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Research Article

Screening Nepalese Rice (*Oryza sativa* L.) Landraces for Drought Tolerance Using Polyethylene Glycol (PEG 6000)-Induced Osmotic Stress at Germination and Early Seedling Stages

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ABSTRACT

Osmotic stress during germination and early seedling development is a major constraint to rice establishment. This study evaluated genotypic variation in 154 rice genotypes under polyethylene glycol (PEG 6000)-induced osmotic stress to identify promising sources of tolerance. Genotypes were assessed under control, 15%, and 20% PEG concentrations for germination, seedling growth, biomass-related traits, and relative water content. Factorial analysis of variance revealed highly significant ($P < 0.001$) effects of genotype, PEG concentration, and their interaction across all 14 traits, demonstrating substantial genetic variability and genotype-specific stress responses. Increasing PEG concentration to 20% reduced germination percentage from 75.55% to 44.10%, germination index from 5.57 to 2.38, shoot length at 14 days from 5.50 to 1.56 cm, and relative water content from 41.03% to 17.59%, while mean germination time increased from 3.72 to 5.24 days. Root-shoot ratios increased markedly under severe stress, reflecting differential allocation to root and shoot growth. An eight-trait standardized composite screening identified TOLI ANDAHI as the most tolerant genotype, with the highest stress tolerance index (STI = 1.712), followed by NGRC02872 (1.456), NGRC01815 (1.423), ANADI (1.320), and NGRC02865 (1.296). Principal component analysis showed that the first two components explained 68.48% of total variation, distinguishing genotypes according to germination and seedling-growth responses. Descriptive characterization of contrasting genotype groups further confirmed superior germination and root development among tolerant entries compared with sensitive genotypes, including MARSHI TANGALO and HIMALI. These findings identify promising rice genetic resources for further drought-tolerance evaluation and breeding to improve establishment under water-limited conditions.

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Introduction

Rice (*Oryza sativa* L.) is the principal staple food crop in South and Southeast Asia, including Nepal, supporting both food security and livelihoods of millions of people. A large proportion of Nepal's rice area is rainfed, and recurring dry spells at seed sowing, transplanting, and early growth are a major constraint on yield (Tripathi et al., 2018; Karanjit et al., 2024). As rainfall patterns

become increasingly unpredictable under climate change, the development of drought-resilient rice varieties has become an urgent breeding priority. Besides drought, several biotic stresses occur, including diseases caused by fungal, bacterial, and viral pathogens and damage by insect pests. The interaction between drought stress and insect-pest damage is complex, as water deficit can alter host plant susceptibility and pest population dynamics. Drought stress not only reduces yield directly but also increases rice plants' susceptibility to damage from insect pests and diseases, further worsening crop losses in rainfed production systems (Kandel et al., 2022). Water-deficit stress can alter plant biochemistry and physiology, weaken structural defenses, and modify canopy microclimate, which favor certain pest and disease outbreaks. Drought stress in rice is frequently associated with increased pathogen infection and pest multiplication of several economically important diseases (Kandel et al., 2022), including rice blast (Bidzinski et al., 2016), bacterial leaf blight (Vemanna et al., 2019, Batool et al., 2026), and brown spot (Barnwal et al., 2013), as well as insect pests such as nematodes (Atkinson and Urwin, 2012), brown plant hopper (Liao et al., 2023), and stem borers (Shui et al., 2026). Therefore, identifying rice landraces with combined tolerance to drought and resistance/tolerance to major biotic constraints is a priority for integrated crop protection in rainfed rice ecosystems. Consequently, breeding for drought tolerance is an integral component of sustainable plant protection strategies in rice-based cropping systems.

Traditional rice landraces, cultivated by farmers over many generations under diverse and often marginal environments, constitute a valuable but underexploited reservoir of genetic variability for stress tolerance (Kandel and Shrestha, 2018). Because these landraces have persisted under recurring water-deficit conditions without formal selection, they are expected to harbor useful alleles for drought adaptation that are not always present in modern, high-yielding cultivars. Nevertheless, the ability of a landrace to persist in a particular production environment does not necessarily demonstrate physiological drought tolerance. Therefore, systematic evaluation under controlled stress conditions is required to characterize differences among landraces and identify promising genetic resources for subsequent breeding research. Systematic screening of large landrace collections is therefore an important first step for identifying donors that plant breeders can use in drought-tolerance improvement programs, which in turn contribute to integrated pest and disease management in water-limited environments.

Because full-cycle, field-based drought screening of large germplasm collections is slow, land-intensive, and difficult to standardize across seasons, laboratory-based screening at the germination and early seedling stage is widely used as a rapid proxy. Polyethylene glycol with a molecular weight of 6000 (PEG 6000) is the most extensively used osmoticum for this purpose because, unlike ionic osmolytes such as NaCl or mannitol, it is a large, largely non-penetrating polymer that lowers the water potential of a solution without inducing significant ion toxicity, allowing researchers to impose a controlled, reproducible level of water stress on germinating seeds (Michel and Kaufmann, 1973). However, PEG-induced osmotic stress represents a laboratory simulation of reduced water availability and does not fully reproduce the physical, chemical, and biological conditions of drought in soil. Therefore, responses observed under PEG treatments should be interpreted as preliminary indicators of osmotic stress responses rather than direct evidence of field-level drought tolerance. Numerous recent studies have used PEG 6000 solutions of varying concentrations to differentiate rice genotypes for germination percentage, germination index, germination energy, seedling vigor, and biomass accumulation under simulated drought (Karanjit et al., 2024; Joseph and Makwinja, 2026). In the Nepalese context, several recent studies have demonstrated that rice landraces differ substantially in their response to simulated and field-imposed drought at various growth stages, from germination through to the reproductive stage, and that this variability can be exploited to identify tolerant donor lines (Kandel et al., 2022; Lamichhane et al., 2025; Yogi et al., 2025). However, most published PEG-screening studies in Nepal have evaluated relatively small sets of landraces or released varieties (typically 12 to 25 genotypes). Screening larger collections at the germination stage offers a more powerful basis for capturing the genetic diversity available within national germplasm holdings before advancing a manageable subset to more resource-intensive field evaluation.

Although drought adaptation is an important component of crop resilience, its relationship with plant responses to insect pests and pathogens cannot be established from germination and seedling growth measurements alone. The identification of landraces with contrasting

osmotic stress responses may, however, provide useful genetic material for future studies investigating interactions between water-deficit conditions, host physiology and susceptibility to major rice pests and diseases. Such investigations would require separate assessments involving relevant pathogens or insect pests under controlled water-availability conditions.

The present study was therefore undertaken to (i) quantify the effect of 15% and 20% PEG 6000-induced osmotic stress on germination and early seedling growth traits across a large panel of 154 rice landraces held at the Plant Breeding Laboratory of the Department of Genetics and Plant Breeding, Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal; (ii) evaluate which traits are most sensitive to increasing PEG concentration; and (iii) identify landraces maintaining comparatively high germination performance under osmotic stress as candidate genetic resources for subsequent drought-tolerance evaluation and breeding research. The findings are intended to provide preliminary information on the early-stage osmotic stress responses of Nepalese rice landraces and to support future investigations involving field-based drought evaluation and the interaction between abiotic and biotic stresses in rice production systems.

Materials and Methods

Experimental site and environmental conditions

The experiment was conducted at the Plant Breeding Laboratory, Department of Genetics and Plant Breeding, Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal. Seed germination and early seedling development were evaluated under controlled laboratory conditions using a germinator maintained at a constant temperature of 25°C throughout the experimental period. The relative humidity inside the germinator was 85%, and the photoperiod was 12 h light/12 h dark. These environmental conditions were maintained consistently across the experimental treatments.

Plant materials

A panel of 154 rice landraces was evaluated, comprising named farmer varieties and accessions held in the National Genetic Resources Centre (identified by "NGRC" accession codes). The landrace collection represented diverse traditional rice genetic resources from Nepal. The complete list of landrace names and corresponding accession identifiers is provided in Table 1. Information on seed production year, seed source, and storage conditions was included where available.

Table 1. Germination and early seedling-growth performance of all 154 rice landraces under control and PEG 6000-induced osmotic stress at 20% PEG.

Sr. No.	Genotypes	GP T0 (%)	GP T15 (%)	GP T20 (%)	GP Ret. T20 (%)	SL14 T0 (cm)	SL14 T20 (cm)	RL14 T0 (cm)	RL14 T20 (cm)	RWC T0 (%)	RWC T20 (%)
1	AACHAME	80	60	56	70.00	4.59	3.02	6.44	7.40	24.61	13.49
2	AAMPJHUTTE	84	68	60	71.43	3.45	1.79	5.01	0.73	29.49	11.18
3	AANGHA 1	40	28	24	60.00	6.53	3.27	8.75	10.52	56.83	13.36
4	AKALE PANHELO	64	60	48	75.00	5.86	2.60	6.14	4.89	49.87	10.40
5	AMUHAWA	76	72	40	52.63	5.42	1.43	4.81	4.56	47.73	10.29
6	ANADHI DHAN	80	72	66	82.50	4.59	1.61	5.48	1.12	57.68	11.79
7	ANADI	92	68	58	63.04	4.71	2.85	7.16	9.32	39.89	13.18
8	ANADI 1	76	64	74	97.37	5.84	2.54	6.09	8.11	68.77	14.26
9	ASORJOG	72	68	18	25.00	6.04	0.61	5.04	5.18	56.72	13.18
10	BAGARNE	80	36	32	40.00	4.32	0.59	5.34	2.87	69.84	10.92
11	BALAMSARI	90	72	64	71.11	5.84	2.53	6.18	4.56	41.36	17.58
12	BANKAE	76	74	66	86.84	5.74	0.74	3.14	4.62	67.49	12.40
13	BANSPATHI	82	72	54	65.85	4.55	0.70	4.95	2.68	11.99	43.99
14	BASMATI	76	44	36	47.37	4.61	0.63	7.09	8.76	13.40	30.47
15	BASMATI NOKHI	86	66	52	60.47	4.22	0.42	15.43	1.98	11.32	27.98
16	BASMATI PASCHIM	68	56	12	17.65	4.02	0.18	5.73	3.54	63.51	11.06
17	BASNADHAR KALO	54	26	20	37.04	6.32	1.08	8.64	3.99	27.84	13.22
18	BHATTE	80	84	60	75.00	5.88	1.71	5.01	1.23	39.49	12.46
19	BHATTE DHAN	62	58	40	64.52	6.73	3.52	7.07	4.20	35.75	10.07
20	BHOLA	88	68	34	38.64	4.85	0.46	8.68	2.18	62.38	18.13

21	BHULING JHAR	86	74	26	30.23	5.35	0.57	6.97	3.24	47.64	11.14
22	BRAMHAFUL	82	72	50	60.98	8.61	2.51	6.18	7.68	55.04	10.51
23	BYARNI	84	68	58	69.05	4.32	2.85	9.17	6.34	30.86	11.89
24	CHOTTE	62	50	8	12.90	4.28	1.06	3.81	3.43	42.53	11.49
25	CHOTTU	78	70	60	76.92	6.45	2.32	5.52	4.72	58.46	21.50
26	DAILE MASINO	82	74	28	34.15	5.50	0.44	4.28	3.43	30.00	11.21
27	DEVRANI	82	74	68	82.93	7.62	1.88	4.81	8.61	55.99	10.40
28	DHAUDO	70	66	56	80.00	5.41	2.24	6.77	5.35	35.95	10.34
29	DHIKATHA	84	74	58	69.05	5.68	0.99	8.07	9.76	58.00	11.14
30	DODHI DHAN	66	54	36	54.55	3.15	3.37	3.93	6.28	28.99	35.69
31	DOLKHE MASINO	90	76	62	68.89	5.53	2.45	4.02	5.20	51.22	10.53
32	DUDHE DHAN	78	56	56	71.79	5.50	2.41	6.87	4.74	34.52	10.65
33	EKLE	78	58	16	20.51	7.48	0.30	5.30	7.32	52.24	11.17
34	GAJALI	86	66	58	67.44	5.25	3.50	4.20	8.32	39.59	25.73
35	GAJAR GAULA	74	62	16	21.62	4.40	2.80	4.85	4.08	25.78	25.28
36	GHUI KUMARI	86	74	58	67.44	6.67	1.60	5.83	6.28	33.18	13.95
37	GUDRIBHOG	86	78	66	76.74	6.69	2.68	9.80	9.92	36.54	10.95
38	HAMADI	72	68	48	66.67	5.64	1.60	4.26	7.55	40.82	10.23
39	HIMALI	64	54	16	25.00	4.65	1.13	5.69	0.70	48.20	45.72
40	HIMALI CHOTO	88	68	66	75.00	5.57	2.32	4.73	4.78	27.84	13.43
41	INDRABELI	74	70	16	21.62	4.00	0.34	6.77	0.31	28.70	11.99
42	JALUNAR	76	68	48	63.16	6.55	2.13	7.01	1.60	74.31	13.69
43	JAUDI KHOLE	84	72	68	80.95	5.76	3.23	8.46	4.85	35.06	51.67
44	JAULE	60	62	54	90.00	9.37	3.80	3.14	7.88	46.69	12.27
45	JAULI	80	78	62	77.50	5.11	1.43	6.85	7.90	52.69	11.25
46	JEERA MASINO	66	60	32	48.48	5.92	0.79	6.30	0.42	45.28	13.02
47	JETHO BUDO	82	74	56	68.29	6.20	0.49	5.30	5.82	52.53	23.49
48	JHILINGI	66	66	44	66.67	5.80	1.93	6.24	4.26	31.43	11.82
49	JHINIDHAN	66	34	28	42.42	5.88	1.48	6.58	6.03	11.10	30.90
50	JHINUWA	76	52	56	73.68	5.45	2.40	7.62	7.01	28.00	18.45
51	JHINUWA 1	68	54	50	73.53	7.17	1.33	4.87	5.20	27.39	11.59
52	JHINUWA BASMATI	78	66	60	76.92	4.36	0.70	5.20	6.73	39.07	11.16
53	JHINUWA DHAN	82	76	50	60.98	5.68	0.84	6.52	4.97	51.38	21.34
54	JHINUWA MASINO	76	68	68	89.47	5.58	3.09	5.97	4.58	24.71	24.30
55	KALA CHAMAL	82	70	28	34.15	3.67	0.53	7.56	3.85	15.01	18.83
56	KALANAMAK	82	66	64	78.05	6.28	1.62	7.38	7.01	17.63	29.23
57	KALO JHINUWA	86	66	62	72.09	4.73	2.03	7.97	7.49	28.05	11.78
58	KALO JUNGE	82	70	44	53.66	5.70	0.49	5.40	2.72	44.17	10.80
59	KALO MACHYA	72	60	52	72.22	5.17	1.03	5.26	4.20	37.32	12.07
60	KALO TAKMURO	80	60	52	65.00	4.83	0.93	4.75	5.10	31.60	27.49
61	KARIYA KAMODH	68	30	34	50.00	5.92	1.33	6.81	2.66	43.64	10.40
62	KATHE JHINUWA	80	66	40	50.00	10.84	1.69	5.50	4.47	42.41	27.34
63	KHAIRENITAR	82	58	46	56.10	5.19	0.72	5.75	5.82	49.11	10.90
64	KHAJIYA	82	64	56	68.29	4.32	1.62	5.28	9.55	46.30	10.93
65	KRISHNABHOG	86	66	58	67.44	4.51	1.95	5.06	7.76	33.21	10.76
66	LAAMCHO DHAN	74	64	52	70.27	5.07	2.73	4.51	5.78	27.40	18.22
67	LALAYATH	90	70	56	62.22	8.72	1.16	7.85	2.12	13.77	18.95
68	LOCAL JEERA	74	50	28	37.84	5.94	0.53	6.09	3.04	62.23	11.13
69	LUMRU	78	66	40	51.28	5.25	0.34	6.18	7.18	32.83	11.67
70	MANHU DHAN	78	62	48	61.54	6.02	0.87	6.58	3.45	54.74	17.09
71	MANSARA	34	20	24	70.59	5.54	1.89	8.38	8.17	38.40	10.96
72	MANSARA 1	86	60	32	37.21	7.19	0.36	5.81	1.54	44.18	11.10
73	MARSHI TANGALO	58	26	12	20.69	4.61	0.91	5.77	2.70	31.27	10.80
74	MEDESHE MASINO	90	70	64	71.11	5.92	0.74	5.16	4.49	53.14	20.26
75	MOTI	86	58	56	65.12	5.64	0.84	7.48	0.21	41.00	12.23
76	NEPALI PUWA	78	62	36	46.15	4.83	0.32	6.48	3.66	69.20	11.45

