

Modeling root development of rice (*Oryza sativa*L) under varying drought stress

ABSTRACT

In the current era, where drought occurrences are frequent and devastating, extensive study on drought tolerance mechanisms is imperative. Root growth under water stress is a key indicator of drought tolerance. Modeling a crop's root-length under such conditions aids in predicting seminal root length (SRL) and, consequently, its tolerance level. Under consecutive stress using Poly-ethylene-glycol (PEG), root length of a landrace originating from the drought-prone area of West Bengal, India was measured through image analysis and then mathematically modulated using an equation. The highest root length under stress was predicted at 6% PEG stress which aligned with the experimental readings. On the 15th day of stress, predicted SRL values were 104.67mm and experimental values were 90.00mm. Despite some over and underestimations with a low root mean square error of 14.68 mm, the equation provides insight into root elongation trends under various stress levels, offering a basis for predicting SRL and yield capabilities under water stress for diverse crop species.

Keywords: Rice, drought stress, root length, modeling

1. INTRODUCTION:

Rice being one of the most demanding food crops in the world, especially in South and South-East Asia faces a range of obstacles to its production (Samal et al., 2018, De et al., 2022). Due to global warming and other pollution-related reasons, environmental extremities are increasing, negatively affecting crop yield. Drought is such a constraint in rice production, the occurrence, and severity of which is unpredictable and is causing severe damage in rice fields all over the globe (Turrall et al., 2011; Pandey and Shukla, 2015). Root plays an important role in plant growth and production under water stress conditions. Several studies reveal that underwater scarcity shoot growth is inhibited whereas root growth is promoted to increase the supply of water and other nutrients (Sharp et al., 2004). Root extension under such situations helps in analyzing drought resistance of crops and helps in predicting the crop yield (Mishra and Salokhe, 2011; Yang et al., 2012). To determine the drought resistance capability of a crop in laboratory condition, artificial drought stress is imposed on the plants, mostly done by using Poly-Ethylene Glycol (PEG). It acts as a non-penetrating osmotic agent resulting in an increase of solute potential (Ψ_s) and blockage of absorption of water by the root system (Chutia and Borah, 2012). Under PEG-induced osmotic stress, effects on root morphology and root hair characteristics were observed in rice (Hannan et al., 2020; Nupur et al., 2020), wheat (Ayalew et al., 2015; Robin et al., 2021), and other cereals (Comeau et al., 2010) during the vegetative growth stage which were genotype-specific. The optimum concentration of PEG for the evaluation of drought resistance remains a challenge. Halimursyadah et al, suggested that 18.1%

PEG concentration could be used as screening drought-tolerant lines, whereas Diana et al., (2017) imposed stress upto 30% of PEG and found that the highest weight of unhulled rice/clump and fully unhulled rice/clump was observed in a specific genotype at 30% PEG stress. Quantification of root growth (especially root length) under such a situation helps to understand the underlying mechanism against drought stress.

Mathematical modeling of root length by using equations can be utilized to simulate root growth. It might be very effective since it quantifies the differences in responses between cultivars and stress conditions (Susilawati et al., 2022). Hirooka et al., (2016) used mathematical formulae to calculate leaf area expansion of many rice cultivars under various situations, as well as simulated dry matter production and yield. Similarly, modeling of seminal root growth under drought stress proved to be an effective method to evaluate a germplasm, Nipponbare where PEG stress at consecutive levels showed varied root lengths which were quantitatively assessed by using mathematical equation by Susilawati et al., (2022). In our study extensive collection of landraces from various parts of Purulia and Bankura districts in West Bengal, India was done and they were subjected to drought stress artificially by PEG. Despite of having higher genetic base, these landraces are almost on the verge of extinction. Different root responses were observed for different germplasms, out of them one landrace, Kalpana had a different rooting pattern and was used as our test material. Slightly modified version of the equations suggested by Susilawati et al., 2022 fitted well with its root growth and the experimental and equational data were almost at par with each other. This equation can be further used to decipher root extension in other germplasms showing similar kind of rooting pattern and as the ultimate seed yield is directly correlated with root length, it can be predicted as well.

