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Modeling Root Development of Rice (*Oryza sativa* L) under Varying Drought Stress

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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

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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 [1,2]. 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 [3,4]. Root plays an important role in plant growth and production under water stress conditions. Several studies reveal that under water scarcity shoot growth is inhibited whereas root growth is promoted to increase the supply of water and other nutrients [5]. Root extension under such situations helps in analyzing drought resistance of crops and helps in predicting the crop yield [6,7]. 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 (Ψs) and blockage of absorption of water by the root system [8]. Under PEG-induced osmotic stress, effects on root morphology and root hair characteristics were observed in rice [9,10] wheat [11,12] and other cereals [13] 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, [14] suggested that 18.1% PEG concentration could be used as screening drought-tolerant lines, whereas Diana et al., [15] imposed stress up to 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 [16]. Hirooka et al., [17] 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., [16]. 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. [16] 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 AND 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, Sidho Kanho Birsa University, Purulia, W.B. India, in the year of 2022 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 [18].

$$'\psi = -(1.18 \times 10^{-2})\, C - (1.18 \times 10^{-4})\, C^2 + (2.67 \times 10^{-4})\, CT + (8.39 \times 10^{-7})\, C^2T \quad [1]$$

Where C is the concentration of PEG-6000 in g/kg of H_2O and T= Temperature i.e., 25^{O}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 where a randomized design was followed. 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.

An equation mentioned below with a slight modification led by Susilawati et al., [16] was used to measure the root length mathematically.

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

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

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

Table 1. Water potential at each stress level

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

(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]

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. MATLAB version 2019 was used for simulation.

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.

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 [19,16]. Elongation of root length under lower osmotic potential is thus explained [20,9].