77	NGRC01705	72	60	54	75.00	5.35	3.39	7.83	5.89	63.83	26.48
78	NGRC01716	72	62	50	69.44	6.59	3.35	4.75	6.20	39.95	13.09
79	NGRC01740	82	80	36	43.90	6.38	0.55	5.81	2.83	40.35	11.64
80	NGRC01757	86	74	64	74.42	6.38	1.41	5.01	1.66	47.74	12.29
81	NGRC01810	96	66	66	68.75	5.98	2.13	8.13	3.43	57.56	16.21
82	NGRC01815	88	66	60	68.18	4.51	3.15	6.54	10.21	14.26	10.33
83	NGRC01876	78	62	56	71.79	5.09	2.07	4.02	6.34	45.34	13.16
84	NGRC02133	76	70	30	39.47	4.93	2.95	5.85	4.68	14.34	10.41
85	NGRC02822	80	74	58	72.50	4.26	1.37	4.91	1.98	29.80	26.41
86	NGRC02831	90	68	66	73.33	4.57	1.48	6.89	1.14	38.55	11.75
87	NGRC02865	82	76	70	85.37	5.99	2.74	3.89	5.41	22.94	11.92
88	NGRC02872	82	64	62	75.61	5.31	3.96	6.42	6.49	54.76	26.41
89	NGRC03206	82	72	46	56.10	4.77	1.79	6.34	5.37	21.10	11.65
90	NGRC03207	80	74	54	67.50	5.29	3.44	5.56	7.45	15.01	13.88
91	NGRC03212	78	62	16	20.51	7.68	0.53	5.79	3.81	10.86	40.77
92	NGRC03305	74	62	36	48.65	5.11	0.17	5.40	3.97	32.82	63.88
93	NGRC03369	80	72	38	47.50	5.64	2.76	10.21	5.55	11.76	11.44
94	NGRC03380	86	58	52	60.47	4.63	1.67	6.30	4.53	25.70	19.73
95	NGRC03381	70	62	56	80.00	5.88	2.76	5.08	5.49	35.72	11.01
96	NGRC03389	74	36	18	24.32	2.90	0.53	3.22	2.15	46.64	10.49
97	NGRC03420	68	28	12	17.65	4.99	0.28	5.52	3.02	37.49	51.37
98	NGRC03422	74	66	40	54.05	4.99	0.72	5.99	4.16	36.83	60.66
99	NGRC04939	70	66	26	37.14	5.17	2.53	5.71	7.38	22.78	11.11
100	NGRC04999	82	70	44	53.66	4.93	0.46	8.21	2.48	52.99	15.57
101	NGRC05024	74	58	32	43.24	5.50	0.23	6.58	1.68	31.58	17.36
102	NGRC05040	82	70	32	39.02	5.05	0.70	6.07	1.77	33.14	23.51
103	NGRC05725	82	70	60	73.17	6.53	0.46	4.67	2.91	58.76	13.88
104	NGRC07860	78	70	48	61.54	5.41	0.40	4.97	3.95	51.51	10.85
105	NGRC07861	74	66	28	37.84	5.35	0.68	9.58	1.08	50.28	13.86
106	NGRC07866	70	30	16	22.86	4.30	0.58	5.24	1.87	34.83	20.83
107	NGRC07869	88	60	28	31.82	4.83	0.63	4.93	2.25	29.17	21.87
108	NGRC07870	78	66	48	61.54	6.08	0.38	7.13	2.41	55.57	12.70
109	NGRC07875	76	62	58	76.32	4.71	0.17	5.73	3.66	47.59	11.01
110	NGRC07900	62	56	10	16.13	3.86	0.28	4.10	1.14	52.08	59.57
111	NGRC08273	78	74	40	51.28	5.07	0.38	5.44	2.35	49.91	16.96
112	NGRC08276	70	62	28	40.00	5.50	0.34	2.53	3.54	42.97	10.08
113	NGRC08278	76	68	64	84.21	5.33	0.49	4.55	3.99	52.44	13.49
114	NGRC08289	74	70	48	64.86	6.02	0.21	7.89	2.29	50.68	10.84
115	NGRC08300	74	70	24	32.43	5.58	1.81	4.06	7.49	26.46	11.16
116	NGRC08372	58	54	20	34.48	5.74	1.24	5.63	5.43	48.00	13.42
117	NIRTACHAN	82	66	28	34.15	4.57	0.59	4.91	1.08	37.66	20.19
118	OR	58	56	28	48.28	6.02	0.32	6.28	0.94	55.41	10.60
119	PADANI	90	64	66	73.33	5.75	2.43	6.05	3.95	35.34	12.13
120	PANGALI	82	66	56	68.29	4.26	0.84	8.21	5.25	39.29	13.01
121	PANHELI	84	68	62	73.81	5.01	2.45	6.48	6.45	68.07	18.07
122	PANHELO	70	64	48	68.57	6.61	3.57	7.48	4.68	46.38	61.97
123	PIPAL MARSI	82	74	66	80.49	5.05	3.06	6.09	5.70	40.86	11.20
124	POKHARA MASINO	84	76	64	76.19	5.31	3.27	3.93	3.64	34.28	10.56
125	PURANO MANSULI	86	70	62	72.09	4.36	2.41	4.91	3.70	41.14	11.55
126	PUSA BARSA	70	66	28	40.00	6.10	1.06	5.59	0.71	70.17	33.38
127	RAINI MODHO	74	58	24	32.43	3.56	0.89	5.10	2.14	30.30	16.41
128	RAJBHOG	78	66	64	82.05	6.40	3.26	4.87	5.10	53.39	11.19
129	RATA CHCCHE	74	66	36	48.65	4.75	0.68	4.87	4.99	47.47	20.50
130	RATO DHAN	86	78	70	81.40	5.13	2.93	6.87	6.03	46.53	11.17
131	RATO JADHE	82	70	44	53.66	4.18	1.05	5.63	4.06	48.40	10.40
132	RATUWA	70	58	44	62.86	4.46	0.45	5.10	3.30	39.58	17.97

133	SARASWOTI	72	64	48	66.67	5.35	0.65	5.40	6.05	56.25	11.35
134	SAURAV	46	42	8	17.39	5.66	0.63	5.87	0.68	28.77	11.24
135	SETA ANADI	86	56	70	81.40	5.76	2.19	4.30	4.04	23.80	11.23
136	SETO CHUCHEE	80	70	64	80.00	6.18	0.82	10.07	9.44	57.94	32.83
137	SETO DALLE	26	24	8	30.77	5.86	0.47	6.38	1.04	42.99	11.88
138	SETO DHAN	72	62	40	55.56	5.39	2.64	5.59	4.56	52.54	10.61
139	SETO GEDE	70	62	28	40.00	6.99	0.40	8.22	6.99	46.38	10.83
140	SETO JHINUWA	76	74	58	76.32	7.05	3.66	4.59	9.78	38.36	10.50
141	STAHANIYA	78	46	22	28.21	3.47	2.35	6.50	11.23	12.43	14.41
142	SUNAULO	72	66	60	83.33	5.45	2.87	5.59	4.70	33.86	11.32
143	SUNGADHI	54	50	28	51.85	6.49	3.39	7.38	8.22	44.13	10.34
144	SYAUDO	58	46	20	34.48	5.35	1.46	5.41	3.22	66.77	30.85
145	TAICHIN	18	8	4	22.22	6.44	0.53	5.89	1.14	57.98	12.68
146	TAULI	70	44	28	40.00	5.07	2.45	7.62	7.28	38.02	11.41
147	THAPACHINIYA	62	54	38	61.29	5.54	2.02	7.46	11.04	67.42	11.00
148	THARU ANADHI 1	58	50	16	27.59	5.61	0.87	4.83	0.04	43.34	58.37
149	THARU ANADHI 2	70	58	20	28.57	6.00	0.57	6.97	2.89	75.00	45.22
150	TILAKI	74	62	28	37.84	4.37	0.39	4.69	0.52	32.72	11.86
151	TIRABFHUL	84	58	56	66.67	6.26	0.57	5.89	4.12	21.59	10.72
152	TOLI ANDAHI	86	70	66	76.74	5.76	4.60	6.99	7.51	21.88	19.87
153	TULSI	72	66	50	69.44	5.23	1.74	8.54	5.20	47.63	13.00
154	YOBO	76	64	48	63.16	7.15	0.53	6.62	3.41	26.14	10.28

Notes: GP = germination percentage; T0 = control (0% PEG); T15 = 15% PEG; T20 = 20% PEG; GP Ret. T20 = germination percentage at 20% PEG expressed as a percentage of the same landrace's control (T0) germination; SL14 = shoot length at 14 days after sowing (cm); RL14 = root length at 14 days after sowing (cm); RWC = relative water content (%). Landraces are ranked from highest to lowest GP Ret. T20 (most to least drought-tolerant by this criterion). The complete set of all recorded traits for every landrace and treatment (including 21-day measurements, biomass, germination index, mean germination time, and root: shoot ratios) is provided in the accompanying supplementary spread sheet.

Preparation of PEG 6000 solutions and experimental treatments

Osmotic stress was induced using polyethylene glycol (PEG 6000). Three treatments were imposed: a non-stressed control (distilled water only, denoted T0) and two levels of PEG 6000-induced osmotic stress, 15% (T15) and 20% (T20). The 15% solution was prepared by dissolving 150 g of PEG 6000 granules in 500 mL of distilled water and making up the volume to 1000 mL with further distilled water; the 20% solution was prepared analogously using 200 g of PEG 6000 in a final volume of 1000 mL. This gravimetric (w/v) approach to preparing PEG 6000 solutions of defined concentration followed the widely adopted convention for simulating water-deficit stress in germination assays (Michel and Kaufmann, 1973).

Seed sterilization, sowing, and germination assessment

For each landrace, 25 seeds were selected for each treatment and replication. Seeds were surface-sterilized using 75% (v/v) ethanol and subsequently rinsed three times with distilled water to remove residual ethanol. Two layers of filter paper were placed at the bottom of each Petri dish, and 25 sterilized seeds were distributed evenly on the filter paper. This protocol was adapted from Zhang et al. (2026).

Each Petri dish received 10 mL of the respective treatment solution. Thus, 10 mL of distilled water was applied to the control dishes, whereas 10 mL of 15% or 20% PEG 6000 solution was applied to the corresponding stress-treatment dishes. The Petri dishes were maintained in a germinator at 25°C. Daily germination counts were recorded for seven consecutive days, from Day 1 to Day 7. To maintain adequate moisture throughout the germination period, an additional 5 mL of distilled water for the control and 5 mL of treatment solution at the respective concentrations were added to each Petri dish on the third and sixth days after sowing (DAS). A seed was considered germinated when the radicle reached approximately 2 mm in length (ISTA, 1996).

Germination parameters

Germination was monitored daily for seven days, and the number of newly germinated seeds was recorded for

each Petri dish. The following germination parameters were evaluated:

Germination percentage (GP %)

GP % was calculated as the proportion of seeds germinated by the end of the observation period relative to the total number of seeds sown (ISTA, 1996) as followed.

$$\text{GP (\%)} = \frac{\text{Total number of germinated seeds}}{\text{Total number of seeds sown}} \times 100$$

Germination energy (GE %)

GE % was calculated as the percentage of seeds germinated by the fifth day after sowing relative to the total number of seeds sown. It was used to assess the rapidity and uniformity of germination during the early phase of the germination period (ISTA, 1996) as followed.

$$\text{GE (\%)} = \frac{\text{Total number of germinated seeds at 5th day}}{\text{Total number of seeds sown}} \times 100$$

Germination index (GI)

GI was used to characterize germination speed based on the daily number of newly germinated seeds. It was calculated using the following equation:

$$\text{GI} = \sum \frac{\text{ni}}{\text{ti}}$$

Where ni is the number of seeds newly germinated on day (ti), and (ti) is the number of days from the beginning of the germination test.

Mean germination time (MGT days)

MGT was calculated using the daily number of newly germinated seeds and the corresponding observation time (Ellis and Roberts, 1981) as followed.

$$\text{MGT} = \frac{\sum (\text{ni ti})}{\sum \text{ni}}$$

Where ni is the total number of seeds newly germinated on day (ti), and (ti) is the number of days from the beginning of the germination test.

Seedling growth and physiological measurements

Seedling growth was assessed at 14 and 21 DAS. Shoot length (SL) and root length (RL) were measured and expressed in cm. Measurements were made separately for the 14- and 21-day assessment periods.

Ten seedlings from each treatment were used for the measurement of fresh weight, turgid weight, and dry weight. Fresh weight was recorded immediately after sampling. The seedlings were then soaked in distilled water at 25°C for 4 h to achieve full turgidity and subsequently weighed to determine turgid weight (TW). The samples were then oven-dried at 80°C for 24 h and weighed to determine dry weight (DW). Relative water content (RWC) was calculated using the given below equation (Barrs and Weatherley, 1962); higher RWC values indicated greater water retention and better maintenance of seedling water status under stress conditions.

$$\text{RWC (\%)} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

Where FW is fresh weight, DW is oven-dried weight, and TW is fully turgid weight after rehydration.

The root-to-shoot length ratio (R) was calculated at 14 and 21 DAS as followed:

$$\text{R} = \frac{\text{Root length}}{\text{Shoot length}}$$

Calculation of stress-response indices

Germination retention (%) was calculated to express germination under osmotic stress relative to the corresponding landrace's germination under the control condition:

$$\text{Germination retention (\%)} = \frac{\text{GPs}}{\text{GPc}} \times 100$$

Where GPs is the germination percentage under 15% or 20% PEG 6000 and GPc is the germination percentage under the control treatment. Values above 100% indicate that the observed germination percentage under the PEG treatment exceeded that of the corresponding control. This index was interpreted together with absolute germination percentage rather than used alone to classify landraces.

Stress-tolerance index calculation and genotype ranking

The stress-tolerance index (STI) was calculated to integrate germination and early seedling-growth traits into a single composite score for ranking rice genotypes under 20% PEG 6000-induced osmotic stress. Eight traits were considered: shoot length at 14 and 21 days (SL14 and SL21), root length at 14 and 21 days (RL14 and RL21), germination index (GI), germination percentage (GP), germination energy (GE), and mean germination time (MGT). Germination percentage and germination energy were included because they directly quantify the proportion of seeds that germinate and the rapidity of germination under stress, respectively, and are the most widely used indicators of seed-lot performance under water deficit (ISTA, 1996; Karanjit et al., 2024). The germination index and mean germination time were

included to capture the speed and timing of germination, since osmotic stress delays germination even when final germination percentage is maintained; mean germination time was reverse-coded because lower values indicate faster germination (Ellis and Roberts, 1981). SL14 and SL21 were included as measures of early seedling vigor and sustained shoot growth, which are sensitive to reduced water availability and reflect the capacity of seedlings to establish under stress (Sagar et al., 2020). RL14 and RL21 were included because root growth is a key determinant of water acquisition and often responds differently from shoot growth under osmotic stress (Kano et al., 2011). Equal weights were assigned to the eight standardized traits to construct an exploratory composite index without imposing subjective trait-importance coefficients (European Union and Joint Research Centre, 2008). Although shoot and root lengths measured at 14 and 21 days may be correlated, their responses to osmotic stress varied among rice varieties and assessment periods. Therefore, both measurements were retained to capture varietal differences in early and later seedling growth.

To place traits measured on different scales on a common scale, each trait was standardized using its z-score:

$$\mathrm{Zi} = \frac{\mathrm{Xi} - \bar{\bar{x}}}{\mathrm{SDX}}$$

Where Xi is the value of a given trait for a genotype, ($\bar{\bar{x}}$) is the mean across genotypes, and SD is the corresponding standard deviation.

For traits in which higher values indicated better performance (SL14, RL14, SL21, RL21, GI, GP, and GE), positive standardized values contributed positively to the index. Mean germination time was treated as an inverse indicator because lower values represent faster germination; therefore, its standardized score was reverse-coded before index calculation.

The STI was then calculated as the arithmetic mean of the eight standardized trait scores:

$$\mathrm{STI_i} = \frac{1}{8}\sum_{j=1}^{8} Z_{ij}^{*}$$

Where Z_{ij}^{*} represents the standardized value of trait j for genotype i, with MGT reverse-coded.

Genotypes were ranked in descending order of STI, with higher scores indicating comparatively better overall performance under osmotic stress. The five highest-ranked genotypes were considered the most promising candidates for further validation, whereas the lowest-ranked genotypes were considered comparatively sensitive. The five highest-ranked genotypes were selected as a practical shortlist for subsequent evaluation. This cut-off was intended to identify a manageable group of promising candidates rather than to establish a statistically validated threshold separating tolerant and sensitive genotypes.

Plant protection relevance and future screening framework

Although this study focused on germination and early seedling traits under PEG-induced osmotic stress, the landraces identified as drought-tolerant have direct relevance to plant protection. The screening protocol described here can be adapted by plant pathology and entomology laboratories to identify germplasm with potential tolerance to drought-associated biotic stresses. The germination-based screening approach is particularly valuable for plant health diagnostics because it is rapid, requires minimal space and resources, and can be standardized across laboratories. Future screening should incorporate challenge inoculation with major rice pathogens (e.g., *Magnaporthe oryzae*, *Xanthomonas oryzae* pv. *oryzae*) and infestation with key insect pests (e.g., *Nilaparvata lugens*) under both well-watered and drought-stressed conditions to identify landraces with dual tolerance to abiotic and biotic constraints. Such integrated screening approaches contribute to the development of integrated pest management strategies for rainfed rice ecosystems.