2. MATERIALS & METHODS:

Seeds of a landrace, Kalpana, an *indica* cultivar, collected from ARW Society, Bankura, West Bengal, India were used as the testing material. The experiment was conducted in the Dept of Botany, SKBU, using a plant growth chamber where seeds of this landrace were treated with varying concentrations of PEG-6000 solution starting from 0% (no water stress) to 21% (severe stress) at germination stage. Before treatment seeds were surface sterilized with 1% Sodium hypochlorite (NaOCl) for 10 minutes, washed with distilled water and then soaked in water in the dark for 24 hours. After that drought stress was imposed on the seeds. The water potential (ψ) of each PEG solution was estimated following the formula led by Michel and Kaufmann (1973).

$$\psi = -(1.18 \times 10^{-2}) C - (1.18 \times 10^{-4}) C^2 + (2.67 \times 10^{-4}) CT + (8.39 \times 10^{-7}) C^2T \quad \dots\dots\dots[1]$$

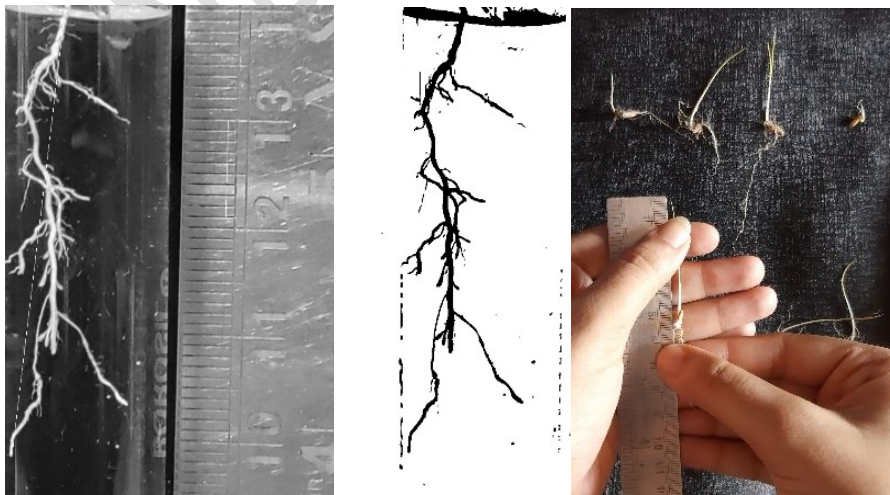
Where C is the concentration of PEG-6000 in g/kg of H₂O and T = Temperature i.e., 25°C

Water potential in each case is mentioned in Table 1. Seeds were treated for fifteen days with varying degree of stress in petriplates and in test tubes with 5 replications each. Their root length was measured each day till the 15th day using a measuring scale/ruler for the samples in petriplates as well as through non-contact method using Image J software for the samples in test tubes [Fig: 1]. The

average data of root length was recorded each day. Then the seeds were left in their respective treatments and measured at 5 days intervals till the 25th day.

PEG concentration (%)	Water potential (bar)
0	0
1	-0.06095
2	-0.14131
3	-0.24107
4	-0.36024
5	-0.49881
6	-0.65679
7	-0.83417
8	-1.03096
9	-1.24715
10	-1.48275
11	-1.73775
12	-2.01216
13	-2.30597
14	-2.61919
15	-2.95181
16	-3.30384
17	-3.67527
18	-4.06611
19	-4.47635
20	-4.90600
21	-5.35505

Table 1: Water potential at each stress level



(a) (b) (c)

Fig 1: Measurement of root length (a) Original RGB image and (b) its 8-bit image in Image J software [non-contact method] and (c) using measuring scale [contact method]

An equation mentioned below with a slight modification led by Susilawati et al., 2022 was used to measure the root length mathematically.

$$SRL = K / (1 + C \exp(-rDAS)) \quad \dots\dots\dots[2]$$

where root length is expressed as the function of DAS (Days after seeding) for each PEG concentration and

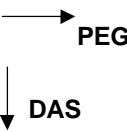
$$K = a(PEG + d)^b \exp\{-c(PEG + d)\} \quad \dots\dots\dots[3]$$

where, K is assumed to be the full length of the root and C, r, a, b, c, and d are regression coefficients whose values were 2.402, 0.165, 5.64, 4.00, 0.677 and 0.0, respectively.

3. RESULTS AND DISCUSSION:

SRL and K values in each stress level estimated using the above equations are shown in Table 2. The experimental values from the average data are displayed in Table 3. There was a similarity in the trend between the approximated SRLs using Eq. 2 and SRLs evaluated using contact and non-contact image analysis from 0 to 15 DAS. Eq. 3 was used to estimate the full root length or K. The treated seedling roots when measured at 25 DAS showed similarity with the expected data of K where the root mean square error was lowest.

