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Polyethylene glycol induced screening for drought tolerance of different rice genotype

Gagendra Singh Rajput, VB Kuruwanshi and Arti Guhey

Abstract
Drought is the major concern of Indian agriculture under changing scenario of climate to sustain the productivity and to fulfill the food requirement of ever increasing population. Hence, the present study was carried out at Research cum Instructional Farm and Department of Plant Physiology, Agricultural Biochemistry, Medicinal and Aromatic Plants, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) during *Kharif* during 2018-19 and 2019-20. The planting materials for experiment consisted of 30 rice genotypes from IIRR, Hyderabad under AICRP on rice programme and 01 local check (Poornima) from IGKV, Raipur (C.G.). In first year (2018-19), 30 rice genotypes were treated with four different levels of PEG namely (T_1- Control (Distilled water), T_2- PEG @ 5%, T_3- PEG @ 10% and T4- PEG @ 15%) in a petri dish under complete randomized design (CRD) design with three replications. The water deficit stress was artificially induced by desired strengths of polyethylene glycol 6000 (PEG-6000; Sigma Chemicals) in growth chamber at 25±0.5 °C and 80%±1 relative humidity to screening the rice genotypes. The PEG study like the germination per cent, germination index, seedling height, seedling dry weight, and relative dry weight of all tested rice genotypes were found decreasing trends with increasing the levels of PEG from 0 to 15% level. Among these rice genotypes, Poornima (LC) followed by RFU 329 > RFU 327 > RFU 304 were performed better in terms of germination per cent, germination index, seedling height, seedling dry weight and relative dry weight under artificially induced stress by using PEG.

Keywords: Polyethylene, glycol, screening, tolerance, genotype

Introduction
Drought “is a major abiotic stress that reduces crop productivity and weaken global food” security, especially “given the current and growing impacts of climate change and increases in the occurrence and severity of both stress” factors. Plants have “developed dynamic responses at the” morphological, physiological and biochemical “levels allowing them to escape and/or adapt to unfavorable environmental” conditions. Nevertheless, even the mildest heat and drought stress negatively affects crop yield. Further, several independent studies have shown that increased temperature and drought can reduce crop yields by as much as 50%. Response to stress is complex and involves several factors including signaling, transcription factors, hormones, and secondary metabolites.

Rice (*Oryza sativa* L.) is the staple food of more than half of the world population. In world rice is cultivated over an area of 156.1 m ha with a production of 680 mt. In world, rice has occupied an area of 154 million hectares, with a total production of 476 million tonnes and productivity 2948.82 kg ha^{-1} (Anonymous, 2012) [3]. Chhattisgarh state is popularly known as “rice bowl of India” because maximum area is covered under rice during *kharif* and contributing major share in national rice production. Rice is cultivated in an area around 3.67 million hectares with the production of 7.49 million tonnes and productivity 2041 kg ha-1 (Anonymous, 2014) [4]. The prime causes of low productivity of rice in Chhattisgarh are inappropriate adoption of agronomical practices, limited irrigation (28.0%), lack of improved drought tolerant varieties with high yielding characteristics suitable to different ecosystems and extension services. Chhattisgarh farmers are mainly depending on climate for rice cultivation. Since the rainfed ecosystem of Eastern India is highly variable and unpredictable, which can range from normal situation to severe drought condition, therefore identification of a stable genotype performing well under water deficit conditions is required.

Drought resistance is complicated processes that involve interaction of multiple pathways, genes and traits. Crop plants follow different drought resistance mechanisms against drought stress that are categorized as drought tolerance, drought escape and drought avoidance (Levitt

J, 1980) [11]. Drought tolerance involves the ability of plants to develop low osmotic potential, cellular elasticity and increase membrane resistance. Drought escape involve the modulation of vegetative and reproductive growth to avoid drought stress by completing the life cycle before onset by either rapid plant growth or little growth during dry season but tremendous growth during wet season. Therefore, there is need to exploit the natural variation of crop plant in drought adaptation as the drought adaption strategy of genotypes within species differ and can provide better in- sight to improve yield and resistance under drought conditions. Plant breeding and generation of transgenic plants through gene manipulation of drought related genes has opened up remarkable opportunities to accelerate the production drought tolerant varieties thus, generation and breeding of drought tolerant genotypes is a key strategy to improve the crop productivity.