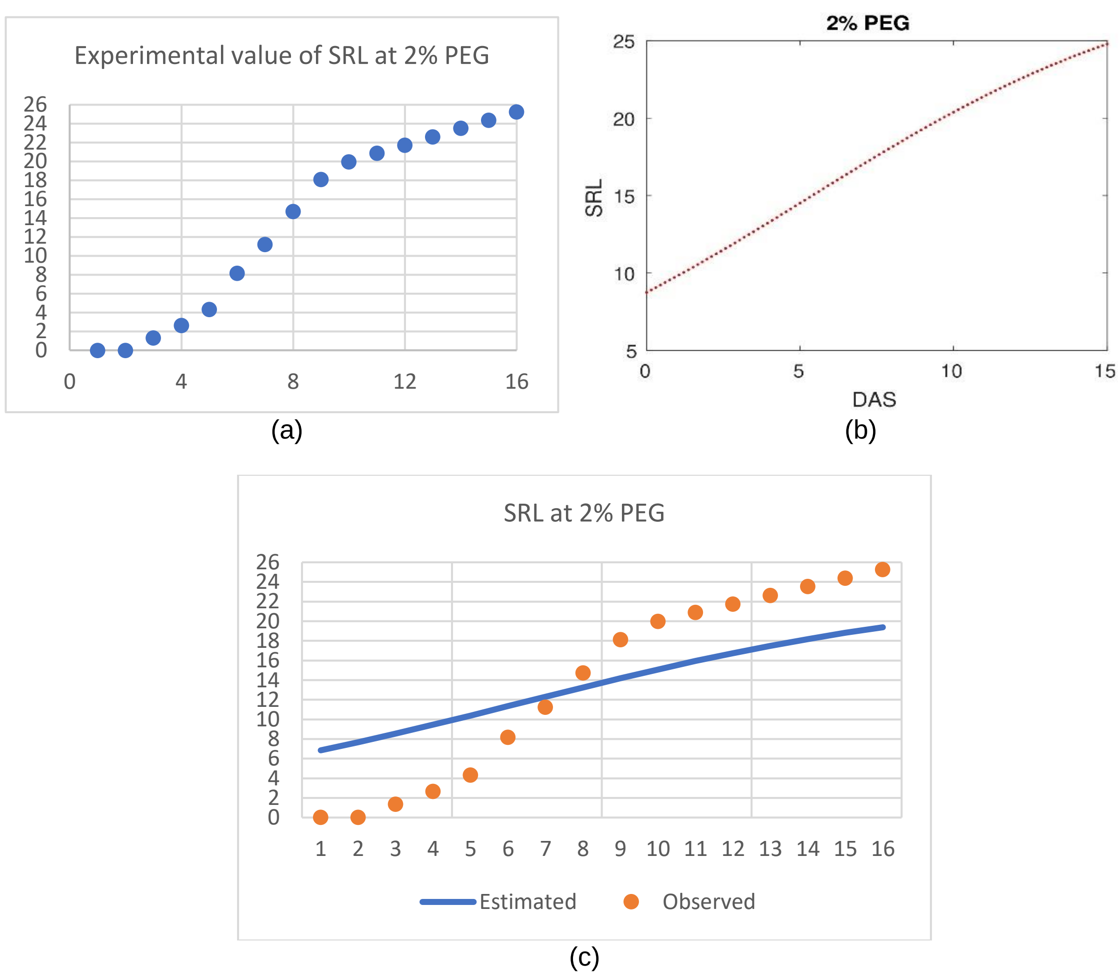


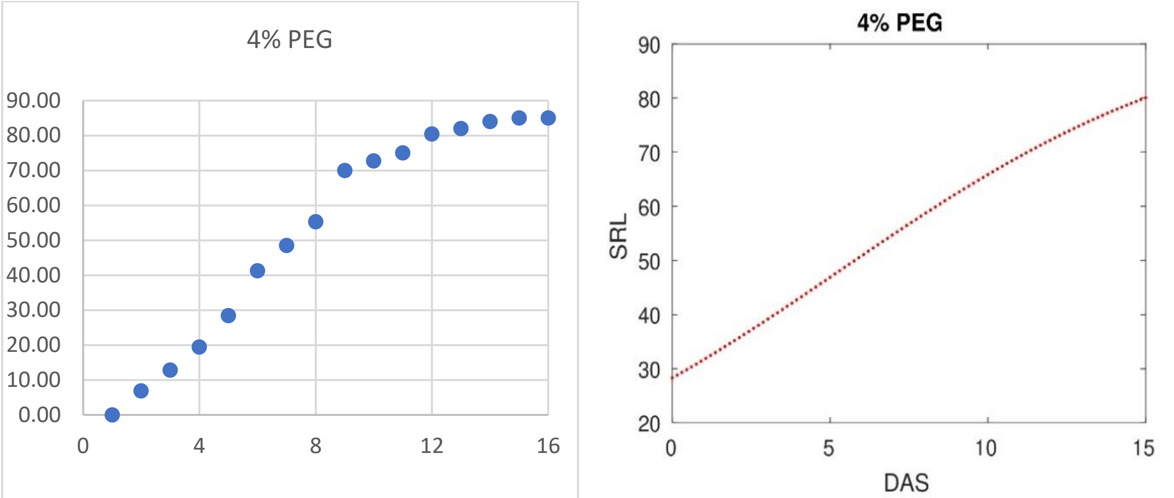
Fig. 2. Increase in root length from 0-15 DAS at 2% PEG concentration (a) experimental values (b) estimated values in MATLAB version 2019 (c) superimposition of both experimental and estimated values

Table 2. Estimated data of Seminal root length (SRL) and full length (K) using Eq. 1 and 2 from 1% to 21% PEG stress where the highest SRL is at 6% stress level