SEMINAL ROOT LENGTH [mm]

 PEG DAS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
0	0.84	6.85	17.6 2	28.3 0	35.1 0	36.9 9	34.8 2	30.1 8	24.5 7	19.0 3	14.1 6	10.1 9	7.13	4.87	3.26	2.15	1.39	0.89	0.56	0.35	0.22
1	0.94	7.67	19.7 4	31.7 0	39.3 3	41.4 4	39.0 1	33.8 1	27.5 2	21.3 2	15.8 6	11.41	7.99	5.46	3.66	2.40	1.56	0.99	0.63	0.39	0.24
2	1.05	8.54	21.9 8	35.3 0	43.7 9	46.1 4	43.4 4	37.6 6	30.6 5	23.7 4	17.6 6	12.7 1	8.90	6.08	4.07	2.68	1.73	1.11	0.70	0.44	0.27
3	1.16	9.46	24.3 2	39.0 6	48.4 6	51.0 6	48.0 7	41.6 7	33.9 2	26.2 7	19.5 4	14.0 6	9.84	6.73	4.51	2.96	1.92	1.23	0.77	0.48	0.30
4	1.28	10.4 0	26.7 4	42.9 5	53.2 8	56.1 4	52.8 5	45.8 1	37.2 9	28.8 8	21.4 8	15.4 6	10.8 2	7.40	4.95	3.26	2.11	1.35	0.85	0.53	0.33
5	1.40	11.35	29.2 0	46.9 0	58.1 8	61.3 0	57.7 1	50.0 3	40.7 2	31.5 3	23.4 6	16.8 8	11.82	8.08	5.41	3.56	2.30	1.47	0.93	0.58	0.36
6	1.51	12.3 1	31.6 7	50.8 6	63.1 0	66.4 9	62.5 9	54.2 6	44.1 6	34.2 0	25.4 5	18.3 1	12.8 2	8.76	5.87	3.86	2.50	1.60	1.01	0.63	0.39
7	1.63	13.2	34.1	54.7	67.9	71.6	67.4	58.4	47.5	36.8	27.4	19.7	13.8	9.44	6.32	4.16	2.69	1.72	1.08	0.68	0.42

		6	2	9	8	3	3	5	7	5	1	3	1								
8	1.75	14.1 9	36.5 1	58.6 4	72.7 4	76.6 5	72.1 6	62.5 5	50.9 1	39.4 3	29.3 3	21.11	14.7 8	10.1 0	6.76	4.45	2.88	1.84	1.16	0.72	0.45
9	1.86	15.0 9	38.8 2	62.3 4	77.3 4	81.4 9	76.7 2	66.5 0	54.1 3	41.9 2	31.1 9	22.4 5	15.7 1	10.7 4	7.19	4.73	3.06	1.96	1.23	0.77	0.48
10	1.96	15.9 4	41.0 2	65.8 7	81.7 2	86.11	81.0 6	70.2 7	57.1 9	44.3 0	32.9 5	23.7 2	16.6 0	11.35	7.60	5.00	3.24	2.07	1.30	0.81	0.50
11	2.06	16.7 5	43.0 9	69.2 0	85.8 4	90.4 5	85.1 5	73.8 1	60.0 8	46.5 3	34.6 2	24.9 1	17.4 4	11.92	7.98	5.25	3.40	2.17	1.37	0.85	0.53
12	2.15	17.5 0	45.0 1	72.2 9	89.6 8	94.4 9	88.9 5	77.11	62.7 6	48.6 1	36.1 6	26.0 3	18.2 2	12.4 5	8.34	5.48	3.55	2.27	1.43	0.89	0.55
13	2.24	18.1 9	46.7 8	75.1 3	93.2 1	98.2 1	92.4 6	80.1 5	65.2 3	50.5 2	37.5 9	27.0 5	18.9 3	12.9 4	8.66	5.70	3.69	2.36	1.49	0.93	0.57
14	2.31	18.8 1	48.4 0	77.7 3	96.4 3	101. 60	95.6 5	82.9 1	67.4 9	52.2 7	38.8 9	27.9 9	19.5 9	13.3 9	8.96	5.90	3.82	2.44	1.54	0.96	0.59
15	2.38	19.3 8	49.8 6	80.0 7	99.3 4	104. 67	98.5 3	85.4 2	69.5 2	53.8 4	40.0 6	28.8 3	20.1 8	13.7 9	9.23	6.07	3.93	2.51	1.58	0.99	0.61
K	2.87	23.3 0	59.9 4	96.2 6	119.4 2	125. 83	118.4 5	102. 68	83.5 8	64.7 3	48.1 6	34.6 6	24.2 6	16.5 8	11.10	7.30	4.73	3.02	1.91	1.19	0.73