In regions where water resources are limited, somaclonal variation offers a means for the production of drought tolerant plants by PEG induced *in vitro* culturing. It has been reported earlier that an increase in drought stress by PEG was accompanied by a steep decline in moisture content of tissues (Adkins *et al.* 1995; El-Tayeb and Hassanein 2000) [1, 7]. Since PEG does not enter into the apoplast, water is withdrawn not only from the cell but also from the cell wall. Thus, in comparison to other low molecular weight osmotica, PEG mimics in a way similar to soil drying and has been used to simulate drought stress in plants and the selection of tolerant cell lines (Nepomuceno *et al.* 1998) [12]. The knowledge about inter-relationship among phenotype, genotype and environmental conditions (Dhondt *et al.,* 2013; Furbank and Tester, 2O11) [6, 9]. Keeping all these points in mind the investigation was undertaken with the following objectives to screening the rice genotypes for drought tolerance using Polyethylene glycol (PEG).

Materials and Methods

Study area

The present study was carried out carried out at Research cum Instructional Farm and Department of Plant Physiology, Agricultural Biochemistry, Medicinal and Aromatic Plants, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) during *Kharif* during 2018-19 and 2019-20.

Climatic conditions

The climate of this region is dry, sub-humid to semi-arid. The average annual rainfall ranges from 1200-1400 mm out of which 80-85 percent is received from middle of June to end of September and very little during October and February. The maximum and minimum temperature goes to 42.6 °C and 9.6 °C, respectively in the months of May and December or January. During crop growth period, the maximum temperature varied between 32.1 °C to 35.5 °C, respectively. The minimum temperature ranged between 24.9 ^{0} C in the first week of July to 16 °C in the fourth week of November. Crop received sunshine hours of 2.5 to 9.6 per day. The maximum and minimum humidity during the crop growth period was 92 and 36 per cent, respectively. A total of 738.6 mm rainfall was received during the crop period.

Source of Rice planting materials

The planting materials for experiment work was consist of 30 rice genotypes taken from IIRR, Hyderabad under AICRP on rice programme and 01 check variety from IGKV, Raipur (C.G.). The 30 rice genotypes taken for present study are following –

Table 1: Details of rice genotypes used in present study

RFU 301	**RFU 306**	**RFU 311**	**RFU 316**	**RFU 321**	**RFU 326**
RFU 302	RFU 307	RFU 312	RFU 317	RFU 322	RFU 327
RFU 303	RFU 308	RFU 313	RFU 318	RFU 323	RFU 328
RFU 304	RFU 309	RFU 314	RFU 319	RFU 324	RFU 329
RFU 305	RFU 310	RFU 315	RFU 320	RFU 325	Poornima (LC)

Experimental details

In first year of experiments 30 rice genotype (Table 1) was treated with four different levels of PEG namely (T_1- Control (Distilled water), T_2- PEG @ 5%, T_3- PEG @ 10% and T4- PEG @ 15%) in a petri dish with three replication under complete randomized design (CRD) design and allocated randomly to different dish plate by using random number from the table of Fisher (1956) [8].

Seeds of each genotype were surface sterilized with 70% ethanol solution for 5 minutes. The seeds were then washed three times with sterilized distilled water. Germination assays were performed by evenly distributing the seeds in a 10-cm-diameter sterilized Petri dish with two layers of what man No. 1 filter paper. Each dish was moistened with 10 ml distilled water or uniform amounts of desired osmotic solutions to mimic drought stress. During screening, water deficit stress is artificially induced by desired strengths of polyethylene glycol 6000 (PEG-6000; Sigma Chemicals). Polyethylene glycol has been used to simulate water stress effects in plants (Swapna and Shylaraj, 2017) [14]. The experiment was laid out in a complete randomized design (CRD) with four levels of drought stress and three replications. Distilled water was used as a control (0 MPa) and osmotic potentials -0.3, -0.6, -0.9 and –1.2 MPa were created by adding PEG-6000 @ 0, 5, 10 and15 g per 100 ml distilled water. Three replicates of 25 seeds of each osmotic potential were used to assess the germination percentage. This experiment was carried out in growth chamber at 25±0.5 °C and 80%±1 of relative humidity. The number of germinated seed was recorded at 24hours interval. The seedling height and seedling dry weights were measured on the 14th day. Seeds were considered germinated when both plumule and radicle extended to more than 2 mm from the seeds. The layout plan is depicted in fig. 1

Fig 1: Layout plan for PEG Treatment

Results and Discussion

Germination per cent: Polyethylene glycol (PEG) is a non-penetrating inert osmoticum that can lower the water potential of nutrient solutions without being taken up or phytotoxic. This approach has been used to simulate drought stress in plants and selection of tolerant genotypes in different crops. A perusal of data presented in Table 2 indicated that effects of PEG 6000 @ control, 5%, 10%, 15% on the germination per cent of rice genotype was found statistically significant. The germination per cent of rice genotype was found in decreasing trends from PEG control to 15% level. Remarkable difference was observed in germination per cent between control and stress environment created by PEG 6000 @15%, reduction in germination percent was 7.32, 16.61, 39.21 at 5, 10 and 15% respectively. Among the rice genotype Poornima (LC) exhibited relatively better germination against drought stress of different level of PEG followed by RFU 329 > RFU 327 > RFU 304 indicating that all these genotypes performed relatively better under drought stress induced by PEG 6000. The results revealed all these four genotypes will further perform better under rainfed environment (Bhadra *et al.* 2018) [5].