Experimental design and statistical analysis

The experiment was conducted using a completely randomized design (CRD) with a two-factor factorial treatment structure comprising 154 rice landraces/genotypes and three polyethylene glycol (PEG 6000) treatments: control (T0), 15% PEG 6000 (T15), and 20% PEG 6000 (T20). Each genotype–treatment combination was replicated twice, resulting in 924 experimental units (154 genotypes × 3 PEG treatments × 2 replications) and approximately 23,100 seeds evaluated across the experiment. Each experimental unit consisted of 25 seeds placed uniformly in a Petri dish and exposed to the respective treatment. Petri dishes were considered the experimental units for statistical analysis, and all units were maintained under identical experimental conditions. Two replications were used because screening the full panel with three or more replications would have exceeded available germinator space, seed quantities for some landraces, and labor for

daily counting and destructive sampling. Although 25 seeds per Petri dish reduce binomial sampling error, seeds within a dish are not independent; the two replications therefore remain the basis for error estimation, and genotype rankings were considered provisional pending validation with higher replication.

For each response variable, treatment means and standard deviations were calculated to summarize the observed variation. Analysis of variance (ANOVA) was used to evaluate the effects of genotype, PEG treatment, and their interaction on germination and early seedling-growth traits. Statistical significance was assessed at $P < 0.05$. To assess genotype-specific germination responses to osmotic stress, germination retention was calculated for each genotype under T20 relative to its corresponding control (T0). Genotypes were ranked according to their germination-retention values to compare their relative responses to PEG-induced osmotic stress. This measure represents the maintenance of germination under stress relative to the control and was interpreted alongside seedling-growth traits when characterizing genotype responses.

Principal component analysis (PCA) was performed to characterize multivariate variation among rice genotypes in germination and early seedling-growth traits under PEG 6000-induced osmotic stress. Nine variables were included: GP, GE, GI, MGT, SL14 and SL21, RL14 and RL21, and RWC. Before PCA, all variables were standardized to a mean of zero and a standard deviation of one to reduce the influence of differences in measurement scales. The resulting principal components were used to examine patterns of multivariate variation and the relationships among measured traits and genotypes.

Results

Genotypic variation and effect of PEG-induced osmotic stress

The factorial analysis of variance revealed highly significant ($P < 0.001$) effects of genotype, PEG concentration, and the genotype × PEG concentration interaction across all 14 seedling and germination traits (Table 2, Table 3). Genotypic variation was prominent for root length at 14 days ($F = 172.33$, $\eta_p^2 = 0.983$), root: shoot ratio at 14 days ($F = 160.07$, $\eta_p^2 = 0.981$), root length at 21 days ($F = 103.58$, $\eta_p^2 = 0.972$), and germination index ($F = 94.93$, $\eta_p^2 = 0.969$). There were substantial differences among the genotypes in root development, biomass allocation, and germination performance. RWC showed the lowest genotypic effect size ($\eta_p^2 = 0.623$), although it remained statistically significant.

Table 2. Effects of genotype, PEG concentration, and genotype × PEG interaction on seedling traits of 154 rice genotypes.

Traits	Genotypes (G)		PEG concentration		G x PEG concentration		CV (%)
	F value	partial η^2	F value	partial η^2	F value	partial η^2	
SL14	58.90***	0.951	19787.36***	0.988	29.97***	0.952	7.14
RL14	172.33***	0.983	3927.30***	0.944	115.58***	0.987	5.18
SL21	27.67***	0.902	13892.64***	0.984	21.73***	0.935	8.57
RL21	103.58***	0.972	3865.62***	0.944	57.35***	0.974	6.11
FW	51.70***	0.945	6538.54***	0.966	19.76***	0.929	8.33
DW	19.56***	0.866	5324.80***	0.958	21.73***	0.935	10.99
TW	32.70***	0.915	1085.03***	0.824	28.24***	0.949	9.83
GI	94.93***	0.969	12986.41***	0.983	18.27***	0.924	6.52
GP	60.33***	0.952	5145.35***	0.957	10.56***	0.875	6.37
GE	76.11***	0.962	9136.98***	0.975	20.22***	0.931	7.97
MGT	194.17***	0.985	17378.57***	0.987	96.35***	0.985	2.28
R at 14 days	160.07***	0.981	10733.78***	0.979	146.62***	0.990	11.43
R at 21 days	26.37***	0.897	1893.32***	0.891	28.63***	0.950	35.40
RWC	4.98***	0.623	647.66***	0.737	5.12***	0.772	27.16

Note: F values represent the effects of genotype (G), PEG concentration, and their interaction (G × PEG). Partial eta-squared (η_p^2) indicates the effect size for each model term. CV = coefficient of variation. *** = P <0.001.

Table 3. Complete factorial ANOVA for all 14 traits

Traits	Effect	df	MS	F value	p value	Error MS	Partial_Eta2	CV (%)
SL14	Genotype (G)	153	3.57	58.90	2.2E-232	0.061	0.951	7.14
	PEG Concentration	2	1200.31	19787.36	0.0E+00	0.061	0.988	7.14
	G x PEG	306	1.82	29.97	2.6E-198	0.061	0.952	7.14
RL14	Genotype (G)	153	15.68	172.33	0.0E+00	0.091	0.983	5.18
	PEG Concentration	2	357.41	3927.30	1.1E-290	0.091	0.944	5.18
	G x PEG	306	10.52	115.58	0.0E+00	0.091	0.987	5.18
SL21	Genotype (G)	153	2.55	27.67	1.1E-163	0.092	0.902	8.57
	PEG Concentration	2	1280.14	13892.64	0.0E+00	0.092	0.984	8.57
	G x PEG	306	2.00	21.73	4.8E-169	0.092	0.935	8.57
RL21	Genotype (G)	153	10.96	103.58	3.5E-286	0.106	0.972	6.11
	PEG Concentration	2	408.83	3865.62	3.3E-289	0.106	0.944	6.11
	G x PEG	306	6.07	57.35	1.4E-259	0.106	0.974	6.11
Fresh_WT	Genotype (G)	153	0.01	51.70	3.5E-220	<0.001	0.945	8.33
	PEG Concentration	2	1.13	6538.54	0.0E+00	<0.001	0.966	8.33
	G x PEG	306	0.00	19.76	1.4E-160	<0.001	0.929	8.33
Dry_WT	Genotype (G)	153	0.00	19.56	3.2E-134	<0.001	0.866	10.99
	PEG Concentration	2	0.71	5324.80	0.0E+00	<0.001	0.958	10.99
	G x PEG	306	0.00	21.73	4.7E-169	<0.001	0.935	10.99
Turgid_WT	Genotype (G)	153	0.03	32.70	1.9E-178	0.001	0.915	9.83
	PEG Concentration	2	0.99	1085.03	2.8E-175	0.001	0.824	9.83
	G x PEG	306	0.03	28.24	7.4E-193	0.001	0.949	9.83
GI	Genotype (G)	153	5.88	94.93	9.1E-278	0.062	0.969	6.52
	PEG Concentration	2	804.11	12986.41	0.0E+00	0.062	0.983	6.52
	G x PEG	306	1.13	18.27	1.0E-153	0.062	0.924	6.52
GP	Genotype (G)	153	898.48	60.33	1.3E-234	14.892	0.952	6.37
	PEG Concentration	2	76623.39	5145.35	0.0E+00	14.892	0.957	6.37
	G x PEG	306	157.29	10.56	1.0E-107	14.892	0.875	6.37
GE	Genotype (G)	153	1122.86	76.11	1.3E-256	14.753	0.962	7.97
	PEG Concentration	2	134800.12	9136.98	0.0E+00	14.753	0.975	7.97
	G x PEG	306	298.37	20.22	1.2E-162	14.753	0.931	7.97
MGT	Genotype (G)	153	2.14	194.17	0.0E+00	0.011	0.985	2.28
	PEG Concentration	2	191.81	17378.57	0.0E+00	0.011	0.987	2.28
	G x PEG	306	1.06	96.35	0.0E+00	0.011	0.985	2.28
RS14	Genotype (G)	153	14.38	160.07	0.0E+00	0.090	0.981	11.43
	PEG Concentration	2	963.94	10733.78	0.0E+00	0.090	0.979	11.43
	G x PEG	306	13.17	146.62	0.0E+00	0.090	0.990	11.43
RS21	Genotype (G)	153	25.51	26.37	1.6E-159	0.967	0.897	35.40
	PEG Concentration	2	1831.12	1893.32	2.6E-223	0.967	0.891	35.40
	G x PEG	306	27.69	28.63	4.1E-194	0.967	0.950	35.40
RWC	Genotype (G)	153	375.47	4.98	7.1E-41	75.402	0.623	27.16
	PEG Concentration	2	48834.55	647.66	9.4E-135	75.402	0.737	27.16
	G x PEG	306	385.80	5.12	9.8E-56	75.402	0.772	27.16

Notes: SL14 = shoot length at 14 days after sowing (cm); RL14 = root length at 14 days after sowing (cm); RWC = relative water content (%). Landraces are ranked from highest to lowest GP Ret. T20 (most to least drought-tolerant by this criterion).

All measured traits also varied strongly with PEG concentration. The PEG concentration effects were largest for shoot length at 14 days (F = 19,787.36, η_p^2 = 0.988), mean germination time (F = 19,378.57, η_p^2 = 0.987), shoot length at 21 days (F = 13,892.64, η_p^2 = 0.984), and germination index (F = 12,986.41, η_p^2 = 0.983). These findings showed that PEG concentration strongly affected germination timing and early seedling development.

The genotype × PEG concentration interaction was significant for every trait, with partial eta-squared values ranging from 0.772 for RWC to 0.990 for root: shoot ratio at 14 days, indicating that genotype responses differed across PEG

concentrations. Especially strong interaction effects were observed for root: shoot ratio at 14 days (F = 146.62, η_p^2 = 0.990), root length at 14 day (F = 115.58, η_p^2 = 0.990), mean germination time (F = 96.35, η_p^2 = 0.985), and root length at 21 days (F = 57.35, η_p^2 = 0.987).

Genotype-specific response to increasing PEG concentration

PEG 6000 concentration markedly altered germination and early seedling traits across the 154 rice genotypes (Table 4). Increasing PEG concentration from control (T0) to 15% (T15) and 20% (T20) was generally associated with progressively greater reductions in germination performance and shoot growth, but root growth and biomass-related traits showed more complex responses.

Table 4. Effect of PEG 6000 concentration on germination and early seedling traits of 154 rice genotypes.

Traits	Control	PEG at 15% concentration	% change	PEG at 20% concentration	% change	SE
SL14	5.50	3.29	40.20	1.56	71.64	0.014
RL14	6.08	6.75	-10.99	4.64	23.67	0.017
SL21	5.31	4.01	24.57	1.31	75.29	0.017
RL21	6.33	5.56	12.25	4.07	35.79	0.019
FW	0.22	0.16	25.83	0.10	55.83	0.001
DW	0.15	0.12	18.53	0.05	64.27	0.001
TW	0.32	0.25	22.81	0.36	-12.27	0.002
GI	5.57	3.50	37.15	2.38	57.22	0.014
GP	75.55	62.04	17.88	44.10	41.62	0.220
GE	70.30	45.60	35.14	28.70	59.17	0.219
MGT	3.72	4.85	-30.39	5.24	-40.86	0.006
R at 14 days	1.14	2.14	-87.20	4.58	-300.70	0.017
R at 21 days	1.21	1.53	-26.53	5.59	-360.70	0.056
RWC	41.03	37.28	9.13	17.59	57.13	0.495

Note: Values are estimated marginal means; SE = standard error. T0, T15 and T20 represent 0, 15 and 20% PEG 6000, respectively.

Germination performance deteriorated consistently as PEG concentration increased (Table 4). Germination percentage decreased from 75.55% to 62.04% and 44.10%, representing reductions of 17.88% and 41.62%. Germination index decreased from 5.57 in the control to 3.50 at T15 and 2.38 at T20, corresponding to reductions of 37.15% and 57.22%. Germination energy exhibited a stronger response, declining from 70.30 to 45.60 at T15 and 28.70 at T20. In contrast, mean germination time increased from 3.72 days in the control to 4.85 and 5.24 days at T15 and T20, respectively, indicating delayed germination under increasing osmotic stress.

Genotype-specific responses were also substantial (Figure 2). NGRC01815, NGRC02872, NGRC02865, and TOLI ANDAHI had more than 80% germination (88, 82, 82, and 86, respectively) and also showed a reduction under T15 to 66, 64, 76 and 70 respectively and further reduced in T20 but maintained above 60% for each genotype (60, 62, 70, and 66%, respectively). In contrast, several genotypes exhibited low germination under severe stress. CHOTTE declined from approximately 62% under T0 to 50% under T15 and 8% under T20. NGRC03389 decreased from 74% to 36% and 18%, while NGRC07900 declined from 62% to 56% and 10%. NIRTACHAN BARDIYA showed a sharp reduction at T20, reaching approximately 28% compared with 82% under T0. SYAUDO similarly declined from 58% under T0 to 46% under T15 and 20% under T20.

Shoot length declined greatly with increasing PEG concentration (Table 2). Shoot length at 14 days decreased from 5.50 under control conditions to 3.29 at T15 and 1.56 at T20, corresponding to reported reductions of 40.20% and 71.64%, respectively. Similarly, shoot length at 21 days decreased from 5.31 to 4.01 and

1.31, representing reductions of 24.57% and 75.29%. Root responses differed from those of shoots. Root length at 14 days increased from 6.08 under control conditions to 6.75 at T15, before declining to 4.64 at T20. Root length at 21 days decreased from 6.33 to 5.56 at T15 and 4.07 at T20, corresponding to reductions of 12.25% and 35.79%.

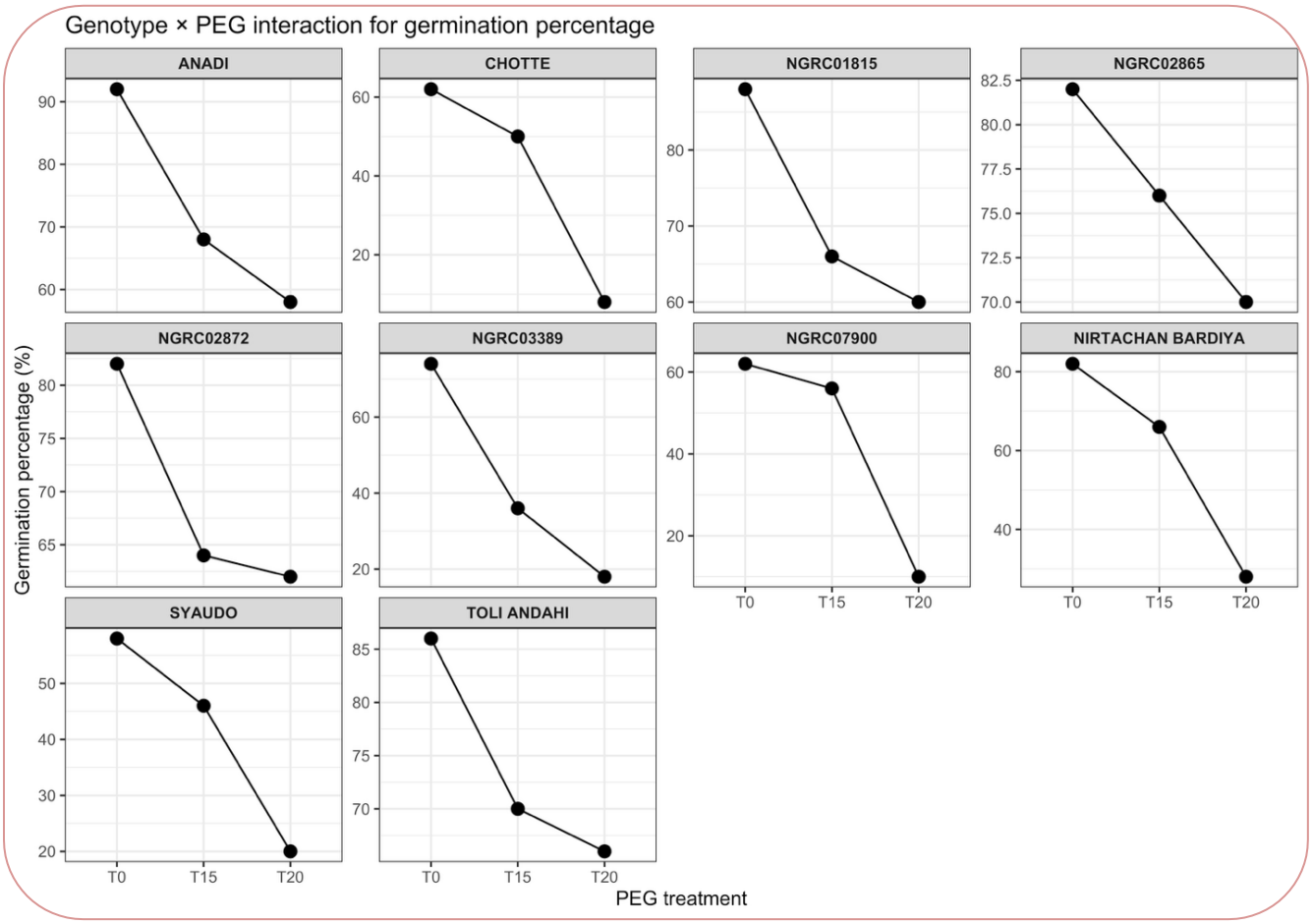


Figure 1. Genotype-specific responses in germination percentage of ten selected rice genotypes exposed to three PEG 6000 treatments (T0, T15, and T20).