7	67.3 3	5.17	14.7 2	48.7 7	55.3 1	66.3 1	65.3 9	52.9 5	32.8 1	26.4 5	11.1 7	8.66	8.34	6.41	2.92	1.00	0.00	0.00	0.00	0.00	0.00	0.00
8	72.0 9	5.99	18.1 0	54.5 2	70.0 0	72.4 5	73.7 1	58.9 9	40.4 4	31.5 6	14.2 0	10.1 1	10.0 4	7.92	3.95	2.27	1.09	0.00	0.00	0.00	0.00	0.00
9	75.8 7	7.02	19.9 8	58.1 7	72.7 2	78.7 1	77.3 2	61.2 9	52.3 3	40.4 8	16.2 9	13.4 1	12.3 4	9.26	5.55	4.43	2.43	0.00	0.00	0.00	0.00	0.00
10	80.2 2	7.77	20.8 9	60.6 3	75.0 0	83.4 9	82.3 1	62.9 1	61.9 8	49.3 2	18.2 9	16.2 1	14.1 2	10.8 2	7.33	5.89	3.24	1.12	0.00	0.00	0.00	0.00
11	84.9 0	8.59	21.7 3	62.4 9	80.4 3	85.7 6	86.4 9	65.5 5	65.4 1	52.2 6	19.2 1	17.1 0	15.7 4	12.3 6	8.00	9.32	3.47	2.34	0.00	0.00	0.00	0.00
12	87.3 1	9.00	22.6 2	64.3 1	82.0 0	86.0 0	89.0 0	79.0 0	68.0 0	54.0 0	19.9 0	17.2 1	16.7 0	14.0 9	9.27	9.88	3.82	3.11	1.04	1.10	1.12	0.00
13	90.6 5	9.53	23.5 1	65.1 0	84.0 0	86.0 0	90.0 0	81.0 0	69.0 0	53.0 0	20.7 2	18.1 0	17.5 1	14.2 8	10.1 1	9.94	4.18	4.38	2.82	1.73	1.54	1.05
14	91.3 3	10.4 4	24.3 8	66.0 7	85.0 0	87.0 0	90.0 0	81.0 0	70.0 0	55.0 0	21.1 1	19.0 4	18.1 1	14.4 3	10.5 9	9.95	5.22	4.89	4.45	3.76	1.84	1.19
15	92.8 1	11.4 6	25.2 5	68.1 1	85.0 0	88.0 0	90.0 0	82.0 0	71.0 0	55.0 0	21.3 9	20.0 0	18.3 9	14.1 2	10.7 4	10.0 1	5.36	5.19	4.62	4.00	1.84	1.38

Table 3: Seminal root length (SRL) at each PEG concentration measured by ruler and Image J software from 0% to 21% PEG stress with the highest SRL at 6 % PEG stress on the 15th day of treatment.

UNDER PEER REVIEW

Fig 2 is the graphical representation of observed SRL at 2% PEG stress and the trend estimated through a mathematical equation which shows overestimation at initial days and underestimation in the later days. The root length was suppressed at 1 and 2% PEG stress, then it consistently increased and was highest at 6%. It again started decreasing gradually and root length was highly suppressed at >10% PEG concentration. When the osmotic potential is lowered from normal conditions, drought-resistance in plants is expressed by maintaining their turgor pressure and expansion of their roots (Kim et al., 2020; Susilawati et al., 2022). Elongation of root length under lower osmotic potential is thus explained.