Table 2: Influence of different levels of PEG on germination (%) and germination index of rice genotypes

Rice genotype	Germination Per cent				Germination index		
	Control	5% PEG	10% PEG	15% PEG	5% PEG	10% PEG	15% PEG
RFU 301	86.93	79.70	71.63	54.05	91.72	82.45	62.16
RFU 302	86.82	79.03	71.63	51.85	91.05	82.54	59.70
RFU 303	85.82	77.70	67.60	40.21	90.57	78.82	46.84
RFU 304	89.32	85.70	79.70	68.04	95.92	89.24	76.17
RFU 305	84.33	76.70	66.60	37.18	90.97	79.01	44.11
RFU 306	86.77	78.70	71.63	51.85	90.70	82.58	59.76
RFU 307	85.88	78.70	70.62	45.40	91.64	82.25	52.91
RFU 308	88.32	82.70	74.65	57.41	93.64	84.56	65.00
RFU 309	87.86	81.26	73.64	57.41	92.52	83.86	65.33
RFU 310	88.77	83.70	76.67	62.00	94.30	86.40	69.82
RFU 311	85.82	77.92	67.60	41.24	90.82	78.82	48.04
RFU 312	87.16	80.70	72.63	54.16	92.61	83.37	62.12
RFU 313	84.82	77.70	66.60	37.18	91.64	78.57	43.82
RFU 314	88.83	82.70	76.67	63.16	93.13	86.35	71.09
RFU 315	86.27	78.70	70.62	48.59	91.25	81.90	56.30
RFU 316	85.14	77.70	67.60	39.20	91.28	79.44	46.04
RFU 317	87.92	81.70	73.64	57.41	92.95	83.80	65.28
RFU 318	85.00	77.70	66.60	39.20	91.43	78.39	46.11
RFU 319	87.83	80.70	73.64	55.17	91.91	83.89	62.79
RFU 320	84.12	76.70	65.60	35.19	91.39	78.18	41.73
RFU 321	88.53	82.82	75.65	59.69	93.60	85.53	67.40
RFU 322	88.56	83.70	75.65	59.69	94.53	85.46	67.38
RFU 323	86.82	79.70	71.63	51.85	91.83	82.54	59.70
RFU 324	85.82	78.70	68.61	45.40	91.73	79.99	52.88
RFU 325	88.16	82.70	74.65	57.41	93.83	84.72	65.10
RFU 326	86.82	79.70	71.63	52.94	91.83	82.54	60.96
RFU 327	89.76	85.95	81.08	69.11	95.76	90.33	76.98
RFU 328	87.83	80.70	73.64	57.41	91.91	83.89	65.35
RFU 329	91.83	88.28	81.72	70.22	96.15	89.03	76.45
Poornima (LC)	93.29	90.80	85.60	73.62	97.32	91.77	78.91
Mean	87.37	80.97	72.85	53.11	92.66	83.34	60.54
CD	2.79	6.03	6.51	4.81	7.80	8.91	4.55
S.Em±	0.99	2.13	2.30	1.70	2.76	3.15	1.61

Germination index

The data presented in Table 3 indicated that effects of PEG 6000 @ various concentration on the germination index was found statistically significant in rice genotypes. The germination index of rice genotype was found in decreasing trends from PEG 5 to 15% level. The remarkable difference was observed in germination index between control and stress environment created by PEG 6000 @ 15%, reduction in germination index was 8.40, 19.06, 45.16 at 5, 10 and 15% respectively. Among the rice genotype Poornima (LC) exhibited relatively better germination index against drought stress of different level of PEG followed by RFU 329 > RFU 327 > RFU 304 genotypes performed relatively better under drought stress induced by PEG 6000. The results also revealed that these four genotypes were performed better under rainfed environment (Bhadra *et al.* 2018 and Islam *et al.* 2018) [5, 10].

