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Exploring Flood Resilience: Analysing Root Images for Physiological Responses to Flood Stress

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ABSTRACT

Background: The experiment followed a randomized block design with two replications, including control and waterlogging treatments. After 40 days of normal growth, waterlogging was applied for two weeks with a 12 cm water level above the soil. Observations were recorded eight days after drainage, identifying four tolerant and four sensitive genotypes based on physiological and anatomical traits.

Methods: The experiment was conducted during the *kharif* season of 2022-23 for one year at the field of All India Coordinated Research Project (AICRP) on Soybean, VNMKV, Parbhani. Among the 27 soybean genotypes evaluated under waterlogging, MAUS 710, JS-20-76, KDS-726 and NRC 257 exhibited high tolerance, showing improved growth and root traits like aerenchyma formation and root diameter. While MAUS 725, MAUS 731, EC250591 and JS 97-52 were susceptible.

Result: Among 27 soybean genotypes tested under waterlogged conditions, MAUS 710, JS-20-76, KDS-726 and NRC 257 observed high tolerance, excelling in traits like aerenchyma formation, root surface area and average root diameter. JS-20-116, KDS-726, JS-20-76 and NRC-257 exhibited moderate tolerance, while MAUS 731 and MAUS 725 were more susceptible. These findings highlight the importance of root traits for flood tolerance and suggest that the tolerant genotypes should be prioritized in breeding programs. Waterlogging resulted in average yield loss, with reduction in root architecture.

Key words: Arenchyma formation, Flood tolerance, Image based root analysis, Root traits, Waterlogging stress.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is a self-pollinated, diploid leguminous crop (2n = 40), originated in China. It is often referred to as the “cow of the field” or “gold from the soil” due to its high oil and protein content (Sivabharathi *et al*., 2025). Soybean belongs to the Leguminosae family and exhibit a symbiotic relationship with many microorganisms, including rhizobium (Budiastuti *et al*., 2025). Soybean contributes about 25% of the world’s edible oil and is a key source of protein for animal feed, especially in poultry and aquaculture. It also provides important raw materials for the food, pharmaceutical and other industries (Alnuaim *et al*., 2025). Reduced tillage improved soybean yield, nutrient uptake, protein, oil content and economics. Applying 100% of the recommended fertilizer dose gave the highest productivity and profitability under the mustard-soybean system (Singh *et al*., 2025).

Brazil leads in soybean production, followed by the United States, Argentina and China, with India ranking fifth-Madhya Pradesh alone contributing 78% of the country’s output (SOPA, 2022). Despite its adaptability, soybean faces significant challenges from abiotic stresses, particularly flooding and drought, which adversely affect growth and yield (Khatoon *et al.,* 2012). Flooding is a critical stressor, impacting about 10% of the Earth’s land area. (Leng *et al*., 2019; Gibbs and Greenway, 2003). As climate change projected to increase flood risks, the development of resilient soybean varieties is crucial. Nevertheless, certain soybean varieties exhibit flood tolerance through adaptations such as aerenchyma formation and enhanced root elongation, mediated by phytohormones like ethylene and abscisic acid (Bailey-Serres and Voesenek, 2008).

This study aimed to understand how soybean plants adapt to flooding stress, focused on analyzing root responses to flood stress using image analysis. The goal of study was to identify flood-tolerant genotypes for sustainable farming and food security.

MATERIALS AND METHODS

The experiment was conducted during the *kharif* season of 2022-23 for one year at the field of All India Coordinated

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Research Project (AICRP) on Soybean, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani. The site is located at 19°16′N latitude, 76°47′E longitude and an altitude of 409 meters above mean sea level. The experiment followed a randomized block design with two replications, comprising of 27 genotypes (Table 1) under control and waterlogging treatments. After 40 days of normal growth, a waterlogging treatment was imposed for two weeks, maintaining a water level of 12 cm above the soil surface. Observations were recorded eight days' post-drainage, leading to the identification of four tolerant and four sensitive genotypes based on physiological and anatomical traits.

Pot experiment

Parallel to the field study, a pot experiment was conducted using plastic bags filled with a 50:1 (v/v) mixture of vertisols and farmyard manure. Each genotype was represented by ten bags, with two subjected to waterlogging and two as controls. Plants were thinned to three per bag at 21 days post-sowing and treated similarly to the field experiment. Soil moisture was maintained at 60-90% field capacity.