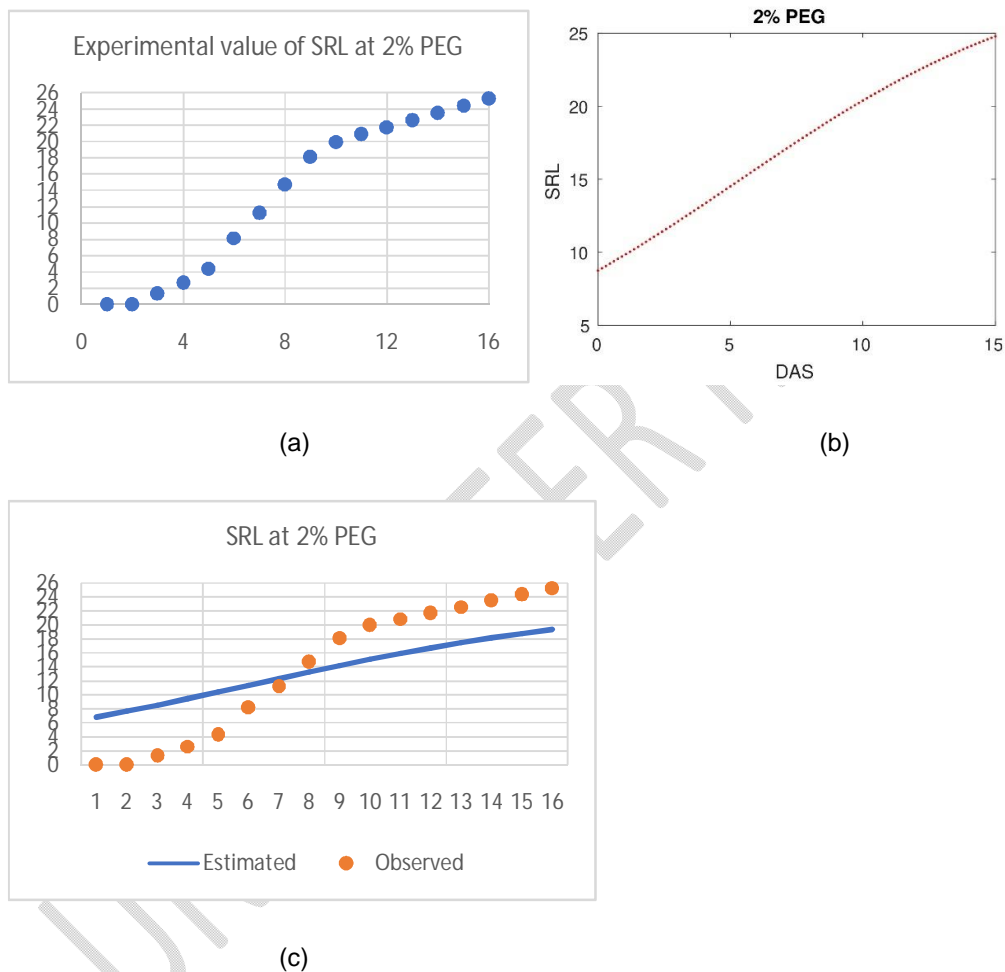
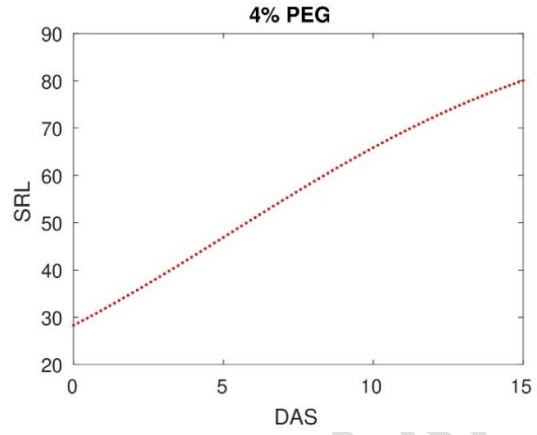
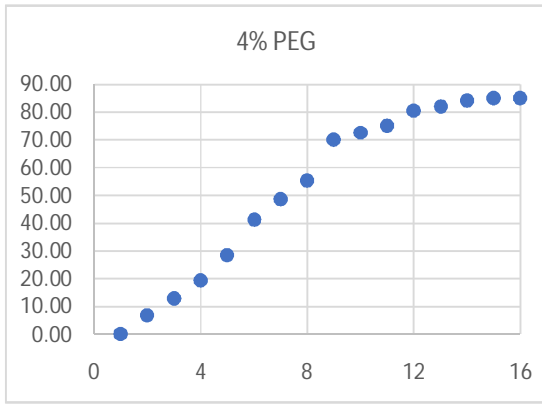
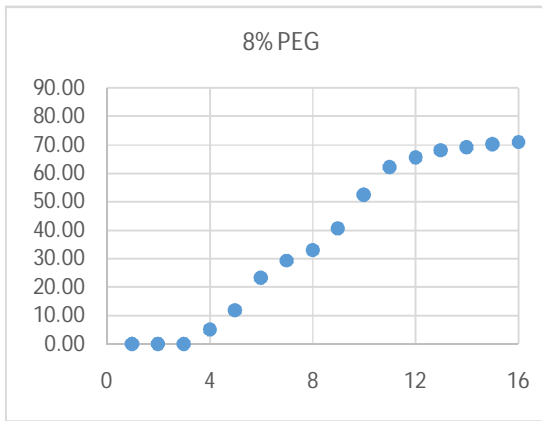


Fig 2: Increase in root length from 0-15 DAS at 2% PEG concentration (a) experimental values (b) estimated values in MATLAB version 2019 (c) superimposition of both experimental and estimated values