Seedling height (cm)

The data presented in fig 2 indicated that effects of PEG 6000 @ control, 5%, 10%, 15% was found statistically significant on the seedling height of rice genotype. The seedling height of rice genotype was exhibited decreasing trends from PEG control to 15% level. The remarkable difference was observed in Seedling height between control and stress environment created by PEG 6000 @15% and the reduction in seedling height was 5.83, 10.63, 14.91 at 5, 10 and 15% respectively. Among the rice genotype Poornima (LC) exhibited relatively better seedling height against drought stress of different level of PEG followed by RFU 329 > RFU

327 > RFU 304 indicating that all these genotypes performed relatively better under drought stress induced by PEG 6000. Bhadra *et al.* (2018) [5] and Islam *et al.* (2018) [10] reported that inhibition of radical emergence is mainly because of decrease in water potential gradient between the external environment and seed and consequently impairs seedling height under water stress condition (Sokoto and Muhammad 2014) [13].

Seedling dry weight (gm)

A perusal of data illustrated in fig 3 presented that the effects of PEG 6000 @ control, 5%, 10%, 15% on the seedling dry weight of rice genotype was found statistically significant. The seedling dry weight of rice genotype was found in decreasing trends from PEG control to 15% level. The seedling dry weight remarkable difference was observed between control and stress environment created by PEG 6000 @15% and reduction was recorded 2.17, 5.15, 8.61 at 5, 10 and 15% respectively. Poornima (LC) rice genotype exhibited relatively better Seedling dry weight against drought stress of different level of PEG followed by RFU 329 > RFU 327 > RFU 304 among all the genotypes. All the tested genotypes performed relatively better under drought stress induced by PEG 6000 (Sokoto and Muhammad 2014; Akte *et al.* 2016; Islam *et al.* 2018) [13, 2, 10].

Relative dry weight (%)

The relative dry weight of a plant reflects its vigour and is considered a good index of its exposure to stresses of all sorts. Influence of different levels of PEG on relative dry weight (%) of rice genotypes summarized in fig 4. The effects of PEG 6000 @ control, 5%, 10%, 15% was found statistically significant on the relative dry weight of rice genotype. It was found in decreasing trends from PEG control to 15% level. The relative dry weight between control and stress environment created by PEG 6000 @15%, reduction was recorded 13.87, 21.12, 51.49 at 5, 10 and 15% respectively. The relative dry weight response of rice seedlings exposed to increasing PEG concentrations, revealed a decrease for seedling height. It may reflect the impact of water stress on root cell development, which would likely impair nutrient uptake as well as having detrimental effects on photosynthesis, essential for biomass accumulation and therefore on shoot and root elongation. Among the rice genotype Poornima (LC) exhibited relatively better relative dry weight against drought stress of different level of PEG followed by RFU 329 > RFU 327 > RFU 304 indicating that all these genotypes performed relatively better under drought stress induced by PEG 6000 (Sokoto and Muhammad 2014; Akte *et al.* 2016 and Islam *et al.* 2018) [13, 2, 10].

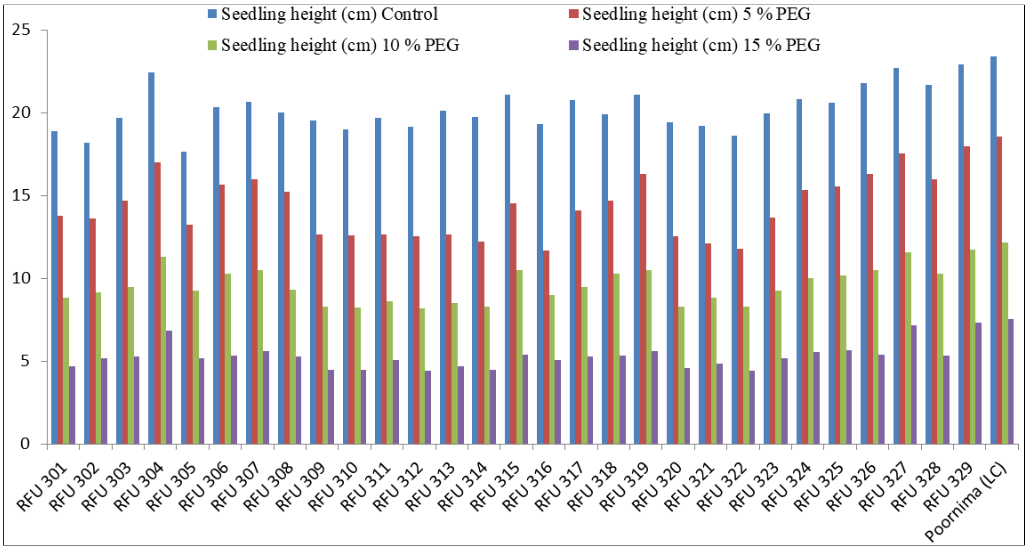

Fig 2: Influence of different levels of PEG on seedling height (cm) of rice genotypes

