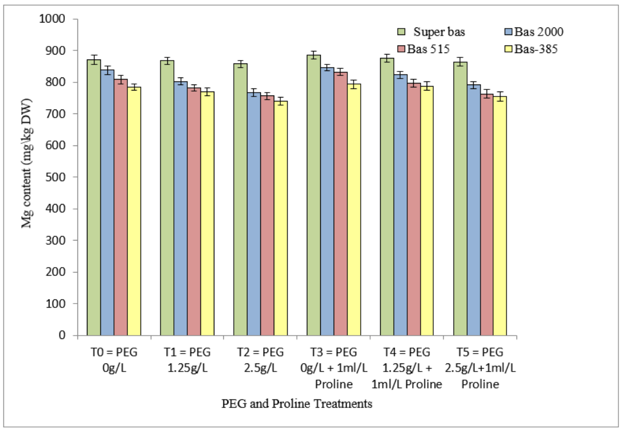

Figure 06: **The main effects of PEG, Proline treatments and their interaction on callus Magnesium (Mg) content (Y-axis), Error bars used in a bar chart is the standard error.**

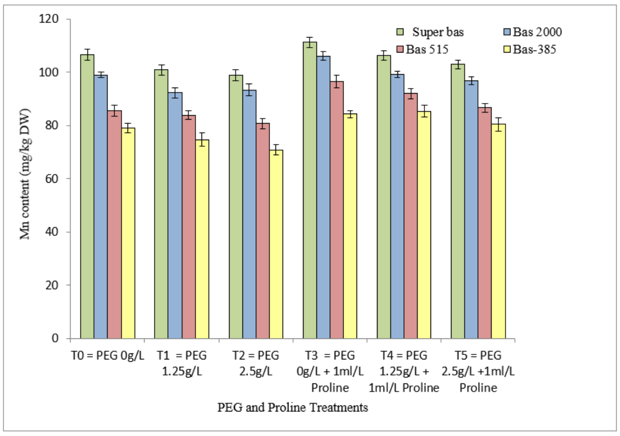

Figure 07: **The main effects of PEG, Proline treatments and their interaction on callus Manganese (Mn) content (Y-axis), Error bars used in a bar chart is the standard error.**

Manganese (Mn): Prol x G revealed significant differences however other interactions like PEG x Prol, PEG x G, and PEG x Prol x G displayed non-significant differences in Table 02. Mn level decreased at all PEG levels, but more decrease was noted at 2.5g/L PEG. Super basmati and Bas-2000 executed better under all PEG treatments than other studied genotypes, while from all observed genotypes, Bas-385 was more sensitive; however, under proline application alone or in combination with all PEG treatments Mn level of all genotypes enhanced. Manganese (Mn) values in Figure 07 depict that the maximum Mn value (106.633mg/Kg) was in Super Basmati and minimum (79.027mg/Kg) in Basmati-385 at treatment (T0). Mn values were consistently decreased for all genotypes in PEG medium treatments 1 and 2, with the maximum Mn (100.903 and 98.997mg/Kg) and minimum (74.767 & 70.89mg/Kg) found in Super Basmati and Basmati-385, respectively. At 1ml/L of proline, the Mn value was raised in treatments 3, 4 & and 5. The results show that Super Basmati had the maximum value (106.46 and 102.997 mg/Kg), in contrast, Basmati-385 obtained the minimum value (85.46 and 80.513mg/Kg), both at treatments 4 and 5. This study revealed that an addition of a water-reducing agent (PEG) reduced Mn values for all genotypes and showed a dependent behavior. An enhancing effect of proline on Mn value in the PEG medium was observed. The highest value of Mn is found in Super Basmati followed by Basmati-2000.

Iron (Fe): Significant difference was noted between PEG x G while non-significant differences were displayed by PEG x Prol, Prol x G as well as PEG x Prol x G interactions in Table 02. Fe content decreased when the PEG level elevated in media, but high depletion was noted at 2.5g/L PEG. Super basmati and Bas 2000 were better while Bas-385 was perceived more sensitive than other genotypes. Under proline application alone or in combination with all PEG treatments Fe content of all genotypes enhanced. Figure 08 showed that iron (Fe) contents formation in callus for four rice genotypes amongst them, maximum values (505mg/Kg) and minimum (471.667mg/Kg) were observed in Super basmati and Basmati-385 respectively, at treatment (T0). Fe decreased in all genotypes under PEG treatments (2 and 3). Super basmati contained the maximum Fe (471.667mg/Kg), and the minimum (448mg/Kg) in Basmati-385 at treatment (T2). Maximum Fe (492.667 and 482.333mg/Kg) was found in Super basmati callus while minimum (464.667 and 458.333mg/Kg) in Basmati-385 when proline was added in PEG media at T4 and T5). Callus Fe content was observed at various treatments but more reduction was noted at T2 (2.5g/L). Super basmati performed better while Basmati-385 found a more sensitive genotype under all treatments. The iron content of the callus of all genotypes improved when proline was applied in combination with PEG levels (Fig.08).