Assessments

Root length

Root lengths of the main roots were measured with the help of measuring scale after uprooting the plants (Chintey *et al*., 2025).

Average diameter of the root

Root images were analysed using ImageJ software by calibrating the scale with a reference marker, converting the images to binary and using the measurement tools to determine root area and length, from which average diameter was automatically obtained (Schneider *et al*., 2012).

Root surface area

After calculating root length and root diameter, root surface area was calculated by following formula:

$$\text{Root surface area} = \text{Root length} \times \text{Root average diameter} \times \pi$$

Number of seminal roots

The number of seminal roots per plant was visually counted after carefully uprooting the seedlings and gently washing the roots to remove soil without causing damage.

Assessment of root anatomy: TS of root

Medium-sized, freshly harvested *Glycine max* roots were manually sectioned into extremely thin cross-sections, stained with Acetocarmine stain (45% w/v) for 30 to 90 seconds and images were taken under a compound light microscope (Rajan *et al*., 2025).

Statistical analysis

The data collected from the experiment were statistically analyzed using a completely randomized design, following the procedure described by Panse and Sukhatme, 1954; (Ramanadane *et al*., 2025).

RESULTS AND DISCUSSION

Impact of waterlogging on soybean growth

Waterlogging reduces soil oxygen, negatively impacting soybean growth and yield. Tolerant genotypes adapt through increased root formation and aerenchyma (Shimamura *et al*., 2010), while early reproductive stages are particularly sensitive, affecting pod and seed development (Ploschuk *et al*., 2022).

Aerenchyma formation in soybean roots

Flood stress induced a notable enhancement of aerenchyma formation in soybean roots, indicating an adaptive response (Fig 1). Cross-sectional analysis showed significant aerenchyma in tolerant genotypes (MAUS 710, JS 20-76, KDS-726, NRC-257) and minimal to absent in susceptible ones (MAUS 725, MAUS 731, EC 250591, JS 97-52). Extending from cortex to medulla, aerenchyma supports oxygen transport under flooding (Shimamura *et al*., 2010). Ethylene accumulation during waterlogging restricts gas diffusion, increases ROS and triggers cell death, forming aerenchyma lacunae (Hayashi *et al*., 2013). This adaptation enhances flood tolerance in soybeans, supported by earlier studies (Hossain and Uddin, 2011; Hingane *et al*., 2015; Ryser *et al*., 2011; Visser

Table 1: List of genotypes included in the study.

Sr. no.	Genotypes
1	NRC 186
2	NRC 192
3	NRC 189
4	NRC 256
5	NRC 257
6	A-20-3/Sel 3-21
7	A-20-2/Sel 3-79
8	TGX 317-37E
9	JS 20-76
10	EC-602288
11	Hardee
12	EC-457464
13	EC-528623
14	EC-250591
15	EC-528622
16	Check JS 97-52
17	Check JS 20-69
18	MAUS 71
19	MAUS 158
20	MAUS 162
21	MAUS 612
22	JS 95-60
23	JS 20-116
24	KDS-726
25	MAUS 725
26	MAUS 731
27	MAUS 710

et al., 1997; Sakazono *et al*., 2000; Hayashi *et al*., 2013; Solaiman *et al*., 2007).

Root length responses to flood stress

Significant reduction in root length was observed under flood stress compared to control across genotypes (Table 2). It highlights the impact of flood stress on soybean root length. Under control conditions, root lengths ranged from 37 cm (MAUS 725) to 49.1 cm (NRC 256), while waterlogging reduced them significantly-down to 10 cm in EC 457464 and EC 250591 and 22.6 cm in NRC 257. These findings align with earlier studies showing that flooding during the reproductive stage limits root growth due to anaerobic conditions (Jhorar *et al*., 1995; Sorte *et al*., 1996;