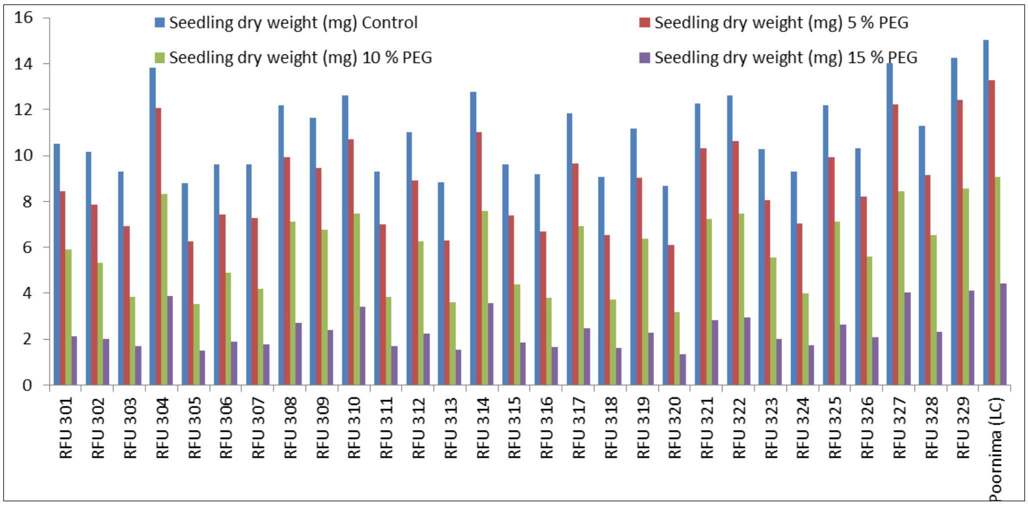

Fig 3: Influence of different levels of PEG on Seedling dry weight (mg) of rice genotypes

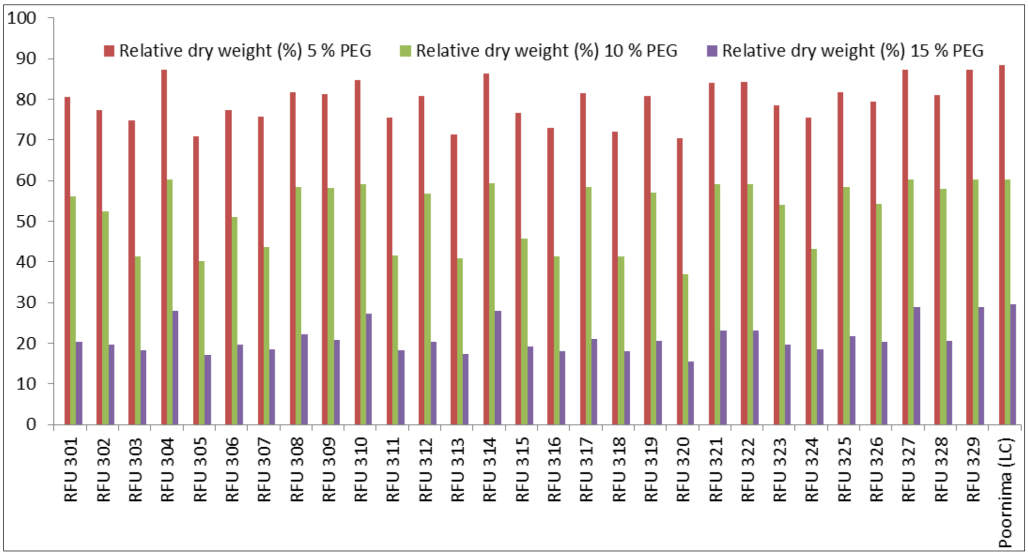

Fig 4: Influence of different levels of PEG on relative dry weight (%) of rice genotypes

Conclusion

The PEG study concluded that the germination per cent, germination index, seedling height, seedling dry weight, and relative dry weight of all tested rice genotypes were found decreasing trends with increasing the levels of PEG from 0 to 15% level. Among these rice genotypes, Poornima (LC) followed by RFU 329 > RFU 327 > RFU 304 were performed better in terms of germination per cent, germination index, seedling height, seedling dry weight and relative dry weight under artificially induced stress by using PEG.

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