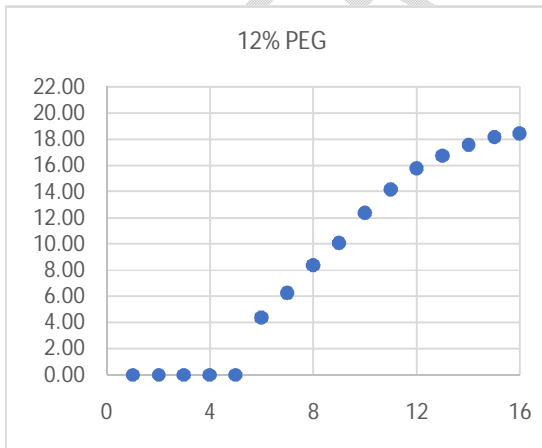
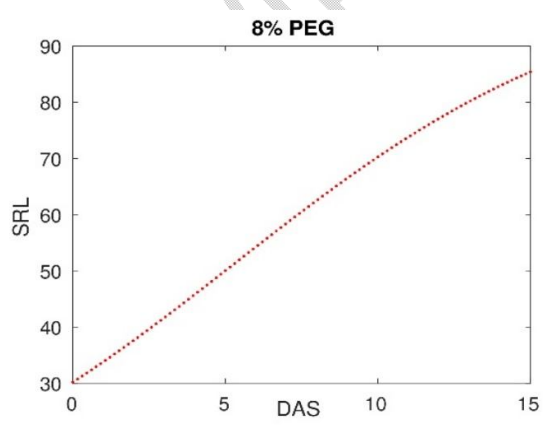
The SRL values of both experimental and equational data at 4%, 8%, 12%, 15% and 20% of PEG concentration are shown in Fig 3. The trend was almost similar in both the data sets up to 10% stress. In stress levels >10% PEG concentration, the predicted value showed an overestimated SRL at the initial days i.e., from the 4th day to 12th day.



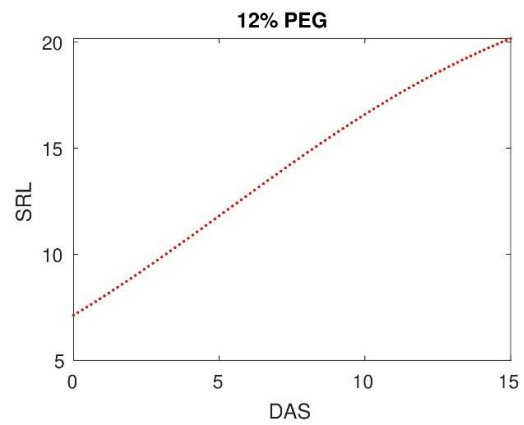
(a)

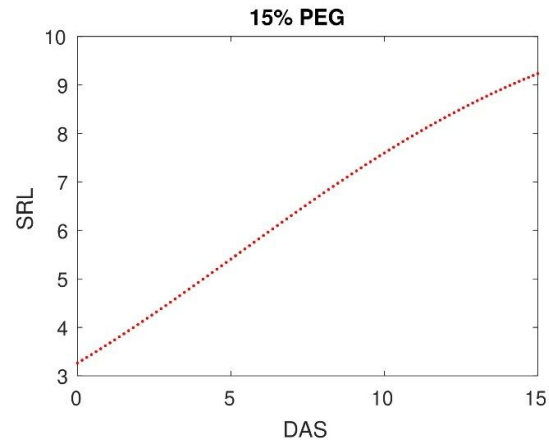
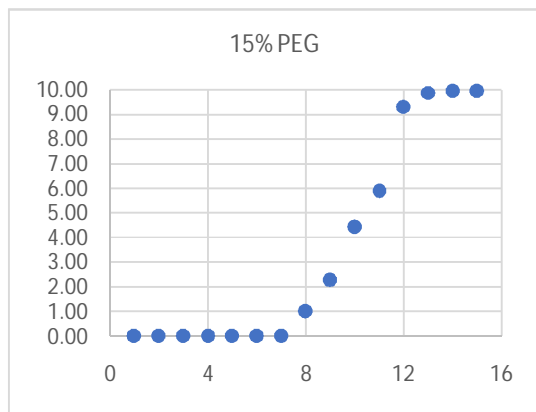


(b)

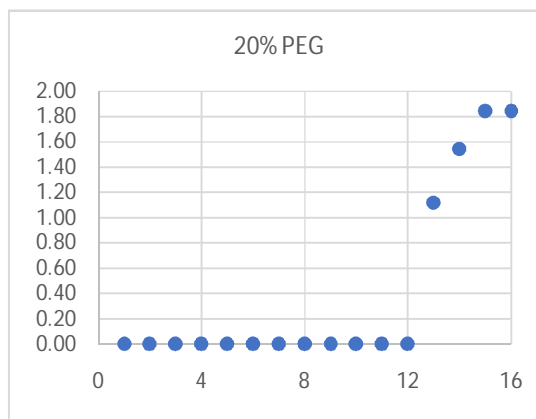


(c)





(d)



(e)

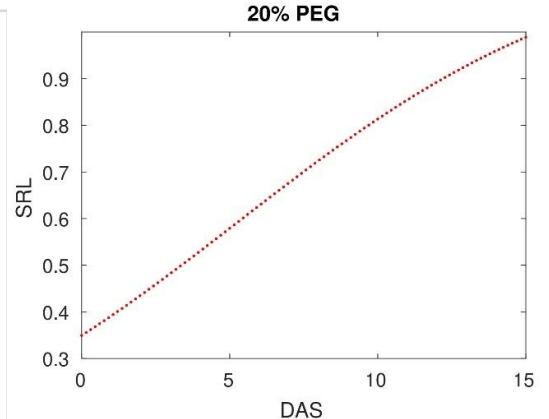


Fig 3: a- 4%, b- 8%, c- 12%, d- 15%, e- 20% PEG stress. Estimation of SRL up to 8% PEG stress was quite similar to the actual data, whereas at >10% PEG stress there was an overestimation on the initial days.

