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Antioxidant defense system response in groundnut under waterlogging and recovery conditions

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Abstract
Waterlogging ranks among the most damaging abiotic stresses for groundnut, yet antioxidant recovery kinetics after water withdrawal remain poorly documented. This research examined enzymatic and non-enzymatic antioxidant responses of groundnut (Arachis hypogaea cv. Giza 6) under 2, 4, and 6 days of waterlogging followed by 2 and 4 days of recovery at Alexandria College of Agriculture, Egypt, during 2023. SOD, CAT, and APX activities peaked at 4 days of waterlogging (SOD: 112.4 U/mg, CAT: 51.8 µmol/min/mg) before declining at 6 days. MDA increased steadily from 6.8 to 31.4 nmol/g FW over 6 days. During recovery, enzyme activities returned toward baseline within 4 days though full recovery was incomplete. These results indicate that the 4-day threshold marks a turning point beyond which oxidative damage exceeds defense capacity.

Keywords: Antioxidant defense, groundnut, waterlogging, recovery, oxidative stress, superoxide dismutase, catalase, malondialdehyde, reactive oxygen species, Arachis hypogaea

Introduction
Waterlogging affects an estimated 16% of global groundnut acreage through soil saturation events [1]. Unlike rice, groundnut lacks aerenchyma for internal gas transport [2]. When soil becomes saturated, oxygen diffusion drops roughly 10,000-fold, and roots shift to fermentation producing toxic metabolites [3]. A secondary consequence is the burst of reactive oxygen species (ROS) during both the anaerobic phase and the transition back to aerobic conditions [4].

Plants counter ROS through enzymatic components (SOD, CAT, APX, GR, POD) and non-enzymatic antioxidants (ascorbate, glutathione) [5, 6]. The balance between ROS production and scavenging determines survival [7]. Tolerant genotypes mount faster antioxidant responses [8]. In groundnut, however, temporal dynamics during both stress and recovery phases have not been clearly defined. This research tracked enzymatic and non-enzymatic antioxidant responses under controlled waterlogging durations and measured recovery rate following water removal.

Material and Methods
Material
The experiment was conducted in the Plant Physiology Glasshouse, Alexandria College of Agriculture (31.21° N, 29.92° E), March-June 2023. Groundnut cv. Giza 6 was sown in plastic pots (30 cm diameter) with sandy loam:sand (3:1). A completely randomized design with six treatments (control, WL 2/4/6 days, recovery 2/4 days after 4-day WL) and four replications was used. Waterlogging was imposed at flowering (45 DAS) by maintaining water 2 cm above soil surface [9].

Methods
Leaf tissue from the third trifoliate was harvested, frozen in liquid nitrogen, and stored at −80°C. SOD was assayed by NBT photoreduction [10]; CAT by H_2O_2 decomposition at 240 nm; APX by ascorbate oxidation at 290 nm [11]. MDA was estimated by thiobarbituric acid reaction [12]. H_2O_2 by potassium iodide method. Ascorbate by DCPIP and glutathione by DTNB method [13]. Proline by ninhydrin method [14]. Data were analyzed by one-way ANOVA with Duncan's test at P = 0.05.

Extraction Protocol

Frozen leaf tissue (0.5 g) was ground in liquid nitrogen and homogenized in 5 ml ice-cold 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 1% PVPP, and 0.1 mM PMSF. The homogenate was centrifuged at 15,000 × g for 20 min at 4°C. For ascorbate and glutathione, tissue was extracted in 5% TCA. All extractions were completed within two hours of thawing [13].

Results

Table 1: Antioxidant enzyme activities in groundnut leaves under waterlogging (WL) and recovery (Rec)

Treatment	SOD (U/mg)	CAT (µmol/min/mg)	APX (µmol/min/mg)	MDA (nmol/g FW)
Control	42.3	18.7	8.4	6.8
WL 2 days	78.6	34.2	16.8	14.3
WL 4 days	112.4	51.8	28.3	22.7
WL 6 days	98.7	44.6	23.7	31.4
Rec 2 days	86.1	38.1	19.4	18.6
Rec 4 days	58.4	24.3	12.1	10.2
SEm(±)	4.37	2.18	1.42	1.08
CD (P=0.05)	13.11	6.54	4.26	3.24

SOD rose from 42.3 U/mg in control to 112.4 at 4 days WL (2.66-fold increase). CAT and APX peaked similarly at 4 days (Table 1). At 6 days, enzyme activities declined while MDA continued rising to 31.4 nmol/g FW, indicating defense system overload.

Table 2: Non-enzymatic antioxidants and H_2O_2 levels under waterlogging stress

Treatment	Ascorbate (µmol/g FW)	Glutathione (nmol/g FW)	H_2O_2 (µmol/g FW)	Proline (µmol/g FW)
Control	2.84	312.6	0.42	3.7
WL 2 days	3.67	478.3	0.89	8.4
WL 4 days	4.21	624.1	1.48	14.6
WL 6 days	3.48	543.7	2.13	18.2
Rec 2 days	3.12	421.8	1.14	12.3
Rec 4 days	2.91	348.2	0.68	6.8
SEm(±)	0.19	28.4	0.11	0.87
CD (P=0.05)	0.57	85.2	0.33	2.61

Ascorbate peaked at 4.21 µmol/g FW and glutathione at 624.1 nmol/g FW, both at 4 days WL (Table 2). H_2O_2 accumulated progressively to 2.13 µmol/g FW at 6 days (5.07-fold increase). Proline rose nearly 5-fold by 6 days.