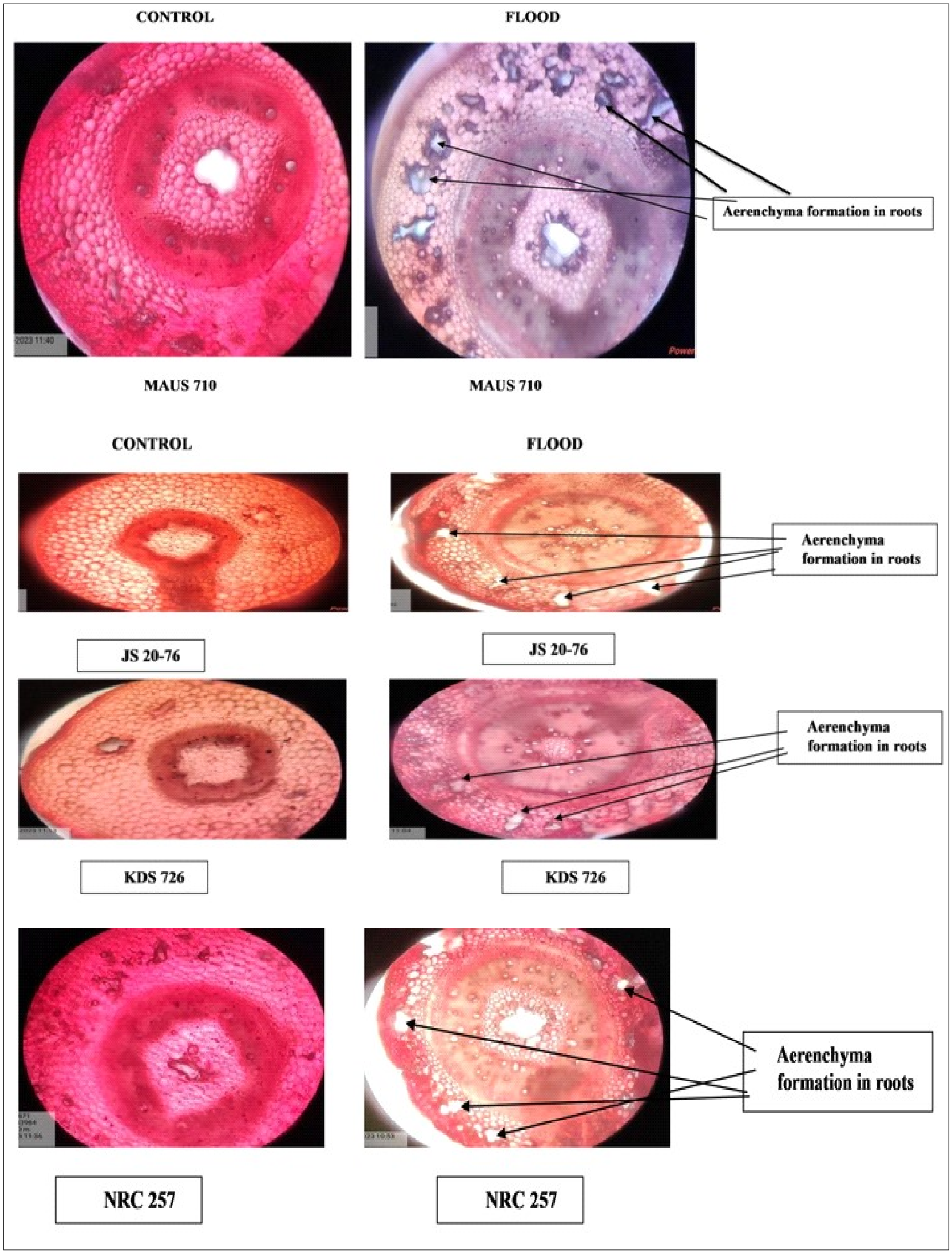

Fig 1: TS of root-Aerenchyma formation in roots.

Table 2: Root morphological traits and yield performance of soybean genotypes under control and flood conditions.