Phosphorus (P): Table 02 presented that the influence of Prol x G and PEG x G exhibited highly significant differences whereas PEG x Prol revealed a significant while PEG x Prol x G showed a significant difference. P content was depleted when PEG levels elevated in media but more reduction was noted at 2.5g/L PEG treatment. Super basmati and Bas-2000 performed better at all PEG treatments while Bas-385 was found as the most sensitive genotype; however, under proline application alone or in combination with all PEG treatments P content of all genotypes improved. Phosphorus (P) obtained from the callus of four rice genotypes under study responded variably at all treatments. The maximum value of P was observed in all rice genotypes under study at treatment (T0) as compared to other four treatments as in Super basmati (505mg/L) and Basmati-385 (471.667mg/L) (Fig. 09). It was highly decreased in PEG medium for all genotypes however Basmati-385 most affected (448mg/L) at 2.5g/L PEG medium at T2. In addition to proline, P levels recovered but in Basmati-385 slightly low recovered (482mg/L) as compared to Super basmati (511mg/L) as well as other genotypes at T3. When proline was added in treatments 4 and 5 with PEG medium it had little effect on the recovery of P values in Super basmati (492.667 and 482.333mg/L), Basmati-385 (464.667 and 458.333mg/L) also to all other genotypes. This study predicted that PEG affected P values which can be recovered with proline and depended on rice genotypes. Further, proline played a positive role in the recovery of P in callus.

At the cellular level, in plants accumulation of cations decreases in response to increased drought stress (Ahmad *et al*., 2007). With the increment in osmotic stress the macro cations like “K^+, Mg^+” along with micro cations like “Fe and Mn” level significantly reduced in genotypes of wheat (Farrukh and Ikram, 2008). Our results showed that there was a significant reduction in mineral nutrients uptake “Mg, Mn, Fe and P” although K, in addition to Ca contents slightly increased under water shortage conditions produced utilizing PEG in calli of all genotypes. Hassan *et al*. (2004) reported that under water shortage conditions K contents level improved however P level declined. It also observed that external proline application improved the level of mineral nutrients in calli of all rice genotypes under stress also non-stress environments. Kishor *et al*. (2005) stated that proline prevents the denaturation of macro-molecules and also reduces the cell’s acidity.

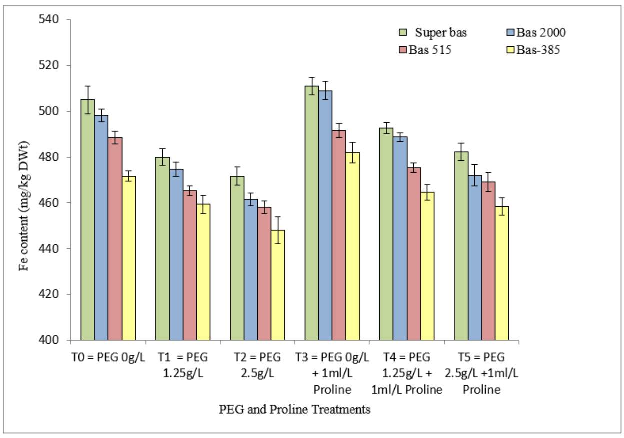

Figure 08: **The main effects of PEG, Proline treatments and their interaction on callus Iron (Fe) content (Y-axis), Error bars used in a bar chart is standard error.**

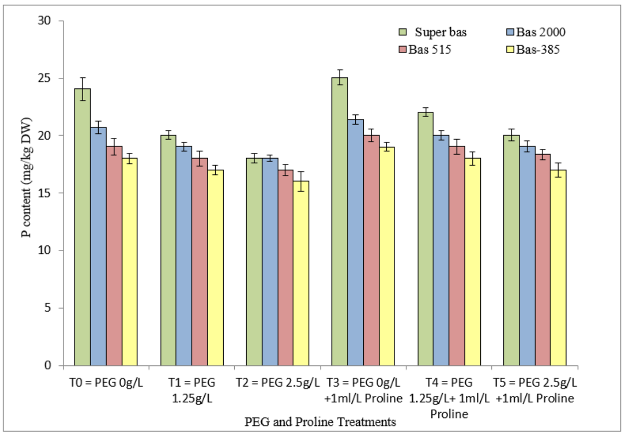

Figure 09: **The main effects of PEG, Proline treatments and their interaction on callus Phosphorus (P) content (Y-axis), Error bars used in a bar chart is the standard error.**

CONCLUSION

Rice depicts great sensitivity concerning drought by employing the stress-stimulating agent PEG in growth media. This study focused on checking the influence of polyethylene glycol tempted drought stress on rice genotype's callus in growing media; moreover, to estimate the effects of external proline application to overcome the adverse influences of drought on basmati rice genotypes. PEG causes reduced fresh weight of the callus whereas the dry weight of the callus to some extent increases at a high PEG level, under water deficit conditions callus accumulates several compatible solutes which causes to increased dry weight of the callus. This study also contributes to the knowledge that the percentage of callus decreased as polyethylene glycol application increased while exogenous proline application improves growth rate by minimizing the adversarial influences of PEG on callus tissues. Bas-385 displayed more sensitivity concerning drought stress as compared to all other genotypes. Biochemical examination revealed that macro-micro nutrients "except K and Ca" as well as total soluble sugars level decreased in water shortage environments; in contrast, exogenously applied proline enhanced these nutrients as well as proteins level. PEG causes reduced growth rate at callus as well as at the cellular level; however, proline retard the adverse effects of PEG by improving fresh and callus weight.

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