Shoot length at 14 DAS varied substantially across the genotypes (Figure 2). NGRC02865, NGRC02872, TOLI ANDAHI, and NGRC01815 exhibited relatively high shoot lengths under T0 (approximately 6.0, 5.3, 5.8, and 4.5 cm, respectively), which declined under increasing PEG concentration. Under T15, shoot lengths decreased to approximately 4.9, 4.8, 5.0, and 3.3 cm, respectively. Under T20, further reductions were observed but not too extensive (2.7 cm in NGRC02865, 4.0 cm in NGRC02872, 4.6 cm in TOLI ANDAHI, 3.2 cm in NGRC01815) relative to T15. In contrast, NGRC07900, NIRTACHAN BARDIYA, NGRC03389, and CHOTTE showed marked shoot-growth inhibition under severe stress, with shoot lengths declining to approximately 0.2, 0.6, 0.5, and 1.1 cm, respectively, under T20. SYAUDO also showed a pronounced decline, from approximately 5.4 cm under T0 to 4.0 cm under T15 and 1.5 cm under T20. These results indicate substantial genotypic variation in shoot-growth responses to increasing PEG concentration, with NGRC02872 and TOLI ANDAHI maintaining comparatively greater shoot elongation under T20, while NGRC07900, NIRTACHAN BARDIYA, and NGRC03389 exhibited greater sensitivity.

Genotype-specific responses in root length at 14 DAS were also pronounced (Figure 3). Moderate osmotic stress may stimulate root elongation in some genotypes, whereas severe stress generally restricts root growth. NGRC01815 and TOLI ANDAHI showed strong root elongation under increasing PEG concentration from T0 to T15, increasing from approximately 6.5 to 8.4 cm and

from 7.0 to 11.7 cm, respectively. ANADI also exhibited an increase in root length from approximately 7.2 cm under T0 to 9.6 cm under T15, followed by a slight decrease to 9.3 cm under T20. Similarly, NGRC02865 increased from approximately 3.9 cm under T0 to 7.1 cm under T15, before declining to 5.4 cm under T20. NGRC02872 showed a comparable pattern, with root length increasing from 6.4 to 7.6 cm under T15 and subsequently decreasing to 6.5 cm under T20. CHOTTE, NGRC03389, and SYAUDO also showed marked increases in root length under T15, reaching approximately 10.3, 6.8, and 9.0 cm, respectively, but their root lengths declined substantially under T20. In contrast, NGRC07900 and NIRTACHAN BARDIYA exhibited progressive reductions in root length, particularly under severe stress, reaching approximately 1.1 cm in both genotypes at T20.

Biomass-related traits showed fewer uniform responses (Table 4). Seedling fresh weight declined from 0.22 under control conditions to 0.16 at T15 and 0.10 at T20, while dry weight decreased from 0.15 to 0.12 and 0.05, respectively. Seedling turgid weight declined from 0.32 to 0.25 at T15 but increased to 0.36 at T20, exceeding the control mean. Root: shoot ratios increased with PEG concentration. The ratio at 14 days rose from 1.14 under control conditions to 2.14 at T15 and 4.58 at T20, while the ratio at 21 days increased from 1.21 to 1.53 and 5.59. Relative water content declined from 41.03% in the control to 37.28% at T15 and 17.59% at T20, corresponding to reductions of 9.13% and 57.13%.

TOLI ANDAHI, NGRC02872, NGRC01815, ANADI, and NGRC02865 had favorable multi-trait performance, although their responses differed across germination and seedling-growth characteristics. NGRC01815 and ANADI maintained relatively high root lengths, whereas NGRC02865 showed favorable germination performance, with a germination percentage and germination energy of 70%. In contrast, CHOTTE, NGRC03389, and NGRC07900 exhibited poor germination performance and longer mean germination times, indicating greater sensitivity to severe osmotic stress. These contrasting trait profiles demonstrate that genotypic responses cannot be adequately characterized by a single trait.

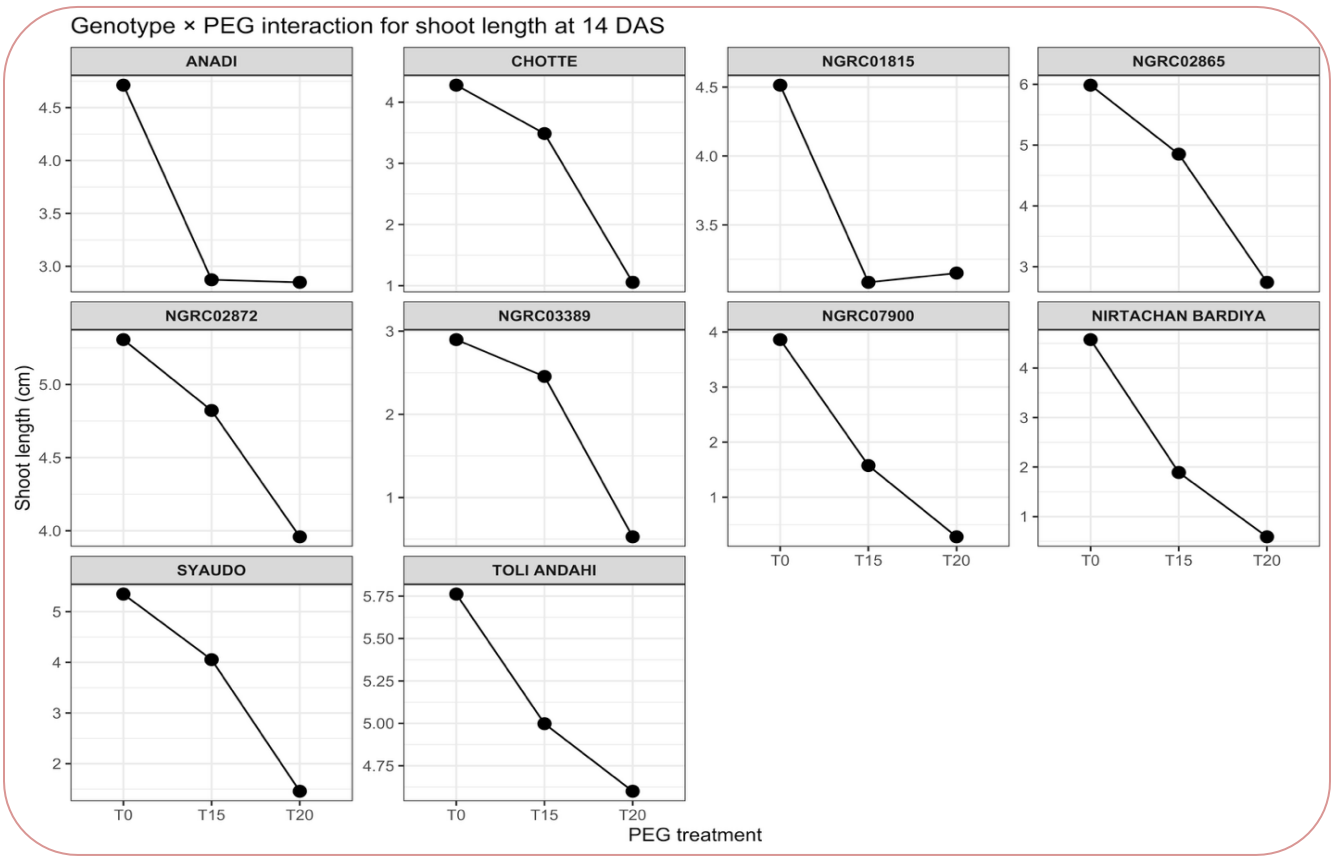


Figure 2. Genotype-specific responses in shoot length at 14 DAS of ten selected rice genotypes exposed to three PEG 6000 treatments (T0, T15, and T20).

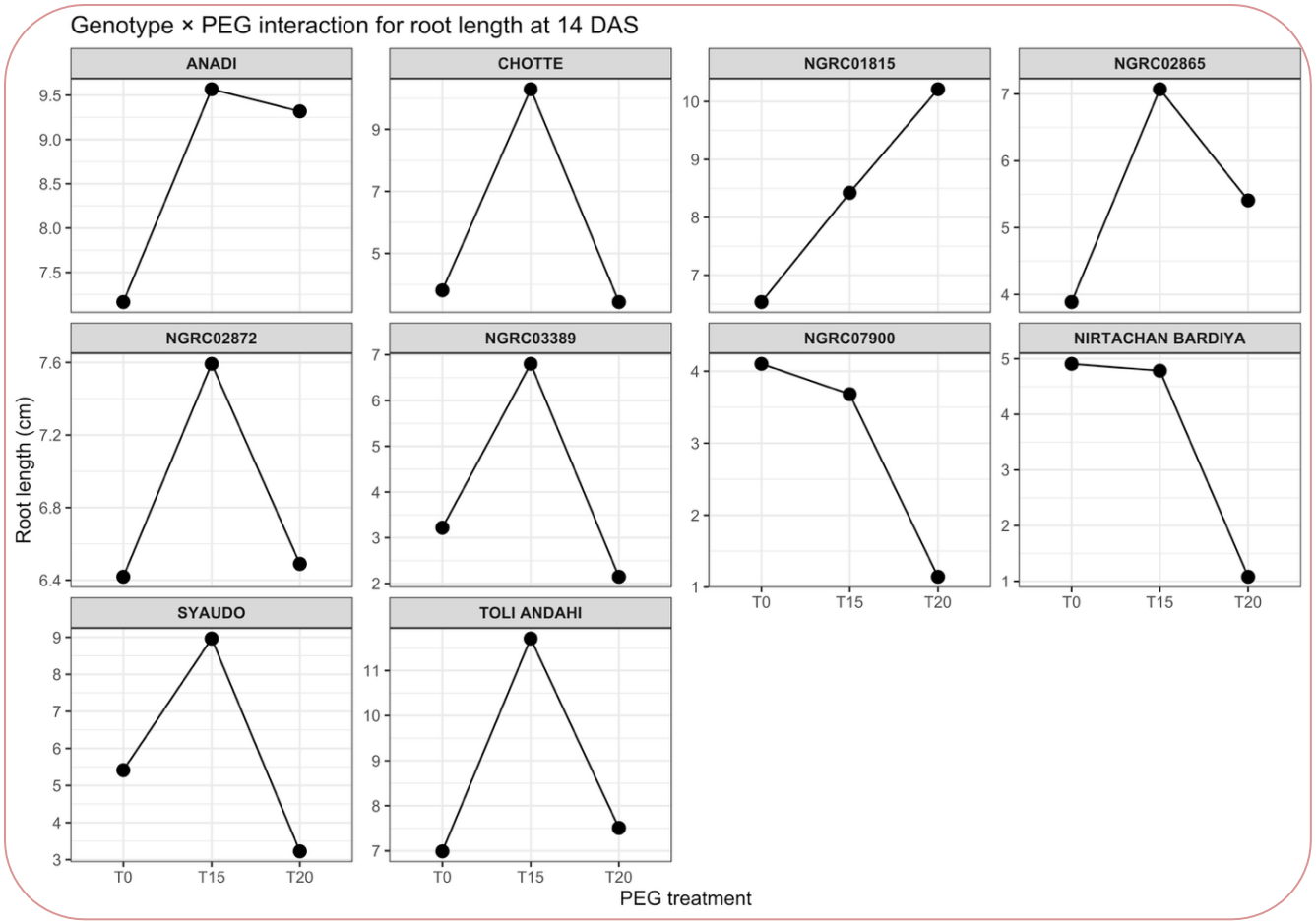


Figure 3. Genotype-specific responses in root length at 14 DAS of ten selected rice genotypes exposed to three PEG 6000 treatments (T0, T15, and T20).

Genotype × PEG interaction reveals contrasting stress responses

The heat map showed the considerable genotypic variation in germination and early seedling growth under 20% PEG 6000-induced osmotic stress (Figure 4; Figure 5).

Identification of osmotic-stress-tolerant and sensitive genotypes

The integrated multi-trait screening identified substantial variation among rice genotypes in their performance under 20% PEG 6000-induced osmotic stress (Table 5; Table 6). TOLI ANDAHI ranked first, with the highest stress tolerance index (STI = 1.712), followed by NGRC02872 (1.456), NGRC01815 (1.423), ANADI (1.320), and NGRC02865 (1.296). TOLI ANDAHI maintained relatively high shoot and root growth, with shoot lengths of 4.60 and 4.26 cm at 14 and 21 days, respectively, and root lengths of 7.51 and 8.25 cm. NGRC02872 also exhibited comparatively balanced performance, with shoot lengths of 3.96 and 3.83 cm, root lengths of 6.49 and 5.58 cm, and germination percentage and energy of 62% under T20. NGRC01815 showed particularly strong root growth, with root lengths of 10.21 cm at 14 days and 9.16 cm at 21 days, accompanied by a germination percentage and energy of 60%. ANADI also maintained relatively high root lengths of 9.32 and 7.98 cm, with a germination percentage of 58%. Meanwhile, NGRC02865 exhibited the highest germination index (4.975) and maintained the highest germination percentage and energy among the top five genotypes, both at 70%.

Other promising genotypes included GUDRIBHOG, ANADI 1, PIPAL MARSI, and RATO DHAN, which ranked seventh to tenth, with STI values of 1.276, 1.205, 1.144, and 1.113, respectively. ANADI 1 showed the highest germination percentage among the listed genotypes (74%), while GUDRIBHOG maintained a germination percentage and an energy of 66%. PIPAL MARSI also

demonstrated comparatively favorable germination performance, with a germination percentage of 66% and germination energy of 62%. These results indicate that promising performance under osmotic stress was associated with different combinations of germination and seedling-growth characteristics rather than a single trait. The lower-ranked entries within the top 20, including PANHELI, DEVRANI, BYARNI, KALO JHINUWA, NGRC01810, and SETO CHUCHEE, had STI values ranging from 0.894 to 0.963.

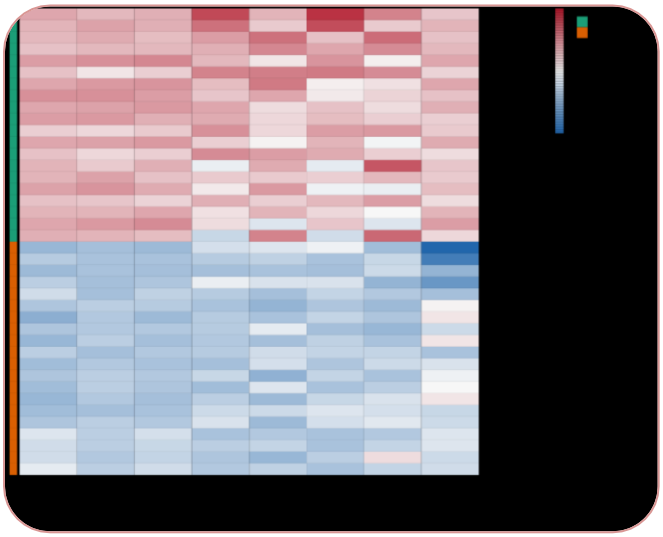


Figure 4. Heat map of eight germination and early seedling-growth traits of 154 rice genotypes under 20% PEG 6000-induced osmotic stress.
Notes: The traits include shoot length at 14 and 21 days after sowing (SL14 and SL21), root length at 14 and 21 days after sowing (RL14 and RL21), germination index (GI), germination percentage (GP), germination energy (GE), and mean germination time (MGT).

Multivariate classification of rice genotypes

Principal component analysis (PCA) also revealed substantial multivariate variation among the rice genotypes in germination and early seedling-growth responses under PEG 6000-induced osmotic stress (Figure 6; Tables 7, 8, and 9). The first two principal components (PC1 51.84% and PC2 16.64%) jointly explained 68.48% of the total variance, and the first three components explained 79.62%, and the first five components accounted for 94.00% of the total variance. PC1 primarily represented a coordinated germination and seedling-growth response. Germination index, germination energy, germination percentage, and root length at 14 days had the highest absolute loadings on PC1 (-0.410, -0.406, -0.372, and -0.296, respectively), followed by shoot length at 14 days (-0.345), shoot length at 21 days (-0.348), and root length at 21 days (-0.281).