	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	17.62	28.30	35.10	36.99	34.82	30.18	24.57	19.03	14.16	10.19	7.13	4.87	3.26	2.15	1.39	0.89	0.56	0.35	0.22
1	0.94	7.67	19.74	31.70	39.33	41.44	39.01	33.81	27.52	21.32	15.86	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	21.98	35.30	43.79	46.14	43.44	37.66	30.65	23.74	17.66	12.71	8.90	6.08	4.07	2.68	1.73	1.11	0.70	0.44	0.27
3	1.16	9.46	24.32	39.06	48.46	51.06	48.07	41.67	33.92	26.27	19.54	14.06	9.84	6.73	4.51	2.96	1.92	1.23	0.77	0.48	0.30
4	1.28	10.40	26.74	42.95	53.28	56.14	52.85	45.81	37.29	28.88	21.48	15.46	10.82	7.40	4.95	3.26	2.11	1.35	0.85	0.53	0.33
5	1.40	11.35	29.20	46.90	58.18	61.30	57.71	50.03	40.72	31.53	23.46	16.88	11.82	8.08	5.41	3.56	2.30	1.47	0.93	0.58	0.36
6	1.51	12.31	31.67	50.86	63.10	66.49	62.59	54.26	44.16	34.20	25.45	18.31	12.82	8.76	5.87	3.86	2.50	1.60	1.01	0.63	0.39
7	1.63	13.26	34.12	54.79	67.98	71.63	67.43	58.45	47.57	36.85	27.41	19.73	13.81	9.44	6.32	4.16	2.69	1.72	1.08	0.68	0.42
8	1.75	14.19	36.51	58.64	72.74	76.65	72.16	62.55	50.91	39.43	29.33	21.11	14.78	10.10	6.76	4.45	2.88	1.84	1.16	0.72	0.45
9	1.86	15.09	38.82	62.34	77.34	81.49	76.72	66.50	54.13	41.92	31.19	22.45	15.71	10.74	7.19	4.73	3.06	1.96	1.23	0.77	0.48
10	1.96	15.94	41.02	65.87	81.72	86.11	81.06	70.27	57.19	44.30	32.95	23.72	16.60	11.35	7.60	5.00	3.24	2.07	1.30	0.81	0.50
11	2.06	16.75	43.09	69.20	85.84	90.45	85.15	73.81	60.08	46.53	34.62	24.91	17.44	11.92	7.98	5.25	3.40	2.17	1.37	0.85	0.53
12	2.15	17.50	45.01	72.29	89.68	94.49	88.95	77.11	62.76	48.61	36.16	26.03	18.22	12.45	8.34	5.48	3.55	2.27	1.43	0.89	0.55
13	2.24	18.19	46.78	75.13	93.21	98.21	92.46	80.15	65.23	50.52	37.59	27.05	18.93	12.94	8.66	5.70	3.69	2.36	1.49	0.93	0.57
14	2.31	18.81	48.40	77.73	96.43	101.60	95.65	82.91	67.49	52.27	38.89	27.99	19.59	13.39	8.96	5.90	3.82	2.44	1.54	0.96	0.59
15	2.38	19.38	49.86	80.07	99.34	104.67	98.53	85.42	69.52	53.84	40.06	28.83	20.18	13.79	9.23	6.07	3.93	2.51	1.58	0.99	0.61
K	2.87	23.30	59.94	96.26	119.42	125.83	118.45	102.68	83.58	64.73	48.16	34.66	24.26	16.58	11.10	7.30	4.73	3.02	1.91	1.19	0.73