Genotypes	Root length (cm)		Root average diameter		Root surface area (cm²)		Number of seminal roots (roots/plant)		Average yield (g/row)	
	Control	Flood	Control	Flood	Control	Flood	Control	Flood	Control	Flood
NRC 186	44.50	21.00	0.35	0.57	45.23	39.00	7	16	106.50	89.50
NRC 192	44.50	19.00	0.33	0.53	46.64	31.5	10	14	218.00	86.50
NRC 189	42.24	20.06	0.28	0.46	37.62	28.98	9	14	153.00	77.90
NRC 256	49.10	19.00	0.36	0.52	57.54	29.00	12	16	248.00	98.40
NRC 257	44.50	22.60	0.36	0.56	49.74	39.50	10	16	168.45	100.00
A-20-3/Sel 3-21	41.00	21.05	0.24	0.46	30.78	30.50	8	16	137.00	86.00
A-20-2/Sel 3-79	43.50	19.50	0.33	0.53	47.34	32.73	10	14	171.00	76.15
TGX 317-37E	47.00	15.00	0.36	0.50	54.27	22.50	12	16	242.00	78.85
JS 20-76	38.50	11.00	0.26	0.45	31.62	14.22	6	18	130.00	106.00
EC 602288	46.50	13.50	0.34	0.50	50.18	20.80	11	15	228.50	87.00
Hardee	46.50	14.00	0.24	0.49	36.16	21.00	8	13	149.00	79.55
EC 457464	41.00	10.00	0.22	0.44	27.63	12.50	9	14	158.00	71.25
EC 528623	46.00	17.00	0.35	0.53	49.42	29.50	9	14	127.00	79.00
EC 250591	40.50	10.00	0.23	0.44	28.89	13.50	9	16	127.50	68.10
EC 525622	42.00	18.16	0.30	0.53	41.45	30.50	9	14	158.00	72.30
MAUS 71	42.00	21.96	0.23	0.43	31.76	29.00	6	14	155.50	95.50
MAUS 158	44.11	11.03	0.25	0.45	31.58	16.38	6	12	145.00	77.90
MAUS 162	44.00	13.00	0.26	0.48	35.11	19.50	9	12	201.50	70.00
MAUS 612	41.50	16.98	0.24	0.44	31.99	24.32	11	14	149.50	79.00
JS 95-60	39.00	17.80	0.25	0.47	30.43	26.15	7	14	83.00	75.00
JS 20-116	45.49	12.08	0.25	0.52	36.26	21.60	12	15	155.50	99.50
KDS-726	43.61	21.75	0.33	0.50	46.88	35.34	12	15	165.00	108.50
MAUS 725	37.00	20.00	0.22	0.51	33.00	27.07	6	13	61.00	38.50
MAUS 731	45.00	19.50	0.24	0.46	33.83	28.45	9	14	184.00	32.50
MAUS 710	45.50	16.60	0.24	0.48	33.91	25.00	9	19	130.50	112.50
JS 97-52 (Check)	41.17	10.38	0.38	0.43	50.53	14.04	10	17	146.00	65.85
JS 20-69 (Check)	38.66	12.00	0.24	0.47	28.85	17.50	11	12	150.00	79.65
CD at 5%	4.47	4.00	0.06	0.05	7.41	5.92	2.08	N/A	10.60	6.42
SE ±	1.53	1.37	0.02	0.02	2.53	0.71	0.71	1.44	3.63	2.20
CV at 1 %	5.02	11.77	9.42	5.35	9.19	10.70	10.70	13.51	3.30	3.83