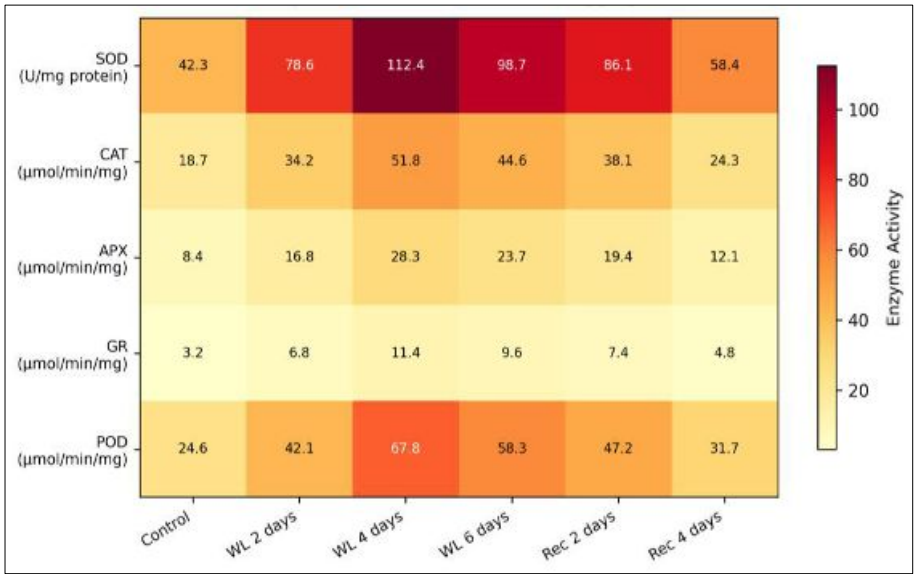

Fig 1: Heatmap of antioxidant enzyme activities across waterlogging and recovery treatments showing peak at 4 days

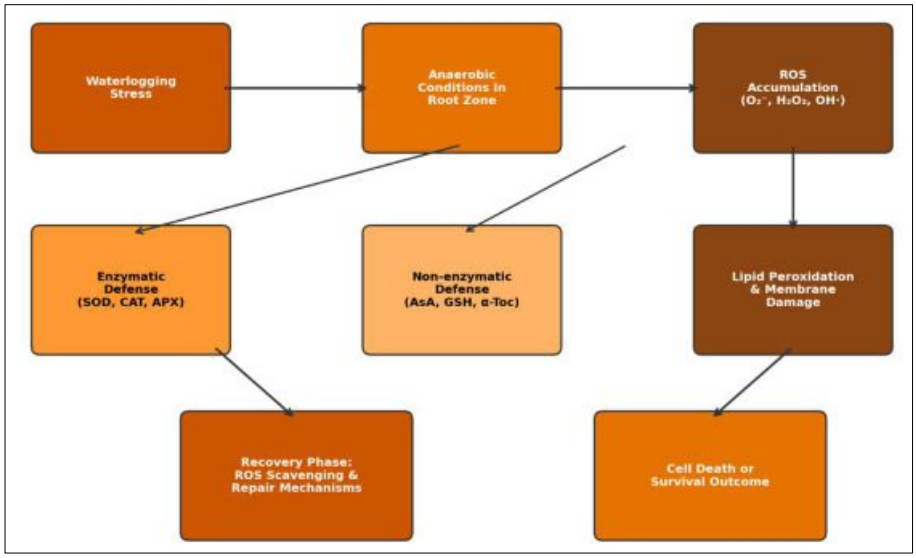

Fig 2: Oxidative stress pathway and antioxidant defense response under waterlogging in groundnut

Comprehensive Interpretation

After 2 days of recovery from 4-day WL, SOD decreased from 112.4 to 86.1 U/mg and MDA fell from 22.7 to 18.6 nmol/g FW. By 4 days recovery, most parameters were within 30-40% of control values, though none reached full baseline. Persistent elevated MDA suggests some membrane damage is not fully repaired within this timeframe.

Discussion

The pattern of enzyme rise followed by decline despite ongoing stress has been reported in other legumes and is interpreted as defense capacity being overwhelmed [5, 7]. The 4-day threshold represents a tipping point where ROS scavenging shifts to net oxidative damage, supported by continued MDA and H_2O_2 rise even as SOD and CAT fell. That APX showed the largest fold increase (3.37×) is consistent with findings in soybean where the ascorbate-glutathione cycle dominates H_2O_2 detoxification under hypoxia [11]. The recovery kinetics are useful for crop management. The observation that enzyme activities approached but didn't reach baseline within 4 days suggests short events (2-4 days) are tolerable, but repeated flooding-drainage cycles could cause cumulative damage. Farmers in waterlogging-prone areas would benefit from drainage allowing water removal within 3-4 days [14].

Conclusion

All enzymatic and non-enzymatic antioxidants were significantly upregulated during waterlogging, peaking at 4 days. Beyond this point, enzyme activities declined while damage markers continued rising. Recovery was partial but meaningful within 4 days of water removal. These findings define a 4-day tolerance threshold for groundnut cv. Giza 6 and identify the ascorbate-glutathione cycle as the most responsive pathway, which could serve as a selection criterion in breeding for waterlogging tolerance.

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