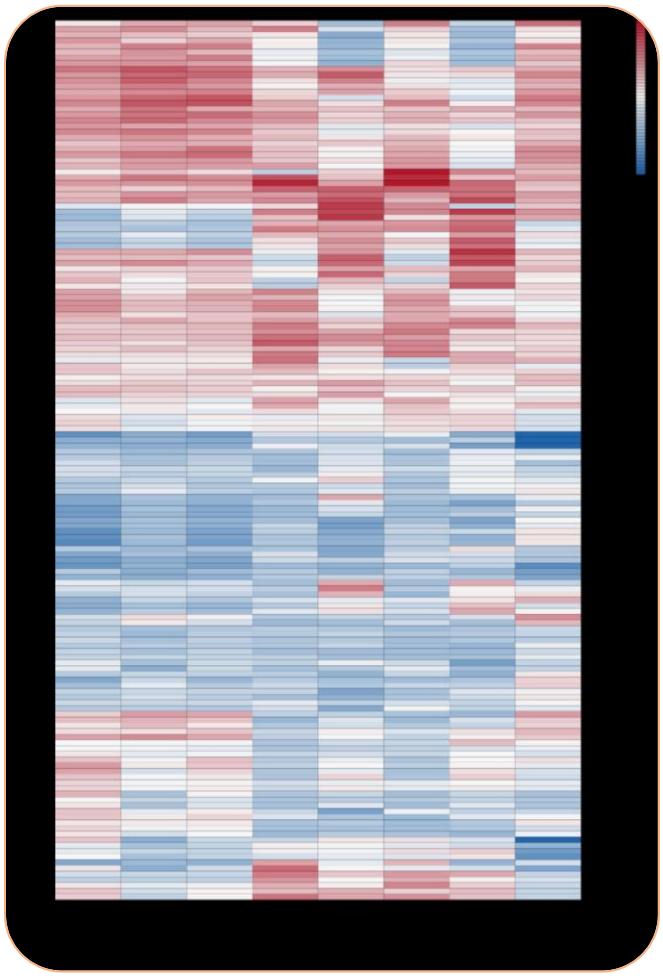


Figure 5. Complete genotype × PEG response heatmap for all 154 rice genotypes.
Notes: The traits include shoot length at 14 and 21 days after sowing (SL14 and SL21), root length at 14 and 21 days after sowing (RL14 and RL21), germination index (GI), germination percentage (GP), germination energy (GE), and mean germination time (MGT).

Mean germination time loaded in the opposite direction (0.322), suggesting a contrast between germination performance and the time required for germination. PC2 was primarily associated with seedling elongation, particularly root length at 14 and 21 days, which exhibited positive loadings of 0.476 and 0.487, respectively. Shoot length at both assessment times also loaded positively, whereas germination percentage, germination energy, and

germination index showed negative loadings. Relative water content was distinguished mainly by PC3, on which it had a strong loading of -0.876; PC3 explained 11.14% of total variance. This pattern suggests that variation in RWC was relatively distinct from the dominant germination and growth gradients captured by PC1 and PC2.

Table 5. Top 20 genotypes showing contrasting performance under 20% PEG 6000 based on integrated multi-trait screening.

Rank	Genotypes	SL14	RL14	SL21	RL21	GI	GP (%)	GE (%)	MGT	STI
1	TOLI ANDAHI	4.600	7.509	4.255	8.250	4.000	66	54	4.333	1.712
2	NGRC02872	3.959	6.49	3.831	5.583	4.058	62	62	3.935	1.456
3	NGRC01815	3.150	10.21	2.113	9.158	3.667	60	60	4.196	1.423
4	ANADI	2.849	9.318	2.522	7.979	3.835	58	54	3.993	1.32
5	NGRC02865	2.743	5.408	2.817	4.328	4.975	70	70	3.657	1.296
6	SETO JHINUWA	3.663	9.776	3.176	7.96	3.325	58	38	4.621	1.291
7	GUDRIBHOG	2.680	9.922	1.522	4.791	4.625	66	66	3.636	1.276
8	ANADI 1	2.538	8.112	1.581	5.313	4.476	74	70	4.243	1.205
9	PIPAL MARSI	3.060	5.699	2.132	4.927	4.518	66	62	3.818	1.144
10	RATO DHAN	2.933	6.032	2.226	5.468	4.033	70	66	4.485	1.113
11	NGRC01705	3.392	5.886	2.676	7.4	3.361	54	42	4.371	1.003
12	HIMALI CHOTO	2.321	4.784	2.344	3.787	4.542	66	62	3.756	0.986
13	GAJALI	3.503	8.32	2.443	5.526	3.326	58	42	4.76	0.976
14	JAULI	1.435	7.904	1.103	9.834	3.968	62	48	4.323	0.973
15	PANHELI	2.448	6.448	1.931	6.24	3.575	62	62	4.388	0.963
16	DEVRANI	1.878	8.611	1.221	3.439	4.15	68	68	4.118	0.923
17	BYARNI	2.849	6.344	2.305	7.245	3.203	58	50	4.806	0.917
18	KALO JHINUWA	2.032	7.488	1.832	3.941	4.200	62	56	3.876	0.909
19	NGRC01810	2.131	3.432	2.069	2.898	4.875	66	66	3.454	0.908
20	SETO CHUCHEE	0.823	9.443	0.788	9.254	3.667	64	56	4.688	0.894

Note: SL14 and SL21 = shoot length at 14 and 21 days; RL14 and RL21 = root length at 14 and 21 days; GI = germination index; GP = germination percentage; GE = germination energy; MGT = mean germination time; STI = stress tolerance index. All length measurements are in cm; GP and GE are expressed as percentages (%); STI and GI are unitless; MGT is expressed in days.

Table 6. Complete multi-trait tolerance-index calculations and ranking of all 154 genotypes.

Genotypes	SL14	RL14	SL21	RL21	GI	GP	GE	MGT	**S**TI	Rank
TOLI ANDAHI	4.60	7.51	4.26	8.25	4.00	66.00	54.00	4.33	1.71	1
NGRC02872	3.96	6.49	3.83	5.58	4.06	62.00	62.00	3.94	1.46	2
NGRC01815	3.15	10.21	2.11	9.16	3.67	60.00	60.00	4.20	1.42	3
ANADI	2.85	9.32	2.52	7.98	3.83	58.00	54.00	3.99	1.32	4
NGRC02865	2.74	5.41	2.82	4.33	4.98	70.00	70.00	3.66	1.30	5
SETO JHINUWA	3.66	9.78	3.18	7.96	3.33	58.00	38.00	4.62	1.29	6
GUDRIBHOG	2.68	9.92	1.52	4.79	4.63	66.00	66.00	3.64	1.28	7
ANADI 1	2.54	8.11	1.58	5.31	4.48	74.00	70.00	4.24	1.20	8
PIPAL MARSI	3.06	5.70	2.13	4.93	4.52	66.00	62.00	3.82	1.14	9
RATO DHAN	2.93	6.03	2.23	5.47	4.03	70.00	66.00	4.49	1.11	10
NGRC01705	3.39	5.89	2.68	7.40	3.36	54.00	42.00	4.37	1.00	11
HIMALI CHOTO	2.32	4.78	2.34	3.79	4.54	66.00	62.00	3.76	0.99	12
GAJALI	3.50	8.32	2.44	5.53	3.33	58.00	42.00	4.76	0.98	13
JAULI	1.43	7.90	1.10	9.83	3.97	62.00	48.00	4.32	0.97	14
PANHELI	2.45	6.45	1.93	6.24	3.58	62.00	62.00	4.39	0.96	15
DEVRANI	1.88	8.61	1.22	3.44	4.15	68.00	68.00	4.12	0.92	16
BYARNI	2.85	6.34	2.30	7.25	3.20	58.00	50.00	4.81	0.92	17
KALO JHINUWA	2.03	7.49	1.83	3.94	4.20	62.00	56.00	3.88	0.91	18
NGRC01810	2.13	3.43	2.07	2.90	4.87	66.00	66.00	3.45	0.91	19
SETO CHUCHEE	0.82	9.44	0.79	9.25	3.67	64.00	56.00	4.69	0.89	20

AANGHA 1	3.27	10.52	2.73	9.51	1.58	24.00	24.00	3.83	0.89	21
JAULE	3.80	7.88	2.74	4.79	3.14	54.00	34.00	4.93	0.87	22
CHOTTU	2.32	4.72	2.29	3.94	4.37	60.00	52.00	3.73	0.86	23
AACHAME	3.02	7.40	2.88	4.89	3.23	56.00	40.00	4.79	0.86	24
KHAIRENITAR	0.72	5.82	4.46	7.44	2.80	46.00	42.00	4.44	0.81	25
NGRC01716	3.35	6.20	2.90	6.26	2.80	50.00	36.00	4.80	0.80	26
SUNAULO	2.87	4.70	2.50	3.19	3.77	60.00	52.00	4.20	0.79	27
JAUDI KHOLE	3.23	4.85	2.27	5.62	3.41	68.00	44.00	5.30	0.79	28
DHAUDO	2.24	5.35	2.50	3.50	3.84	56.00	52.00	3.86	0.79	29
SETA ANADI	2.19	4.04	1.67	4.06	4.18	70.00	62.00	4.40	0.78	30
DOLKHE MASINO	2.45	5.20	2.13	4.62	3.53	62.00	54.00	4.52	0.76	31
KHAJIYA	1.62	9.55	1.75	7.65	3.06	56.00	36.00	5.00	0.74	32
KALANAMAK	1.62	7.01	1.58	3.59	3.83	64.00	60.00	4.31	0.74	33
JHINUWA MASINO	3.09	4.58	2.57	3.88	3.46	68.00	44.00	5.29	0.70	34
BALAMSARI	2.53	4.56	2.48	4.44	3.71	64.00	40.00	4.76	0.69	35
LAAMCHO DHAN	2.73	5.78	2.72	4.50	2.95	52.00	44.00	4.65	0.68	36
DHIKATHA	0.99	9.76	0.59	8.67	3.15	58.00	44.00	4.79	0.68	37
NGRC01876	2.07	6.34	2.09	4.02	3.48	56.00	48.00	4.25	0.68	38
HAMADI	1.60	7.55	2.05	6.36	3.06	48.00	38.00	4.28	0.65	39
BRAMHAFUL	2.51	7.68	1.99	3.96	2.92	50.00	40.00	4.44	0.61	40
RAJBHOG	3.26	5.10	2.07	3.77	3.38	64.00	32.00	5.01	0.60	41
JALUNAR	2.13	1.60	2.86	2.51	3.73	48.00	48.00	3.29	0.55	42
PADANI	2.43	3.95	1.58	3.13	3.66	66.00	50.00	4.79	0.53	43
POKHARA MASINO	3.27	3.64	1.80	1.60	3.34	64.00	56.00	4.94	0.52	44
NGRC03207	3.44	7.45	2.63	5.60	2.47	54.00	14.00	6.00	0.51	45
GHUI KUMARI	1.60	6.28	1.77	5.51	2.98	58.00	42.00	5.09	0.49	46
THAPACHINIYA	2.02	11.04	2.69	2.40	2.31	38.00	30.00	4.53	0.49	47
PURANO MANSULI	2.41	3.70	2.25	5.51	3.12	62.00	34.00	5.36	0.48	48
KRISHNABHOG	1.95	7.76	1.32	4.71	2.94	58.00	38.00	5.41	0.43	49
STAHANIYA BASMATI	2.35	11.23	1.58	6.76	1.38	22.00	22.00	4.08	0.43	50
DUDHE DHAN	2.41	4.74	1.34	4.85	3.12	56.00	36.00	4.71	0.42	51
SARASWOTI	0.65	6.05	1.75	8.60	2.64	48.00	32.00	4.75	0.41	52
SUNGADHI	3.39	8.22	2.64	8.31	1.28	28.00	8.00	5.57	0.40	53
BHATTE	1.71	1.23	1.38	1.06	4.29	60.00	60.00	3.58	0.40	54
BHATTE DHAN	3.52	4.20	0.67	6.26	2.60	40.00	32.00	4.20	0.38	55
MEDESHE MASINO	0.74	4.49	0.65	4.29	3.65	64.00	56.00	4.62	0.35	56
NGRC03369	2.76	5.55	2.34	4.29	2.03	38.00	34.00	4.79	0.34	57
JHINUWA	2.40	7.01	1.78	5.74	2.46	56.00	20.00	5.99	0.32	58
JHINUWA BASMATI	0.70	6.73	0.66	7.66	3.16	60.00	28.00	5.20	0.32	59
NGRC03206	1.79	5.37	1.71	4.40	2.78	46.00	34.00	4.44	0.32	60
SETO DHAN	2.64	4.56	2.01	4.17	2.40	40.00	32.00	4.40	0.31	61
NGRC01757	1.41	1.66	1.22	1.55	4.24	64.00	52.00	4.12	0.30	62
AKALE PANHELO	2.60	4.89	2.78	5.20	2.25	48.00	24.00	5.92	0.29	63
DODHI DHAN	3.37	6.28	2.48	6.36	1.64	36.00	16.00	6.00	0.27	64
NGRC02831	1.48	1.14	1.69	1.51	3.73	66.00	54.00	4.55	0.27	65
NGRC03381	2.76	5.49	0.53	5.18	2.63	56.00	32.00	5.50	0.24	66
JHINUWA DHAN	0.84	4.97	0.99	4.71	3.12	50.00	34.00	4.31	0.19	67
NGRC04939	2.53	7.38	2.48	7.92	1.15	26.00	12.00	5.92	0.17	68
MANSARA	1.89	8.17	1.92	8.60	1.22	24.00	16.00	5.50	0.17	69
TAULI	2.45	7.28	1.23	6.61	1.47	28.00	24.00	4.88	0.17	70

NGRC02822	1.37	1.98	0.97	1.89	3.37	58.00	54.00	4.42	0.14	71
ANADHI DHAN	1.61	1.12	1.51	1.82	3.57	66.00	38.00	4.97	0.10	72
JHILINGI	1.93	4.26	1.85	4.85	2.06	44.00	32.00	5.55	0.09	73
TULSI	1.74	5.20	1.66	5.02	2.29	50.00	24.00	5.76	0.09	74
NGRC08300	1.81	7.49	1.64	7.98	1.25	24.00	16.00	5.33	0.08	75
JHINUWA 1	1.33	5.20	1.77	5.12	2.51	50.00	22.00	5.72	0.08	76
BANKAE	0.74	4.62	0.53	4.25	3.15	66.00	32.00	5.48	0.04	77
NGRC03380	1.67	4.53	1.60	4.97	2.34	52.00	20.00	5.69	0.04	78
BANSPATHI	0.70	2.68	0.32	1.24	3.63	54.00	52.00	3.92	0.02	79
AAMPJHUTTE	1.79	0.73	1.52	1.29	3.02	60.00	44.00	5.13	0.00	80
KALO TAKMURO	0.93	5.10	1.06	4.46	2.54	52.00	28.00	5.54	-0.03	81
NGRC07875	0.17	3.66	0.12	3.21	3.23	58.00	46.00	4.62	-0.04	82
NGRC02133	2.95	4.68	2.23	4.23	1.48	30.00	14.00	5.68	-0.05	83
TIRABFHUL	0.57	4.12	0.35	4.06	3.24	56.00	28.00	4.93	-0.06	84
KALO MACHYA	1.03	4.20	1.06	4.06	2.47	52.00	28.00	5.49	-0.09	85
JETHO BUDO	0.49	5.82	0.37	5.22	2.57	56.00	28.00	5.79	-0.09	86
NGRC08278	0.49	3.99	0.32	4.66	2.95	64.00	20.00	5.69	-0.16	87
YOBO	0.53	3.41	0.45	2.55	2.59	48.00	44.00	4.75	-0.17	88
RATUWA	0.45	3.30	0.69	5.70	2.29	44.00	28.00	5.18	-0.19	89
LALAYATH	1.16	2.12	0.13	3.48	2.95	56.00	32.00	5.29	-0.20	90
NGRC01740	0.55	2.83	0.47	3.19	2.42	36.00	36.00	3.78	-0.20	91
BASMATI	0.63	8.76	0.47	4.19	1.80	36.00	20.00	5.44	-0.21	92
PANHELO JHINUWA	3.57	4.68	1.10	3.09	1.74	48.00	0.00	7.00	-0.28	93
MANHU DHAN	0.87	3.45	0.67	4.08	2.29	48.00	24.00	5.67	-0.28	94
LUMRU	0.34	7.18	0.18	6.34	1.80	40.00	16.00	6.00	-0.30	95
NGRC08372	1.24	5.43	0.93	6.47	1.01	20.00	12.00	5.20	-0.33	96
AMUHAWA	1.43	4.56	1.64	5.20	1.53	40.00	16.00	7.20	-0.34	97
BASNADHAR KALO	1.08	3.99	1.02	4.54	1.25	20.00	16.00	4.20	-0.35	98
SETO GEDE	0.40	6.99	0.10	4.37	1.51	28.00	20.00	4.86	-0.38	99
NGRC07870	0.38	2.41	0.20	2.05	2.57	48.00	32.00	5.00	-0.41	100
JHINIDHAN	1.48	6.03	0.35	3.94	1.34	28.00	16.00	5.57	-0.42	101
KALO JUNGE	0.49	2.72	0.59	3.15	2.10	44.00	28.00	5.45	-0.43	102
MOTI	0.84	0.21	1.18	2.38	2.49	56.00	24.00	5.93	-0.43	103
KARIYA KAMODH	1.33	2.66	0.85	2.57	1.68	34.00	16.00	5.28	-0.50	104
KALA CHAMAL	0.53	3.85	0.51	3.65	1.42	28.00	24.00	5.00	-0.51	105
KATHE JHINUWA	1.69	4.47	1.04	2.94	1.57	40.00	8.00	6.70	-0.51	106
BASMATI NOKHI	0.42	1.98	0.22	1.95	2.45	52.00	28.00	5.78	-0.53	107
NGRC05024	0.23	1.68	0.78	1.56	1.85	32.00	32.00	4.37	-0.53	108
GAJAR GAULA	2.80	4.08	2.14	1.09	0.70	16.00	4.00	5.75	-0.54	109
RATA CHCCHE	0.68	4.99	0.49	3.61	1.59	36.00	12.00	5.89	-0.54	110
RATO JADHE	1.05	4.06	0.99	4.56	1.57	44.00	8.00	7.45	-0.58	111
NGRC05725	0.46	2.91	0.26	2.61	2.46	60.00	8.00	6.40	-0.58	112
NGRC03305	0.17	3.97	0.79	3.73	1.68	36.00	16.00	6.00	-0.58	113
RAINI MODHO	0.89	2.14	0.67	2.36	1.30	24.00	24.00	4.67	-0.60	114
NGRC08289	0.21	2.29	0.32	1.18	2.35	48.00	20.00	5.33	-0.61	115
ASORJOG	0.61	5.18	0.67	5.60	0.82	18.00	8.00	5.78	-0.63	116
PANGALI	0.84	5.25	1.57	3.27	1.67	56.00	0.00	8.57	-0.63	117
NGRC07860	0.40	3.95	0.20	3.36	1.98	48.00	8.00	6.33	-0.63	118
BHULING JHAR	0.57	3.24	0.22	3.90	1.29	26.00	20.00	5.37	-0.65	119
NGRC04999	0.46	2.48	0.48	2.48	1.94	44.00	16.00	6.27	-0.68	120