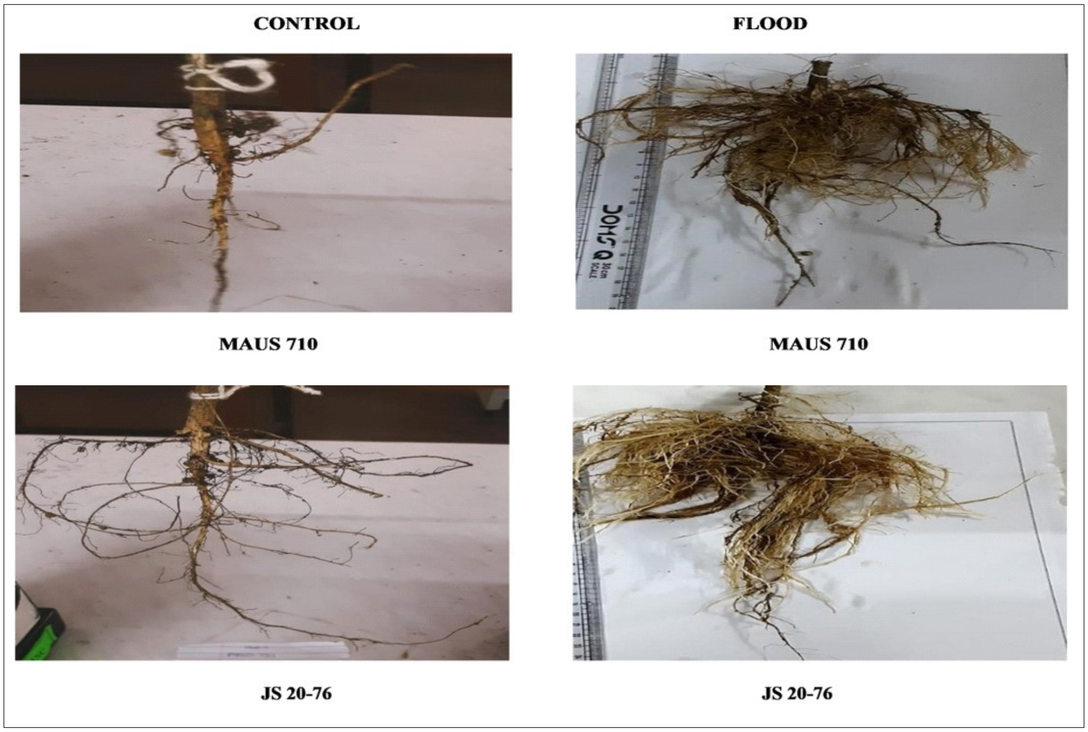

Fig 2: Comparison of number of seminal roots in control and flood condition.

Sallam and Scott, 1987, Jitsuyama, 2015; Figueroa-Bustos *et al*., 2018). Similar reductions were reported by Solaiman *et al*. (2007) and Sakazono *et al*. (2014), with losses ranging from 2.7% to 83.1%.

Root average diameter responses to flood stress

Root average diameter varied among genotypes, with higher values recorded under flood stress relative to control (Table 2). It shows that root diameter varied significantly among soybean genotypes under control and flood conditions. Under control, diameters ranged from 0.22 mm (EC 457464) to 0.36 mm (NRC 256, NRC 257), while flooding increased them to 0.43-0.57 mm in NRC 186, NRC 257. These findings support Keeley (1979), who noted that flood-tolerant genotypes develop larger, more branched roots and align with reports that aerenchymatous roots have greater diameters (Visser *et al*., 2000a, b; Matsui and Tsuchiya, 2006; Grimoldi *et al*., 2005).

Root surface area responses to flood stress

Flood stress caused a notable decline in root surface area, reflecting restricted root development (Table 2). It shows that root surface area varied significantly between control and waterlogged conditions. Under control, it ranged from 27.63 cm^2 (EC 457464) to 57.54 cm^2 (NRC 256), while flooding reduced it to 13.5 cm^2 (NRC 257) to 39.5 cm^2 (NRC 186).

Number of seminal roots responses to flood stress

Significant increase in the number of seminal roots was observed under flood stress compared to control across genotypes (Table 2). It shows that flood-tolerant soybean genotypes increased seminal root numbers under waterlogging (Fig 2). In control conditions, root counts ranged from 6 (JS 20-76, MAUS 71) to 12 (JS 20-116, KDS 726, NRC 256), while flooding increased them up to 19 in MAUS 710 and 18 in JS 20-76. This adaptive response aligns with studies reporting that waterlogging promotes adventitious root and aerenchyma formation for survival (Hossain and Uddin, 2011; Jhorar *et al*., 1995; Keeley, 1979). Ethylene plays a key role in this response by enhancing adventitious and lateral root development under low oxygen (Malik *et al*., 2001; Sauter, 2013).

Average yield (g/row)

Significant reduction in average yield was observed under flood stress compared to control across genotypes (Table 2). It highlights yield differences among soybean genotypes under control and flood conditions. Under control, NRC 256 had the highest yield (248 g/row) and MAUS 725 the lowest (61 g/row). Under flooding, MAUS 710 (112.5 g/row), KDS-726 (108.5 g/row) and JS 20-76 (106 g/row) performed best, while MAUS 731 (32.5 g/row) and MAUS 725 (38.5 g/row) showed the lowest yields. These findings confirm genotype-dependent yield variation under flooding, with tolerant genotypes experiencing less yield loss (Kumar *et al*., 2013; Ren *et al*., 2014; Zhou *et al*., 2021).

CONCLUSION

Waterlogging significantly affects soybean root traits and yield performance. Tolerant genotypes showed welldeveloped aerenchyma, supporting internal oxygen transport. These genotypes also maintained better root length under stress compared to susceptible ones. Flooding led to a notable increase in root diameter, especially in tolerant lines. Root surface area decreased under stress, but tolerant genotypes retained relatively higher values. Seminal root number increased in tolerant genotypes, indicating adaptive root proliferation. Ethylene-induced responses played a role in enhancing root development under waterlogging. Tolerant genotypes also produced higher yields under flood conditions. Yield losses were more pronounced in susceptible genotypes with poor root traits. Root morphological traits proved critical in determining flood tolerance. These findings provide a valuable basis for future breeding programs aimed at developing flood-resilient soybean cultivars.

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Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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