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

	Seminal Root Length [mm]																					
PEG → / DAS ↓	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	2.38	0.00	0.00	0.00	6.82	6.23	5.77	3.43	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	8.19	1.11	1.33	5.19	12.84	14.28	15.02	11.55	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	14.44	2.07	2.64	11.14	19.40	24.91	25.66	19.23	4.98	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	24.34	3.39	4.32	23.55	28.41	33.33	37.29	29.42	11.66	8.61	4.18	4.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	40.42	3.61	8.16	32.49	41.22	47.43	47.20	41.89	23.08	15.29	6.44	5.18	4.33	3.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	54.01	4.23	11.22	41.51	48.49	56.50	58.23	46.10	29.38	21.07	9.38	6.47	6.21	4.37	2.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	67.33	5.17	14.72	48.77	55.31	66.31	65.39	52.95	32.81	26.45	11.17	8.66	8.34	6.41	2.92	1.00	0.00	0.00	0.00	0.00	0.00	0.00
8	72.09	5.99	18.10	54.52	70.00	72.45	73.71	58.99	40.44	31.56	14.20	10.11	10.04	7.92	3.95	2.27	1.09	0.00	0.00	0.00	0.00	0.00
9	75.87	7.02	19.98	58.17	72.72	78.71	77.32	61.29	52.33	40.48	16.29	13.41	12.34	9.26	5.55	4.43	2.43	0.00	0.00	0.00	0.00	0.00
10	80.22	7.77	20.89	60.63	75.00	83.49	82.31	62.91	61.98	49.32	18.29	16.21	14.12	10.82	7.33	5.89	3.24	1.12	0.00	0.00	0.00	0.00
11	84.90	8.59	21.73	62.49	80.43	85.76	86.49	65.55	65.41	52.26	19.21	17.10	15.74	12.36	8.00	9.32	3.47	2.34	0.00	0.00	0.00	0.00
12	87.31	9.00	22.62	64.31	82.00	86.00	89.00	79.00	68.00	54.00	19.90	17.21	16.70	14.09	9.27	9.88	3.82	3.11	1.04	1.10	1.12	0.00
13	90.65	9.53	23.51	65.10	84.00	86.00	90.00	81.00	69.00	53.00	20.72	18.10	17.51	14.28	10.11	9.94	4.18	4.38	2.82	1.73	1.54	1.05
14	91.33	10.44	24.38	66.07	85.00	87.00	90.00	81.00	70.00	55.00	21.11	19.04	18.11	14.43	10.59	9.95	5.22	4.89	4.45	3.76	1.84	1.19
15	92.81	11.46	25.25	68.11	85.00	88.00	90.00	82.00	71.00	55.00	21.39	20.00	18.39	14.12	10.74	10.01	5.36	5.19	4.62	4.00	1.84	1.38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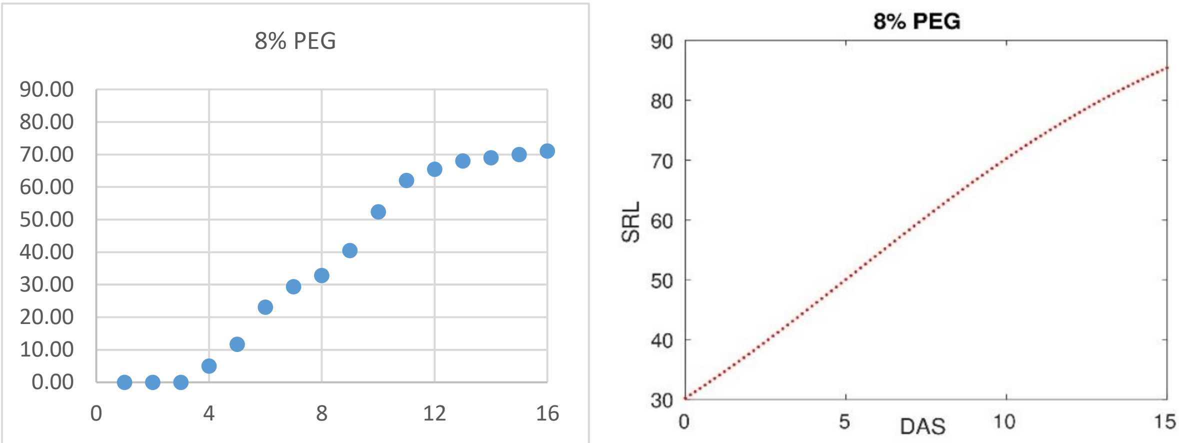
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0
4
8
12
16
4% PEG
SRL
DAS
90
80
70
60
50
40
30
20
0
5
10
15

(a)