Previously Susilawati et al., mathematically deduced that the highest full length (K) was at 5.9% PEG concentration having an osmotic potential of -0.4 bar, and the predicted K data showed similarity with experimental root length at 30 DAS with a root mean square error (RMSE) of 13.7 mm. In our study, the full root length when measured at 25 DAS was highly akin to the predicted K value which was highest at 6% PEG concentration equivalent to 0.6 bar with a root mean square error of 14.68 mm. Low RMSE suggested that the image analysis protocol was quite accurate and can be adapted in other studies.

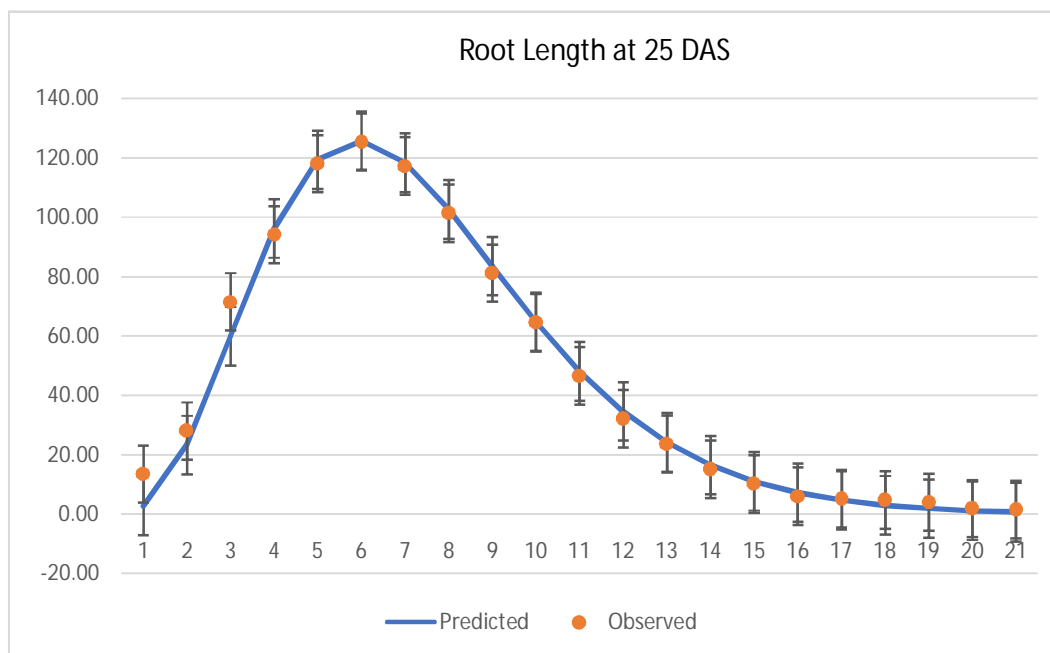


Fig 4: The equational values of K i.e., the full length (line) was plotted with SRL at 25 DAS (symbols) at each stress level where the similarity between observed and expected data was found.

The analogy between the present study in Kalpana, a landrace grown in the eastern part of India and the experiment by Susilawati et al., 2022 in Nipponbarea japonica varieties shows that this equation is very much adaptable in simulation of root growth of rice subspecies in different water stress situation.

4. CONCLUSION:

This study shows that under field conditions where mild to severe drought is prevalent this equation can be used to simulate the root length to get an estimation of the root elongation and yield under fluctuating water potential. The successful evaluation of rice root growth under drought stress based on both contact and non-contact image analysis makes it a suitable method for estimation in such other studies. This equation can fit well with both indica and japonica rice varieties, so it can be used in other subspecies of rice or might fit with other crop plants like wheat, millet etc. This is a new modified experimental design to simulate root length under varied water stress situations as well as in heat, salinity, submergence, or cold stress.

DATA AVAILABILITY STATEMENT:

All data generated or analysed during this study are included in this published article.