PUSA BARSA	1.06	0.71	1.26	2.45	1.29	28.00	12.00	5.57	-0.73	121
NGRC08276	0.34	3.54	0.35	3.54	1.33	28.00	8.00	5.43	-0.73	122
NGRC03422	0.72	4.16	0.74	0.19	1.69	40.00	8.00	6.00	-0.74	123
JEERA MASINO	0.79	0.42	0.28	3.32	1.52	32.00	16.00	5.37	-0.75	124
OR	0.32	0.94	0.36	2.92	1.43	28.00	20.00	5.00	-0.76	125
NEPALI PUWA	0.32	3.66	0.33	3.84	1.47	36.00	8.00	6.44	-0.76	126
NGRC08273	0.38	2.35	1.10	1.62	1.67	40.00	0.00	6.00	-0.79	127
NGRC05040	0.70	1.77	0.25	1.74	1.57	32.00	16.00	5.50	-0.80	128
NGRC07861	0.68	1.08	0.70	0.89	1.48	28.00	16.00	5.00	-0.80	129
EKLE	0.30	7.32	0.36	1.68	0.76	16.00	8.00	5.50	-0.81	130
DAILE MASINO	0.44	3.43	0.18	3.59	1.18	28.00	4.00	6.00	-0.85	131
LOCAL JEERA	0.53	3.04	0.26	1.24	1.32	28.00	12.00	5.43	-0.86	132
BAGARNE	0.59	2.87	0.41	2.16	1.37	32.00	4.00	6.00	-0.87	133
NGRC07866	0.58	1.87	0.17	1.81	0.85	16.00	16.00	4.75	-0.91	134
BHOLA	0.46	2.18	0.22	1.93	1.46	34.00	8.00	6.17	-0.92	135
TILAKI	0.39	0.52	0.46	4.89	1.16	28.00	4.00	6.29	-0.93	136
NGRC07869	0.63	2.25	0.16	1.90	1.24	28.00	8.00	5.86	-0.94	137
MANSARA 1	0.36	1.54	0.16	1.33	1.55	32.00	8.00	5.88	-0.98	138
HIMALI	1.13	0.70	0.81	3.07	0.68	16.00	8.00	6.25	-1.00	139
MARSHI TANGALO	0.91	2.70	0.95	2.70	0.48	12.00	0.00	6.33	-1.04	140
SAURAV	0.63	0.68	0.65	2.75	0.42	8.00	4.00	5.00	-1.06	141
BASMATI PASCHIM	0.18	3.54	0.16	1.58	0.57	12.00	8.00	5.33	-1.07	142
THARU ANADHI 1	0.87	0.04	0.59	0.99	0.73	16.00	8.00	5.50	-1.11	143
NGRC03420	0.28	3.02	0.25	2.25	0.54	12.00	4.00	6.00	-1.14	144
THARU ANADHI 2	0.57	2.89	0.59	2.05	0.71	20.00	0.00	7.00	-1.15	145
SETO DALLE	0.47	1.04	0.49	1.04	0.40	8.00	8.00	5.00	-1.15	146
NGRC03212	0.53	3.81	0.15	0.35	0.68	16.00	4.00	6.25	-1.18	147
TAICHIN	0.53	1.14	0.60	1.17	0.20	4.00	4.00	5.00	-1.20	148
INDRABELI	0.34	0.31	0.25	0.48	0.78	16.00	8.00	5.25	-1.20	149
NIRTACHAN BARDIYA	0.59	1.08	0.59	1.24	1.00	28.00	0.00	7.14	-1.21	150
SYAUDO	1.46	3.22	0.93	0.12	0.60	20.00	0.00	8.40	-1.27	151
NGRC07900	0.28	1.14	0.13	2.32	0.37	10.00	2.00	7.50	-1.47	152
NGRC03389	0.53	2.15	0.20	2.19	0.54	18.00	2.00	9.27	-1.52	153
CHOTTE	1.06	3.43	1.22	0.68	0.20	8.00	0.00	10.50	-1.61	154

Notes: SL14 = shoot length at 14 days after sowing; RL14 = root length at 14 days after sowing; SL21 = shoot length at 21 days after sowing; RL21 = root length at 21 days after sowing; GI = germination index; GP = germination percentage; GE = germination energy; MGT = mean germination time; STI = stress tolerance index. All length measurements are in cm; GP, GE, and STI are expressed as percentages (%); GI is unitless; MGT is expressed in days.

Table 7. PCA explained variance.

Components	Eigenvalue	Variance per cent	Cumulative per cent
PC1	4.67	51.84	51.84
PC2	1.50	16.64	68.48
PC3	1.00	11.14	79.62
PC4	0.71	7.89	87.51
PC5	0.58	6.49	94.00
PC6	0.26	2.87	96.87
PC7	0.17	1.88	98.76
PC8	0.09	1.05	99.81
PC9	0.02	0.19	100.00

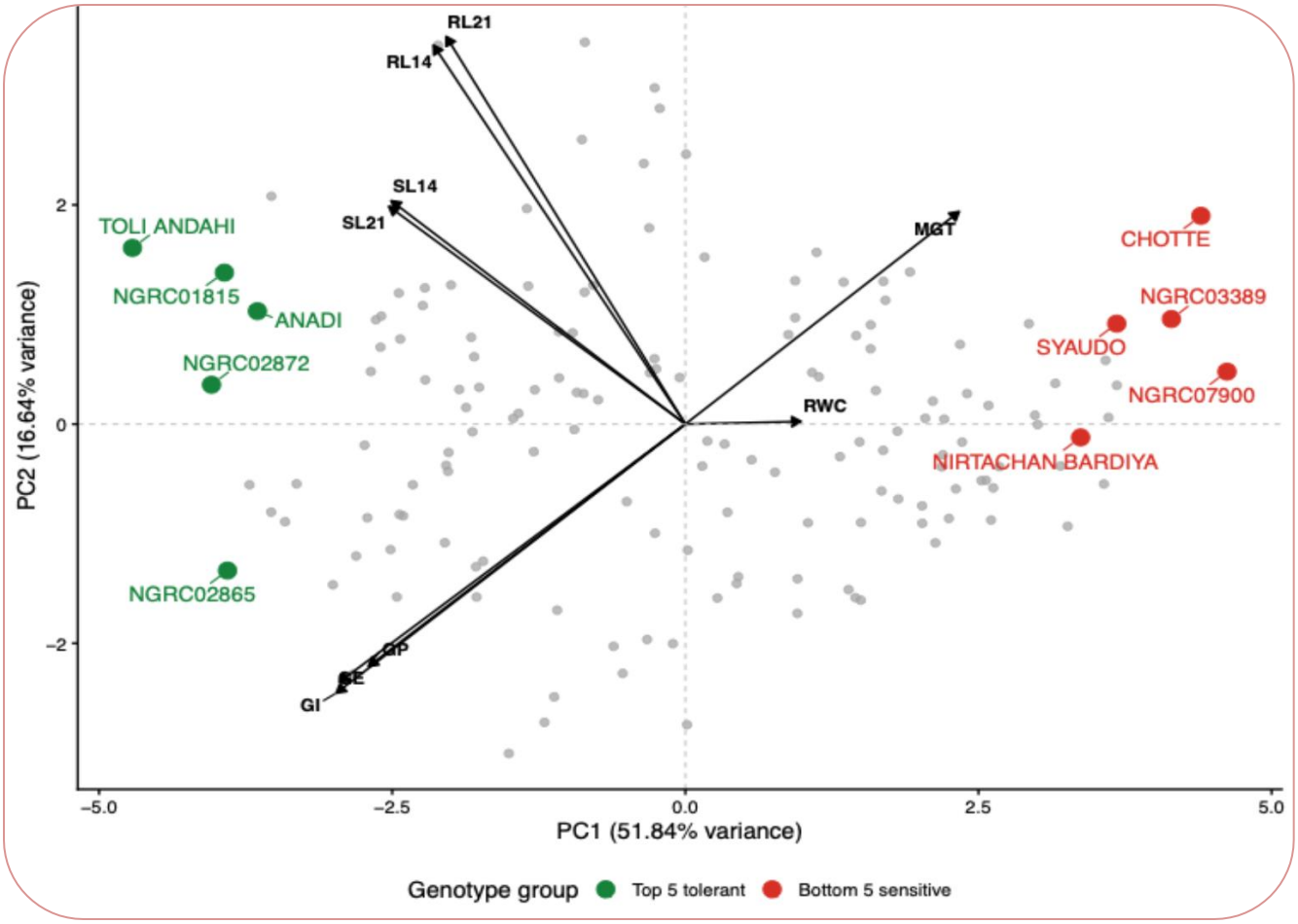


Figure 6. Principal component analysis (PCA) biplot illustrating the relationships among rice genotypes and germination and early seedling-growth traits under 20% PEG 6000-induced osmotic stress.

Notes: SL14 = shoot length at 14 days after sowing; RL14 = root length at 14 days after sowing; SL21 = shoot length at 21 days after sowing; RL21 = root length at 21 days after sowing; GI = germination index; GP = germination percentage; GE = germination energy; MGT = mean germination time; STI = stress tolerance index. All length measurements are in cm; GP and GE are expressed as percentages (%); STI and GI are unitless; MGT is expressed in days.

Table 8. PCA genotype scores.

Genotypes	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
AACHAME	-2.432	0.776	-0.363	-0.752	0.085	0.378	0.280	0.183	0.005
AAMPJHUTTE	-0.326	-1.966	-0.140	-1.306	0.493	-0.189	-0.089	-0.148	0.271
AANGHA 1	-2.110	3.457	0.530	0.178	-1.748	-0.108	-0.146	0.137	0.023
AKALE PANHELO	-0.860	1.203	-0.104	-1.272	0.637	-0.393	0.296	0.087	0.102
AMUHAWA	0.936	1.308	0.407	-0.502	1.340	-0.349	0.279	-0.360	0.088
ANADHI DHAN	-0.612	-2.028	-0.095	-0.966	0.677	-0.318	-0.026	0.333	-0.005
ANADI	-3.649	1.030	0.207	0.563	-0.329	0.013	0.093	-0.141	-0.143
ANADI 1	-3.532	-0.804	-0.001	0.782	0.397	0.649	-0.227	-0.444	0.067
ASORJOG	1.916	1.388	1.068	0.467	-0.442	-0.243	0.068	-0.168	-0.053
BAGARNE	2.360	-0.164	0.790	-0.345	0.267	0.187	-0.085	0.359	-0.072
BALAMSARI	-2.040	-0.377	-0.638	-0.609	0.356	-0.299	0.158	0.334	-0.109
BANKAE	-0.259	-0.996	0.656	0.831	1.106	-0.037	-0.078	0.302	0.093
BANSPATHI	0.014	-2.743	-1.781	1.400	-0.907	0.245	0.018	-0.120	-0.111
BASMATI	0.879	0.816	-0.287	1.792	-0.277	1.049	0.298	0.148	0.020

BASMATI NOKHI	1.450	-1.584	-0.598	0.643	0.561	-0.080	-0.033	0.002	0.106
BASMATI PASCHIM	3.005	-0.005	1.153	-0.201	-0.978	0.605	0.102	-0.066	0.038
BASNADHAR KALO	1.081	0.471	0.884	-0.084	-1.551	-0.296	-0.066	0.170	0.003
BHATTE	-1.506	-3.005	-0.042	-0.820	-0.522	0.020	-0.082	-0.151	-0.229
BHATTE DHAN	-1.077	0.421	0.493	-0.317	-0.742	-0.393	-1.947	-0.139	-0.214
BHOLA	2.563	-0.513	0.240	-0.049	0.359	0.062	-0.116	0.171	-0.039
BHULING JHAR	1.809	-0.065	1.101	0.222	-0.436	-0.154	-0.242	-0.317	0.061
BRAMHAFUL	-1.758	0.335	0.246	-0.351	-0.318	0.873	0.029	0.122	0.064
BYARNI	-2.600	0.702	0.100	-0.101	0.199	-0.500	-0.190	-0.390	0.146
CHOTTE	4.397	1.900	-0.151	-1.794	2.601	0.955	0.683	-1.584	-0.415
CHOTTU	-2.515	-1.145	-0.748	-0.187	-0.513	-0.096	0.227	0.176	-0.410
DAILE MASINO	2.403	0.278	1.047	0.156	0.140	-0.058	-0.197	0.179	-0.080
DEVRANI	-2.808	-1.204	0.482	0.759	0.143	1.382	0.068	-0.390	0.129
DHAUDO	-2.406	-0.838	0.077	-0.744	-0.484	0.250	0.464	0.054	-0.154
DHIKATHA	-1.826	0.791	1.379	2.315	0.369	0.076	-0.107	-0.198	0.041
DODHI DHAN	-0.355	2.377	-1.923	-0.272	-0.093	-0.366	-0.292	0.009	0.026
DOLKHE MASINO	-2.324	-0.553	0.082	-0.458	0.153	-0.023	0.015	-0.243	0.152
DUDHE DHAN	-1.293	-0.252	0.322	-0.224	0.158	-0.109	-0.615	0.148	-0.036
EKLE	2.343	0.727	1.163	0.291	-0.706	1.568	0.408	0.117	0.023
GAJALI	-2.594	0.986	-1.238	0.026	-0.021	0.493	-0.263	0.112	-0.006
GAJAR GAULA	1.689	1.302	-1.260	-1.828	-0.883	0.734	-0.062	0.203	0.032
GHUI KUMARI	-1.423	0.098	0.253	0.359	0.408	-0.065	0.320	-0.091	0.187
GUDRIBHOG	-3.717	-0.554	0.364	0.797	-0.209	1.340	-0.255	-0.183	-0.248
HAMADI	-1.801	0.615	0.752	0.404	-0.417	-0.021	0.489	0.146	-0.127
HIMALI	3.155	0.372	-2.023	0.122	-0.708	-0.831	-0.133	-0.382	-0.025
HIMALI CHOTO	-3.006	-1.466	-0.214	-0.426	-0.212	0.025	0.282	-0.079	-0.216
INDRABELI	3.260	-0.932	0.776	-0.929	-0.920	0.046	-0.099	0.027	0.021
JALUNAR	-1.781	-1.578	-0.337	-1.583	-1.236	-0.576	0.577	0.190	-0.277
JAUDI KHOLE	-1.870	0.151	-3.326	0.705	0.318	-0.655	-0.322	0.001	0.188
JAULE	-2.444	1.195	-0.448	-1.041	0.147	0.659	-0.299	0.293	-0.105
JAULI	-2.683	0.480	1.156	1.946	0.239	-0.847	-0.225	-0.128	-0.315
JEERA MASINO	2.019	-0.746	0.686	-0.331	-0.240	-0.778	-0.516	-0.058	0.021
JETHO BUDO	0.335	-0.182	0.100	1.618	0.808	-0.043	0.059	0.077	0.109
JHILINGI	-0.302	0.468	0.224	-0.598	0.213	-0.338	0.079	-0.286	0.234
JHINIDHAN	1.457	0.807	-0.613	0.922	-0.509	0.531	-0.480	-0.086	0.013
JHINUWA	-0.786	1.266	-0.334	0.125	0.939	0.092	-0.181	0.391	0.084
JHINUWA 1	-0.249	0.503	0.429	-0.096	0.669	-0.275	0.407	0.272	-0.050
JHINUWA BASMATI	-0.868	0.278	1.189	1.654	0.788	-0.535	-0.062	0.334	-0.157
JHINUWA DHAN	-0.500	-0.707	0.132	0.980	-0.413	-0.198	0.184	0.285	-0.189
JHINUWA MASINO	-2.019	-0.259	-1.461	-0.751	0.690	-0.092	-0.040	0.084	0.189
KALA CHAMAL	1.485	-0.163	0.492	0.481	-0.772	-0.034	0.066	-0.219	0.144
KALANAMAK	-2.051	-1.083	-0.985	1.027	-0.146	0.671	0.425	-0.236	0.121
KALO JHINUWA	-2.712	-0.855	0.260	0.260	-0.214	0.783	0.250	0.042	-0.201
KALO JUNGE	1.046	-0.900	0.827	0.065	0.260	-0.179	0.091	-0.121	0.146
KALO MACHYA	0.144	-0.383	0.538	0.131	0.555	-0.118	0.105	0.113	0.123
KALO TAKMURO	0.188	-0.155	-0.539	0.945	0.382	-0.111	0.271	0.136	0.074
KARIYA KAMODH	1.319	-0.297	0.602	-0.705	-0.226	0.023	-0.288	0.174	0.014
KATHE JHINUWA	1.580	0.687	-0.938	-0.095	0.761	0.304	-0.178	0.204	0.018
KHAIRENITAR	-2.239	1.082	0.393	-0.592	-0.533	-1.370	2.611	0.042	0.128
KHAJIYA	-1.999	1.269	0.821	1.130	0.396	0.173	0.336	0.136	-0.026
KRISHNABHOG	-1.284	0.313	0.448	0.376	0.718	0.728	-0.086	-0.025	0.113
LAAMCHO DHAN	-1.928	0.315	-0.646	-0.724	-0.260	0.045	0.308	-0.080	0.146