8% PEG
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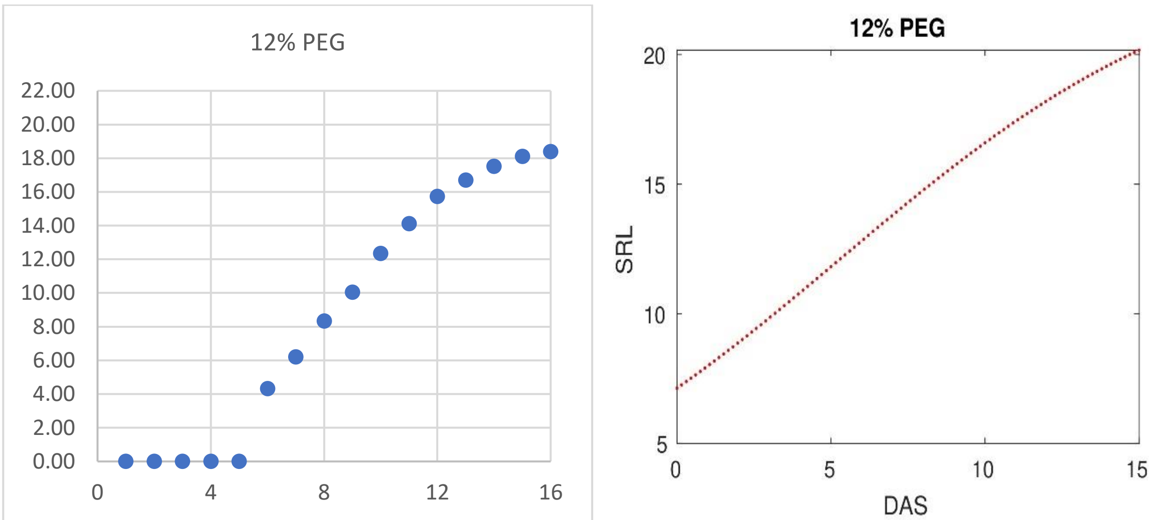
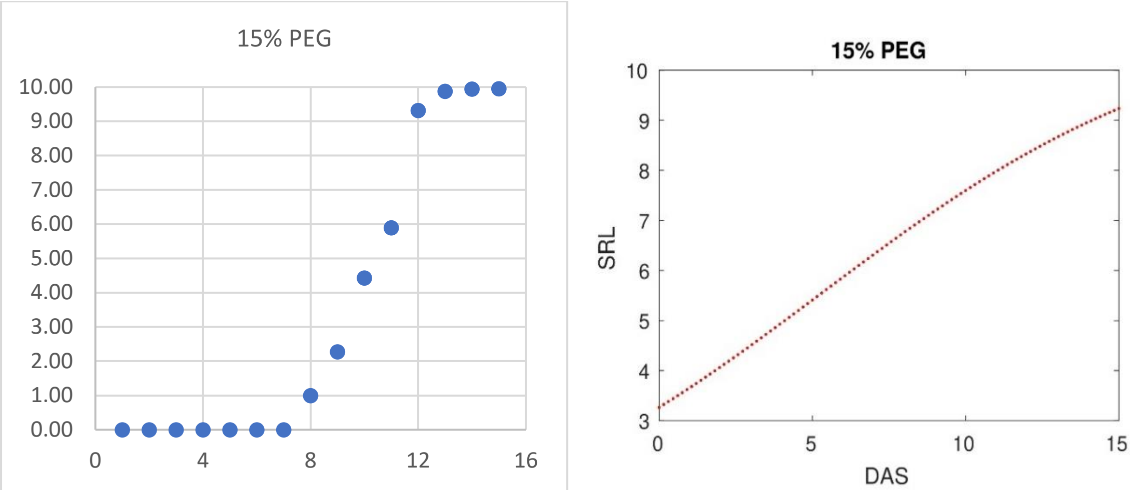
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
0
4
8
12
16
8% PEG
SRL
DAS
90
80
70
60
50
40
30
0
5
10
15

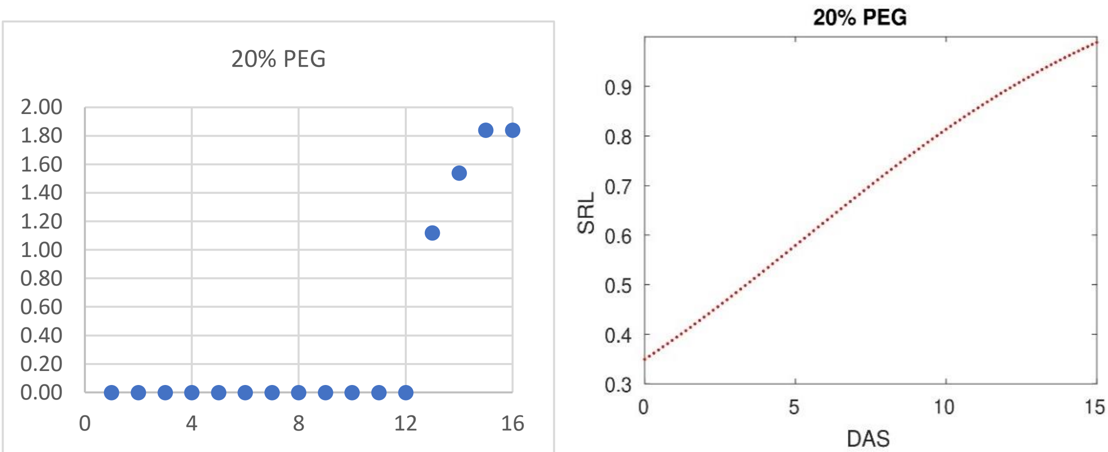
(b)


12% PEG
22.00
20.00
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
0
4
8
12
16
12% PEG
SRL
DAS
20
15
10
5
0
5
10
15

(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

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 4^{th} day to 12^{th} day.