REFERENCES:

1. R Samal, P S Roy, A Sahoo, M K Kar, B C Patra, B C Marndi, and J N R Gundimeda. Morphological and molecular dissection of wild rices from eastern India suggests distinct

- speciation between *O. rufipogon* and *O. nivara* populations. *Scientific reports*, 8(1), 1-13. (2018)
2. De A, Dey A, Raha S and Kar D K. Drought response in rice - A brief review. (2022) *Journal of Scientific Enquiry*. <https://doi.org/10.54280/jse.222215>
 3. H Turrall, J J Burke and J M Faurès. *Climate Change, Water and Food Security*. Food Water Reports. Food and Agriculture Organization of the United Nations. (2011)
 4. V Pandey and A Shukla. Acclimation and tolerance strategies of rice under drought stress. *Rice Sci*, 22(4): 147–161. (2015)
 5. Sharp, R. E. et al. Root growth maintenance during water deficits: Physiology to functional genomics. *J. Exp. Bot.* 55(407), 2343– 2351 (2004).
 6. Mishra A, Salokhe V M. 2011. Rice root growth and physiological responses to SRI water management and implications for crop productivity. *Paddy and Water Environment*, 9, 41-52.
 7. YANG, J. C., Zhang, H., & ZHANG, J. H. (2012). Root morphology and physiology in relation to the yield formation of rice. *Journal of Integrative Agriculture*, 11(6), 920-926.
 8. Jnandabhiram, Chutia & Sailen Prasad Borah. (2012). Water stress effects on leaf growth and chlorophyll content but not the grain yield in traditional rice (*Oryza sativa* Linn.) genotypes of Assam, India II. Protein and proline status in seedlings under PEG induced water stress. *American Journal of Plant Sciences*, 2012.
 9. Hannan A., Hassan L., Hoque M.N., Tahjib-Ul-Arif M., Robin A.H.K. Increasing new root length reflects survival mechanism of rice (*Oryza sativa* L.) genotypes under PEG-induced osmotic stress. *Plant Breed. Biotech.* 2020;8:46–57. doi: 10.9787/PBB.2020.8.1.46.
 10. Nupur J.A., Hannan A., Islam M.A.U., Sagor G.H.M., Robin A.H.K. Root development and anti-oxidative response of rice genotypes under polyethylene glycol induced osmotic stress. *Plant Breed. Biotech.* 2020;8:151–162. doi: 10.9787/PBB.2020.8.2.151.
 11. Ayalew H., Ma X., Yan G. Screening wheat (*Triticum* spp.) genotypes for root length under contrasting water regimes: Potential sources of variability for drought resistance breeding. *J. Agron. Crop Sci.* 2015;201:189–194. doi: 10.1111/jac.12116.
 12. Robin, A. H. K., Ghosh, S., & Shahed, M. A. (2021). PEG-induced osmotic stress alters root morphology and root hair traits in wheat genotypes. *Plants*, 10(6), 1042.
 13. Comeau A., Nodichao L., Collin J., Baum M., Samsatly J., Hamidou D., Picard E. New approaches for the study of osmotic stress induced by polyethylene glycol (PEG) in cereal species. *Cereal Res. Commun.* 2010;38:471–481. doi: 10.1556/CRC.38.2010.4.3.
 14. Halimursyadah, H., Hereri, A. I., & Hafnizar, A. (2013). PENGGUNAAN POLYETHYLENE GLYCOLE SEBAGAI MEDIA SIMULASI CEKAMAN KEKERINGAN TERHADAP VIABILITAS DAN VIGOR BEBERAPA VARIETAS BENIH KACANG TANAH (*Arachis hypogaea* L.) PADA STADIA PERKECAMBAHAN. *Journal Floratek*, 8(2), 73-79.
 15. Diana, Susanti, FirnawatiSakalena, Dora Fatma Nurshanti, and Supli Effendi Rahim. "Drought tolerance estimation of several rainfed rice (*Oryza sativa* L.) Of lokalverietiy using polyethylene glycol (PEG)." In *MATEC Web of Conferences*, vol. 97, p. 01115. EDP Sciences, 2017.

16. Susilawati, P. N., Tajima, R., Giamerti, Y., Yang, Y., Yufdy, M. P., Lubis, I., & Homma, K. (2022). Application of consecutive polyethylene glycol treatments for modeling the seminal root growth of rice under water stress. *Scientific reports*, 12(1), 2096.
17. Hirooka, Y., Homma, K. & Shiraiwa, T. A leaf area-based non-destructive approach to predict rice productivity. *Agron. J.* 113(5), 3922–3934 (2021).
18. Michel, B. E., & Kaufmann, M. R. (1973). The osmotic potential of polyethylene glycol 6000. *Plant physiology*, 51(5), 914-916.
19. Kim, Y., Chung, Y. S., Lee, E., Tripathi, P., Heo, S., & Kim, K. H. (2020). Root response to drought stress in rice (*Oryza sativa* L.). *International journal of molecular sciences*, 21(4), 1513.
20. D Panda, S S Mishra and P K Behera. Drought tolerance in rice: focus on recent mechanisms and approaches. *Rice Science*, 28(2), 119-132. (2021)

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