LALAYATH	0.438	-1.455	0.058	0.493	0.525	-0.363	-0.703	0.020	-0.059
LOCAL JEERA	2.307	-0.591	0.845	-0.332	-0.325	0.558	-0.079	0.170	0.029
LUMRU	0.936	0.970	1.332	1.512	0.598	0.102	-0.020	0.012	-0.032
MANHU DHAN	0.764	-0.440	0.282	0.395	0.496	-0.297	-0.094	0.055	0.044
MANSARA	-0.219	2.881	0.929	0.359	-0.450	-0.423	0.135	-0.208	-0.016
MANSARA 1	2.607	-0.876	0.783	-0.431	0.191	0.108	-0.131	0.233	-0.123
MARSHI TANGALO	2.930	0.916	0.714	-0.972	-0.263	-0.012	0.089	-0.134	-0.107
MEDESHE MASINO	-1.093	-1.698	0.110	1.239	0.275	-0.096	0.099	-0.377	0.106
MOTI	0.956	-1.412	0.169	-0.806	0.988	-0.745	0.152	0.197	0.144
NEPALI PUWA	2.111	0.209	0.941	0.291	0.741	-0.115	0.023	0.088	-0.037
NGRC01705	-2.639	0.952	-1.172	-0.056	-0.430	-0.805	-0.309	0.015	-0.132
NGRC01716	-2.222	1.242	-0.301	-0.946	-0.134	-0.321	-0.094	0.049	0.039
NGRC01740	0.452	-1.393	1.030	0.283	-1.212	-0.148	-0.074	-0.041	-0.027
NGRC01757	-1.202	-2.722	0.071	-0.513	0.055	-0.036	-0.018	0.063	-0.249
NGRC01810	-2.831	-2.280	-0.412	-0.407	-0.430	-0.055	0.194	-0.131	-0.360
NGRC01815	-3.931	1.382	0.540	0.926	-0.098	0.053	-0.383	-0.509	0.051
NGRC01876	-2.024	-0.431	0.066	-0.177	-0.246	0.403	0.324	0.031	-0.021
NGRC02133	0.164	1.524	-0.001	-1.572	-0.170	0.017	-0.302	0.059	-0.010
NGRC02822	-0.534	-2.275	-0.851	0.131	-0.254	-0.063	-0.090	-0.338	0.162
NGRC02831	-1.119	-2.489	-0.093	-0.952	0.312	-0.227	0.255	-0.121	0.163
NGRC02865	-3.905	-1.336	-0.300	-0.596	-0.128	0.009	0.375	-0.224	-0.272
NGRC02872	-4.041	0.359	-1.786	-0.906	-0.565	-0.213	0.345	-0.207	0.009
NGRC03206	-0.947	-0.050	0.419	-0.216	-0.425	0.062	0.106	0.170	-0.052
NGRC03207	-1.351	1.966	-0.480	-0.925	0.912	0.230	-0.255	0.640	-0.038
NGRC03212	3.608	0.060	-1.437	0.507	-0.596	0.915	0.115	-0.033	-0.048
NGRC03305	2.208	0.046	-3.046	2.123	-0.374	-0.434	0.692	0.070	0.012
NGRC03369	-0.961	0.832	0.022	-1.105	-0.583	0.190	-0.022	-0.168	0.209
NGRC03380	-0.049	0.424	-0.274	0.019	0.569	-0.403	0.041	0.365	0.091
NGRC03381	-0.744	0.221	0.366	0.133	0.716	0.210	-1.363	-0.092	0.116
NGRC03389	4.145	0.959	0.520	-0.773	2.146	0.203	0.013	-1.164	-0.275
NGRC03420	3.680	0.353	-1.988	1.145	-1.063	0.003	0.211	-0.135	-0.061
NGRC03422	2.525	-0.516	-3.272	1.175	-0.220	0.751	0.469	0.641	0.063
NGRC04939	-0.262	3.068	0.444	-0.489	-0.131	-0.467	0.116	-0.110	0.016
NGRC04999	1.816	-0.683	0.336	0.012	0.817	-0.063	0.069	0.090	0.016
NGRC05024	1.391	-1.511	0.390	-0.167	-1.087	-0.072	0.416	-0.127	0.176
NGRC05040	2.247	-0.861	-0.185	0.013	-0.304	0.004	-0.237	0.037	0.004
NGRC05725	1.498	-0.898	0.438	0.290	1.562	-0.015	-0.146	0.779	-0.012
NGRC07860	1.687	-0.240	0.895	0.404	1.122	0.101	-0.109	0.469	-0.028
NGRC07861	2.132	-1.084	0.431	-0.834	-0.706	0.045	0.036	0.165	0.035
NGRC07866	2.626	-0.582	0.296	-0.095	-1.424	0.057	-0.248	-0.141	0.114
NGRC07869	2.678	-0.389	0.006	0.007	-0.153	0.106	-0.249	0.118	-0.029
NGRC07870	0.955	-1.727	0.716	0.257	0.092	0.105	-0.079	0.015	0.039
NGRC07875	-0.106	-2.004	1.035	0.962	0.220	0.088	0.026	-0.155	0.090
NGRC07900	4.619	0.478	-2.833	1.070	-0.151	-0.492	0.141	-0.710	-0.194
NGRC08273	2.187	-0.391	0.109	-0.518	0.450	-0.032	0.522	0.835	-0.054
NGRC08276	2.046	0.053	1.187	0.148	-0.264	-0.060	-0.022	0.255	-0.065
NGRC08278	0.359	-0.805	0.717	0.966	1.217	-0.355	-0.169	0.609	-0.023
NGRC08289	1.496	-1.606	0.769	-0.084	0.365	0.274	0.114	0.430	0.017
NGRC08300	0.006	2.463	0.944	0.341	-0.562	-0.383	-0.031	-0.163	-0.028
NGRC08372	1.119	1.567	0.951	0.374	-0.811	-0.433	-0.219	-0.103	-0.022
NIRTACHAN BARDIYA	3.371	-0.121	-0.223	-0.724	0.784	-0.061	0.087	0.013	-0.081
OR	2.020	-0.905	1.039	-0.254	-0.633	-0.558	-0.102	-0.129	0.074

PADANI	-1.725	-1.251	-0.137	-0.565	0.502	0.169	-0.353	-0.091	0.094
PANGALI	1.706	1.130	-0.017	-0.412	2.891	0.291	0.758	0.348	0.136
PANHELI	-2.736	-0.192	-0.238	0.465	-0.048	-0.169	-0.081	-0.572	0.217
PANHELO JHINUWA	1.349	1.295	-4.166	0.437	0.743	0.256	-1.234	0.598	-0.022
PIPAL MARSI	-3.414	-0.891	-0.076	-0.349	-0.127	0.072	-0.357	-0.187	-0.242
POKHARA MASINO	-1.785	-1.302	-0.468	-1.447	0.449	0.657	-0.636	-0.413	0.347
PURANO MANSULI	-1.468	0.052	-0.063	-0.723	0.774	-0.776	-0.066	0.176	0.070
PUSA BARSA	2.196	-0.280	-1.229	-0.424	-0.604	-0.715	0.162	0.092	0.048
RAINI MODHO	1.673	-0.610	0.422	-0.360	-1.160	-0.071	-0.108	-0.200	0.160
RAJBHOG	-1.815	-0.070	-0.315	-1.043	0.610	0.246	-0.581	0.520	-0.034
RATA CHCCHE	1.624	0.306	0.211	0.620	0.186	0.261	0.026	0.196	0.001
RATO DHAN	-3.316	-0.545	-0.092	-0.237	0.396	-0.009	-0.181	-0.521	0.191
RATO JADHE	1.581	0.905	0.544	-0.210	1.725	-0.246	0.039	-0.058	0.025
RATUWA	0.565	-0.326	0.553	0.888	0.011	-0.885	0.080	-0.097	0.015
SARASWOTI	-1.080	0.842	1.119	1.078	-0.069	-1.181	0.656	0.052	-0.012
SAURAV	2.981	0.081	0.963	-0.908	-1.386	-0.585	-0.135	-0.010	-0.007
SETA ANADI	-2.460	-1.578	0.056	-0.210	0.400	-0.121	-0.159	-0.337	0.043
SETO CHUCHEE	-2.218	0.403	-0.270	3.278	0.197	-0.369	0.216	-0.455	0.036
SETO DALLE	3.199	-0.382	0.856	-0.978	-1.422	0.062	0.000	-0.088	0.083
SETO DHAN	-0.926	0.289	0.183	-1.016	-0.714	-0.021	-0.277	0.070	0.012
SETO GEDE	1.138	0.431	1.439	1.091	-0.689	0.694	-0.040	0.021	0.021
SETO JHINUWA	-3.530	2.079	-0.015	-0.311	0.104	0.100	0.006	0.302	-0.053
STAHANIYA BASMATI	-0.882	2.596	0.762	0.747	-1.614	1.038	-0.141	0.148	0.059
SUNAULO	-2.437	-0.824	-0.294	-1.127	-0.153	0.239	0.047	-0.008	-0.027
SUNGADHI	-0.857	3.484	0.320	-0.717	-0.296	-0.319	-0.341	0.220	-0.045
SYAUDO	3.679	0.917	-1.498	-0.942	1.200	0.916	0.128	-0.522	-0.133
TAICHIN	3.361	-0.095	0.802	-1.055	-1.579	0.030	0.035	-0.028	0.054
TAULI	-0.308	1.790	0.735	0.138	-0.787	0.130	-0.659	-0.227	0.090
THAPACHINIYA	-1.340	1.259	0.248	-0.470	-0.725	2.079	1.134	0.446	0.068
THARU ANADHI 1	3.570	-0.547	-2.959	0.383	-1.447	-0.477	-0.036	-0.026	0.043
THARU ANADHI 2	3.584	0.581	-1.895	0.548	0.032	0.039	0.278	-0.099	-0.099
TILAKI	2.585	0.169	0.913	-0.183	0.335	-1.299	-0.206	-0.048	-0.117
TIRABFHUL	0.020	-1.151	0.963	0.772	0.456	-0.089	-0.143	0.404	-0.259
TOLI ANDAHI	-4.715	1.607	-1.363	-1.016	-0.033	-0.742	0.085	-0.017	-0.016
TULSI	-0.263	0.596	0.226	-0.184	0.627	-0.174	0.079	0.138	0.116
YOBO	0.273	-1.588	0.898	0.297	-0.113	0.241	0.069	-0.362	0.217

Table 9. PCA trait loadings.

Trait	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
GP	-0.372	-0.304	-0.083	0.142	0.540	-0.066	-0.063	0.430	0.507
GE	-0.406	-0.324	-0.021	0.086	-0.081	0.072	0.092	-0.796	0.263
GI	-0.410	-0.338	-0.057	0.098	0.199	0.001	0.036	0.075	-0.812
SL14	-0.345	0.281	-0.311	-0.445	-0.023	0.132	-0.697	-0.049	-0.015
RL14	-0.296	0.476	0.097	0.399	0.005	0.695	0.165	0.082	0.009
SL21	-0.348	0.273	-0.232	-0.506	-0.052	-0.184	0.668	0.098	0.043
RL21	-0.281	0.487	0.198	0.407	0.025	-0.670	-0.133	-0.107	-0.036
MGT	0.322	0.267	-0.150	-0.084	0.794	0.045	0.080	-0.381	-0.110
RWC	0.136	0.003	-0.876	0.421	-0.170	-0.076	0.047	0.015	0.004

Notes: SL14 = shoot length at 14 days after sowing; RL14 = root length at 14 days after sowing; SL21 = shoot length at 21 days after sowing; RL21 = root length at 21 days after sowing; GI = germination index; GP = germination percentage; GE = germination energy; MGT = mean germination time; STI = stress tolerance index. All length measurements are in cm; GP and GE are expressed as percentages (%); STI and GI are unitless; MGT is expressed in days.

The genotype scores revealed substantial multivariate variation along PC1 and PC2. Among the genotypes with negative PC1 scores, TOLI ANDAHI, NGRC02872, NGRC01815, ANADI, and NGRC02865 were also identified as promising performers in the composite multi-trait stress-tolerance ranking. Their favorable performance was associated with different combinations of germination and seedling-growth traits under 20% PEG 6000: TOLI ANDAHI showed comparatively strong shoot and root growth, NGRC01815 and ANADI maintained relatively high root lengths, NGRC02872 exhibited favorable overall performance across several traits, and NGRC02865 showed high germination percentage and germination energy. In contrast, NGRC07900, CHOTTE, NGRC03389, and NGRC03420 had strongly positive PC1 scores and were among the genotypes exhibiting poorer performance in key germination or seedling-growth traits under severe osmotic stress. Their responses included reduced germination performance and/or weak seedling growth, consistent with greater sensitivity to the imposed stress.

Characterization of contrasting higher- and lower-ranked genotypes

Five higher-ranked and five lower-ranked rice genotypes were selected from the composite STI ranking for descriptive characterization of their contrasting responses under 20% PEG 6000-induced osmotic stress (Table 10). The higher-ranked genotypes generally maintained higher germination and root growth and exhibited shorter mean germination times than the low-ranked genotypes. Among the tolerant group, NGRC02865 recorded the highest germination percentage and germination energy, both at 70%, followed by TOLI ANDAHI, with a germination percentage of 66% and germination energy of 54%. NGRC02872 and NGRC01815 also maintained relatively high germination percentage and energy (more than 60%). ANADI exhibited a germination percentage of 58% and germination energy of 54%. Root growth further distinguished the tolerant genotypes: NGRC01815 recorded the greatest root lengths at 14 and 21 days after sowing (10.21 and 9.16 cm, respectively), followed by ANADI (9.32 and 7.98 cm) and TOLI ANDAHI (7.51 and 8.25 cm). The mean germination time of the tolerant genotypes ranged from 3.66 days in NGRC02865 to 4.33 days in TOLI ANDAHI, indicating comparatively rapid germination even under severe osmotic stress.

Table 10. Performance of contrasting rice genotypes under 20% PEG 6000-induced osmotic stress.

Genotypes	Group	GP (%)	GE (%)	RL14 (cm)	RL21 (cm)	MGT
TOLI ANDAHI	Tolerant	66	54	7.51	8.25	4.33
NGRC02872	Tolerant	62	62	6.49	5.58	3.94
NGRC01815	Tolerant	60	60	10.21	9.16	4.20
ANADI	Tolerant	58	54	9.32	7.98	3.99
NGRC02865	Tolerant	70	70	5.41	4.33	3.66
MARSHI TANGALO	Sensitive	12	0	2.70	2.70	6.25
HIMALI	Sensitive	16	8	0.70	3.07	5.88
MANSARA 1	Sensitive	32	8	1.54	1.33	5.86
NGRC07869	Sensitive	28	8	2.25	1.90	6.29
TILAKI	Sensitive	28	4	0.52	4.89	6.17

Notes: RL14 = root length at 14 days after sowing; RL21 = root length at 21 days after sowing; GP = germination percentage; GE = germination energy; MGT = mean germination time. All length measurements are in cm; MGT is expressed in days.

In contrast, the sensitive genotypes recorded poorer germination performance and longer mean germination time. MARSHI TANGALO showed the lowest germination percentage (12%) and almost zero recorded germination energy, together with relatively short root lengths of 2.70 cm at both assessment times and a mean germination time of 6.25 days. HIMALI had a germination percentage of 16% and germination energy of 8%, with a root length of only 0.70 cm at 14 days and 3.07 cm at 21 days. MANSARA 1 recorded a germination percentage of 32%, but its germination energy was only 8%, and root length declined from 1.54 cm at 14 days to 1.33 cm at 21 days. Similarly, NGRC07869 exhibited a germination percentage of 28% and germination energy of 8%, with root lengths of 2.25 and 1.90 cm. TILAKI also showed low germination energy (4%) and a relatively long mean

germination time of 6.17 days, although its root length increased from 0.52 cm at 14 days to 4.89 cm at 21 days.