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 could be adapted in other studies.

The analogy between the present study in Kalpana, a landrace grown in the eastern part of India and the experiment by Susilawati et al., [16] in Nipponbare a japonica variety shows that this equation is very much adaptable in simulation of root growth of rice subspecies in different water stress situation [21].

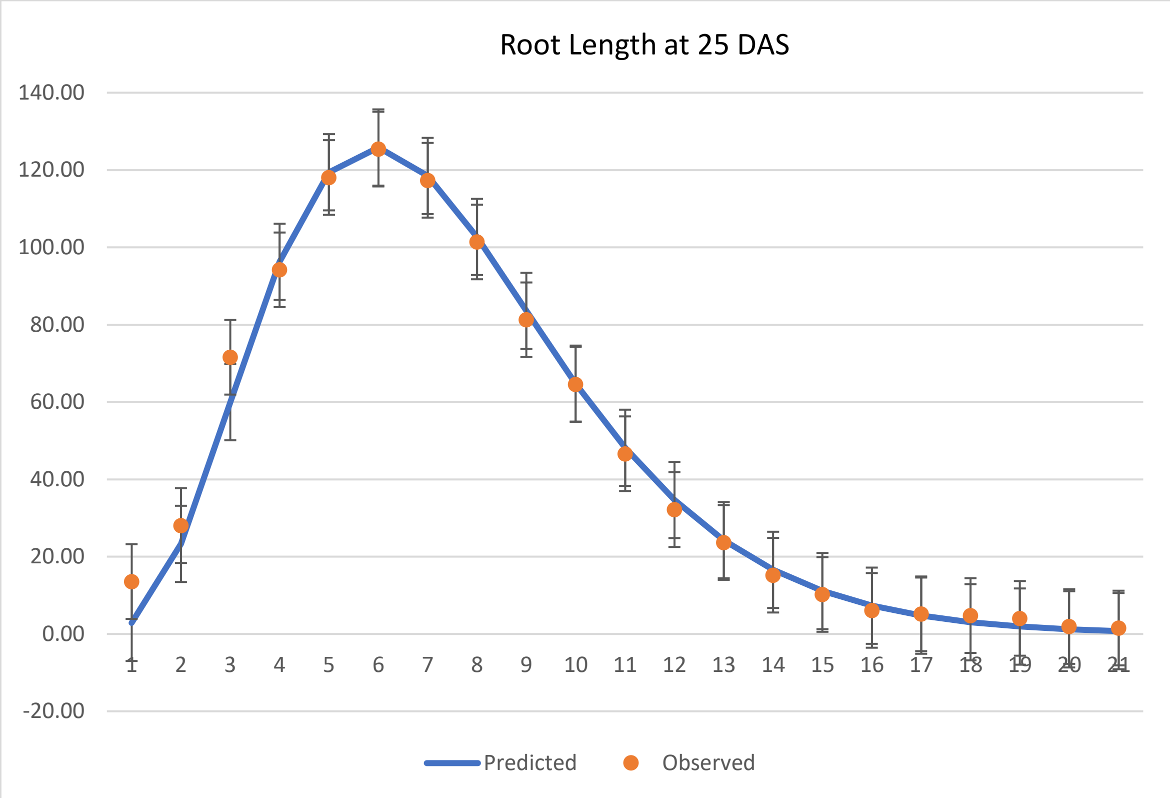


Fig. 4. The equational values of K i.e., the full length (line) was plotted with SRL at 25 DAS (symbols) at each stress level where the similarity between observed and expected data was found

4. CONCLUSION

This study demonstrates that under field conditions with varying degrees of drought, the proposed equation effectively simulates root length, offering reliable estimates of root elongation and yield under fluctuating water potential. The successful evaluation of rice root growth under drought stress, utilizing both contact and non-contact image analysis, highlights the method's robustness for similar applications in future studies. Given that the equation works well for both indica and japonica rice varieties, it may also be applicable to other rice subspecies or crop plants such as wheat and millet. Furthermore, this experimental design, modified to simulate root length under different water stress conditions, can be adapted to study responses to heat, salinity, submergence, or cold stress.

DATA AVAILABILITY STATEMENT

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

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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