Discussion

Effects of PEG-induced osmotic stress on germination and early seedling growth

The progressive decline in germination percentage, germination energy, and shoot growth with increasing PEG 6000 concentration proves the sensitivity of rice germination and early seedling development to reduced water availability (Tables 2 and 4). PEG 6000 lowers the osmotic potential of the medium outside the seed, restricting water uptake by seeds and probably delaying the physiological and biochemical processes required for germination. Insufficient water uptake can affect embryo expansion, reserve mobilization, enzyme activity, and radicle emergence, ultimately reducing germination performance and seedling vigour (Binodh et al., 2022; Dueñas et al., 2024). In the present study, mean germination percentage declined from 75.55% under control conditions to 62.04% under 15% PEG and 44.10% under 20% PEG, while mean germination time increased from 3.72 days to approximately 5.24 days. These changes indicate that increasing osmotic stress not only reduced the proportion of seeds that germinated but also delayed the germination process. Such responses are consistent with previous studies reporting concentration-dependent reductions in germination and early seedling growth in rice landraces exposed to PEG-induced osmotic stress (Karanjit et al., 2024; Joseph and Makwinja, 2026).

The reduction in shoot length under increasing PEG concentrations further indicates that early seedling growth was more strongly constrained under severe than under moderate osmotic stress (Tables 2 and 4). Shoot length at 14 days decreased by approximately 44% under 15% PEG and 69% under 20% PEG relative to the control. Reduced water availability limits cell expansion and shoot elongation due to this stress, as shoot elongation depends on adequate tissue hydration and the maintenance of cellular growth processes (Skirycz and Inzé, 2010). Reduced shoot development during establishment may limit early seedling vigour and the ability of seedlings to develop a competitive canopy under water-limited conditions. Though the present experiment did not directly measure cellular water status, hormonal responses, or metabolic activity; therefore, these processes should be considered potential explanations rather than demonstrated mechanisms.

Root growth showed a different response to increasing osmotic stress, increasing slightly under 15% PEG at 14 days but declining markedly under 20% PEG. This pattern suggests that root development did not respond linearly to the imposed stress and that the response depended on stress intensity. A modest increase in root elongation under mild stress could reflect differences in growth allocation or genotype-specific responses to reduced water availability. However, root elongation alone does not establish improved water acquisition or drought adaptation, particularly in a Petri-dish assay where the roots cannot explore a heterogeneous soil profile. Under the more severe 20% PEG treatment, the decline in root growth indicates that the stress level may have exceeded the capacity of many genotypes to maintain normal seedling development.

Root growth exhibited a nonlinear response to increasing osmotic stress, increasing slightly under 15% PEG at 14 days but declining markedly under 20% PEG (Tables 2 and 4). A modest increase in root elongation under mild stress may reflect reallocation of assimilates toward roots, osmotic adjustment, or genotype-specific compensatory growth responses to reduced water availability (Ogawa and Yamauchi, 2006; Kano et al., 2011). However, greater root elongation under mild PEG stress alone does not necessarily prove it for drought adaptation (Kano et al., 2011; Salehi-Lisar and Bakhshayeshan-Agdam, 2016). Under the more severe PEG stress (20% concentration), the pronounced decline in root growth suggests that the imposed stress exceeded the capacity of many genotypes to maintain normal seedling development. Mahreen et al. (2022) and Susilawati et al. (2022) also reported that high PEG concentrations suppress root elongation and root system development in rice.

The contrasting responses of different genotypes under 15% and 20% PEG also highlight the importance of using multiple stress intensities in germplasm screening. A single severe treatment may identify genotypes that maintain some germination under substantial osmotic constraint, but it cannot adequately characterize the full range of responses occurring under mild and severe stress. Conversely, screening only under mild stress may fail to distinguish genotypes whose performance deteriorates sharply as water availability decreases. The observed non-linear response is due to the fact that a low level of an otherwise inhibitory stressor may stimulate particular biological responses while greater

exposure becomes inhibitory (Siemieniuk et al., 2025). However, the slight increase in root length under 15% PEG is insufficient by itself to demonstrate hormesis; confirmation would require appropriate dose-response evidence and supporting physiological measurements.

Genotypic variation and identification of promising rice germplasm

A major finding of this study was the substantial variation among the 154 Nepalese rice genotypes in their responses to PEG-induced osmotic stress (Tables 2 and 4). Under 20% PEG, germination percentage relative to each genotype's control ranged from approximately 2.63% to 87.10%, indicating marked differences in the maintenance of germination under stress. These differences under PEG exceeded the corresponding control value; such responses should be interpreted cautiously because they may reflect biological variation, differences in seed-lot performance, or experimental variability, rather than necessarily indicating superior drought tolerance. Nevertheless, the wide range of responses demonstrates that the tested germplasm was heterogeneous in its early-stage response to osmotic stress.

To integrate variation across germination and seedling growth, the composite stress-tolerance index combined standardized scores for germination percentage, germination energy, germination index, shoot and root lengths at 14 and 21 days, and mean germination time. This multivariate approach enabled genotypes to be compared using several indicators rather than relying on germination percentage or seedling length alone. The ranking identified TOLI ANDAHI, NGRC02872, NGRC01815, ANADI, and NGRC02865 as the five highest-ranked genotypes under 20% PEG. Their relative performance reflected different combinations of germination and seedling-growth characteristics. For example, TOLI ANDAHI combined relatively strong shoot and root growth with favorable germination performance, whereas NGRC01815 and ANADI exhibited comparatively long roots. NGRC02865 showed particularly favorable germination performance, with germination percentage and germination energy of 70% each. These differences indicate that promising overall performance may arise through different combinations of component traits rather than through uniformly superior values for every trait. In contrast, CHOTTE, NGRC03389, and NGRC07900 were among the lowest-ranked genotypes. Their poor performance was associated with low germination and/or lower seedling growth, with increased mean germination time also evident in some sensitive genotypes. The contrast between these genotypes and the higher-ranked entries provides a useful basis for selecting contrasting materials for subsequent physiological and genetic investigations. However, the ranking represents relative performance within the tested collection and under the specified PEG treatment; it should not be interpreted as an absolute classification of drought tolerance across environments or growth stages. The PCA summarized patterns of variation in the multivariate dataset and helped visualize similarities and differences among genotypes. Agreement between the ranking and the broad patterns observed in the PCA can strengthen confidence that the identified candidates possess distinctive combinations of measured traits.

Previous evaluations of Nepalese rice germplasm have also highlighted variation in responses to abiotic stress and the value of identifying locally adapted genetic resources for further evaluation (Kandel et al., 2022; Lamichhane et al., 2025; Yogi et al., 2025). The present results extend this line of investigation by identifying contrasting germination and early seedling-growth responses under controlled PEG-induced osmotic stress. The promising genotypes identified in the present experiment should be tested for screening and evaluation under soil-based water-deficit conditions. Their performance should also be assessed during vegetative growth, flowering, and grain filling.

Interpretation of seedling biomass and relative water content

Shoot growth declined markedly with increasing PEG 6000 concentration (Table 4), although the magnitude of the response varied among genotypes (Figures 2 and 3). Mean shoot length at 14 days decreased from 5.50 under control conditions to 3.29 at T15 and 1.56 at T20, corresponding to reductions of 40.20% and 71.64%, respectively. Similarly, shoot length at 21 days declined from 5.31 under control conditions to 4.01 at T15 and 1.31 at T20, representing reductions of 24.57% and 75.29%. These results indicate that increasing osmotic stress progressively constrained shoot elongation, particularly under severe stress. Reduced water availability can restrict cell expansion and seedling growth. Comparable reductions in rice seedling growth under increasing PEG 6000 concentrations have been reported by Sagar et al. (2020) and Shereen et al. (2019), who also demonstrated variation in stress responses among rice genotypes.

Root growth showed a contrasting response to increasing PEG concentration. Mean root length at 14 days increased from 6.08 under control conditions to 6.75 at T15 before declining to 4.64 at T20. At 21 days, root length decreased from 6.33 under control conditions to 5.56 at T15 and 4.07 at T20. The increase in root length at 14 days under mild stress suggests that root elongation may respond differently from shoot growth, and it depends on stress intensity and developmental stage. However, this response should not be interpreted as absolute evidence of an adaptive mechanism because root architecture, water uptake, and physiological responses were not directly measured in the present experiment. Studies of rice genotypes exposed to PEG-induced osmotic stress have resulted in similar responses in root and shoot traits (Sagar et al., 2020). The contrasting responses observed in our experiment reinforce the importance of evaluating both root and shoot characteristics rather than relying on a single morphological trait.

Substantial genotypic variation was evident in shoot growth under increasing osmotic stress (Figures 2 and 3). NGRC02865, NGRC02872, TOLI ANDAHI, and NGRC01815 exhibited relatively high shoot lengths under control conditions, at approximately 6.0, 5.3, 5.8, and 4.5 cm, respectively. Under T15, their shoot lengths declined to approximately 4.9, 4.8, 5.0, and 3.3 cm, respectively. Under T20, NGRC02865 and NGRC02872 showed further reductions to approximately 2.7 and 4.0 cm, whereas TOLI ANDAHI and NGRC01815 maintained shoot lengths of approximately 4.6 and 3.2 cm. From these differences, we can suggest that genotypes varied in their capacity to maintain shoot elongation under severe osmotic stress. In contrast, NGRC07900, NIRTACHAN BARDIYA, NGRC03389, and CHOTTE exhibited marked growth inhibition under T20, with shoot lengths of approximately 0.2, 0.6, 0.5, and 1.1 cm, respectively. SYAUDO also showed a substantial decline, from approximately 5.4 cm under control conditions to 4.0 cm under T15 and 1.5 cm under T20. Genotype-dependent differences in seedling growth under PEG-induced stress in other rice collections have also been reported by Sagar et al. (2020), supporting the use of controlled osmotic-stress screening to identify contrasting germplasm for further evaluation.

Importantly, the ability to maintain shoot elongation under T20 did not necessarily correspond to the highest overall stress-tolerance ranking. Although NGRC02872 and TOLI ANDAHI maintained comparatively greater shoot growth under severe stress, the composite stress-tolerance index also incorporated germination percentage, germination energy, germination index, root length, and mean germination time. Consequently, the final ranking reflected a combination of traits rather than shoot length alone. This multi-trait approach is consistent with previous rice-screening research demonstrating the value of combining germination, seedling vigour, root development, and biomass-related indicators to differentiate genotypes under osmotic stress (Joseph and Makwinja, 2026). In the present experiment, PEG-based screening provides an initial assessment of responses to reduced external water potential and was not tested for drought tolerance under soil or field conditions. Thus, the identified genotypes here should therefore be evaluated in soil-based water-deficit experiments and field trials to determine whether their early seedling responses translate into improved establishment and agronomic performance.

Relative water content is an important physiological indicator of plant water status, reflecting the balance between water uptake and water loss and the capacity of plant tissues to maintain hydration under water-limited conditions (Barrs and Weatherley, 1962; González and González-Vilar, 2001). In the present study, mean RWC progressively declined from 41.03% under control conditions to 37.28% under 15% PEG and 17.59% under 20% PEG-induced osmotic stress, indicating a substantial reduction in seedling tissue hydration with increasing stress intensity. This reduction may be associated with the lower external water potential created by PEG 6000, which restricts water uptake and affects cellular hydration, turgor maintenance, and seedling growth (Michel and Kaufmann, 1973; Farooq et al., 2009). However, the comparatively low RWC observed even under control conditions (41.03%) requires careful interpretation, as adequately hydrated seedlings would generally be expected to exhibit higher RWC values. RWC measurements are sensitive to tissue selection, sampling procedures, rehydration duration, and the accuracy of fresh, turgid, and dry weight measurements (Barrs and Weatherley, 1962; González and González-Vilar, 2001).

Implications for plant protection and integrated crop management

From a plant protection perspective, the identification of rice genotypes that maintain germination and early seedling growth under osmotic stress is relevant to crop establishment in rainfed and water-limited production systems. Poor and uneven establishment can create gaps

in crop stands, reduce the uniformity of plant development, and complicate subsequent crop management. Genotypes that establish more consistently under water-limited conditions may therefore offer useful material for developing rice production strategies suited to environments where early-season water availability is unreliable. However, the present experiment assessed germination and seedling-growth responses under PEG-induced osmotic stress; it did not measure pest incidence, disease severity, pathogen infection, or natural-enemy abundance. Any implications for pest and disease management must consequently be considered prospective rather than demonstrated outcomes.

Drought and biotic stresses can interact in complex ways, and the direction and magnitude of these interactions depend on the host genotype, pathogen or pest species, developmental stage, stress intensity, and environmental conditions. Water deficit may alter plant physiology, tissue quality, canopy development, and host defence, but it does not invariably increase pest or disease pressure. Some pests may become more damaging to stressed plants, whereas certain pathogens require environmental conditions that are less favourable under drought. Similarly, a genotype that maintains germination under osmotic stress is not necessarily resistant to insect pests or pathogens. The candidate genotypes identified in this study should therefore be considered resources for future integrated screening, rather than genotypes with established dual tolerance to abiotic and biotic stresses.

At the physiological and molecular levels, plants respond to water deficit and pathogen attack through partially overlapping signalling and defence networks (Fujita et al., 2006; Kissoudis et al., 2014; Ramegowda and Senthil-Kumar, 2015). Abscisic acid (ABA), reactive oxygen species (ROS), and associated signalling pathways contribute to the regulation of water balance and stress responses, while pathogen defence involves additional mechanisms of defence-related responses. The present study did not measure ABA, ROS, defence-related proteins, gene expression, or responses to pathogens; we cannot determine whether the genotypes with higher composite scores possess enhanced defence pathways or cross-tolerance to biotic stress. These mechanisms provide testable hypotheses for subsequent research, not conclusions from the current measurements. TOLI ANDAHI, NGRC02872, NGRC01815, ANADI, and NGRC02865 should be evaluated alongside contrasting genotypes such as CHOTTE, NGRC03389, and NGRC07900. Such studies should compare genotype responses under well-watered and water-deficit conditions and include clearly defined pest or pathogen challenges.

Limitations and future research directions

The present study provides a preliminary laboratory-based assessment of germination and early seedling-growth responses among 154 Nepalese rice genotypes exposed to PEG 6000-induced osmotic stress. Although the large genotype panel enabled substantial variation to be characterized, several limitations should be considered when interpreting the findings. First, PEG-induced osmotic stress is a controlled screening approach and does not fully reproduce the physical, chemical, and biological conditions of water deficit in soil. Differences in soil structure, water distribution, root penetration, temperature, and other environmental factors may influence genotype performance in the field. Second, the assessment focused on germination and early seedling growth, so the results cannot establish performance during later developmental stages or predict grain yield under drought conditions. Third, the composite index provides a relative ranking based on the selected traits and the standardization procedure. Although integrating multiple traits is better as compared to dependence on a single response variable, the ranking is sensitive to trait selection, the direction assigned to each trait, and the method used to combine standardized values. Mean germination time must be coded so that faster germination contributes positively to the index, whereas higher values for favourable growth and germination traits contribute positively.

Future research should first validate the performance of the identified candidates in independent experiments and soil-based drought-screening systems. Subsequent trials should evaluate seedling establishment, root and shoot development, survival, recovery after rewatering, and yield-related traits under contrasting water regimes. Multi-location and multi-season field evaluation will be needed to assess environmental stability and determine whether the observed early-stage responses translate into agronomic benefits.

Finally, a plant protection-focused research programme could investigate whether these genotypes differ in their responses to important rice pests and pathogens under both well-watered and water-deficit conditions. Such work should measure biotic-stress outcomes directly

and distinguish resistance, tolerance to damage, and general plant vigour. Combining validated drought-response data with pest and disease screening would establish whether any of the identified landraces offer benefits across both abiotic and biotic stress domains. Until such evidence is available, the genotypes identified in this study should be regarded as candidates for further evaluation rather than confirmed sources of combined drought and biotic-stress resistance.

Conclusion

PEG 6000-induced osmotic stress reduced germination and shoot growth and delayed germination among the 154 Nepalese rice landraces evaluated, with substantial genotypic variation in stress responses. The standardized composite stress-tolerance index identified TOLI ANDAHI, NGRC02872, NGRC01815, ANADI, and NGRC02865 as promising candidates for further evaluation, whereas CHOTTE, NGRC03389, and NGRC07900 showed comparatively poor performance under severe osmotic stress of PEG 6000. However, the rankings should be interpreted cautiously because of the limited number of independent replications and potential correlations among the component traits. These findings support the use of PEG 6000-based screening as a preliminary tool for selecting rice germplasm for drought-related research. Further validation under soil-based water-deficit and field conditions is essential to confirm their agronomic potential. Their responses to major rice pests and pathogens should also be evaluated before any contribution to integrated pest and disease management can be established.

Authors' Contributions

SD, MPP, and SM conceptualized the study and designed the experiment. SD conducted the investigations and performed the experimental work. MPP provided overall supervision and ensured methodological rigor and analytical consistency throughout the study. KHD and SM served as co-supervisors, providing critical guidance on experimental design, data interpretation, and research refinement at various stages. SD and SM prepared the initial manuscript draft, organized the content, compiled the results, and incorporated preliminary interpretations. All authors subsequently critically reviewed and revised the manuscript, contributed to its intellectual refinement, verified the data and analyses, and approved the final version for submission.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger
SDG 13: Climate Action
SDG 15: Life on Land

Policy Addressed

The findings support policies promoting climate-resilient rice breeding, conservation and utilization of genetic resources, and improved crop establishment under water-limited conditions. Promising genotypes should undergo field validation under drought-prone environments before their integration into national rice improvement programmes. These priorities align with food security, climate adaptation, and sustainable agricultural development.

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