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Oxidative Stress Markers During Germination and Early Seedling Growth of Legumes Subjected to Abiotic Stress

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Abstract: ***India, being predominantly a vegetarian country, is a major producer of cereals and pulses. These crops hold a crucial place in the human diet, particularly in addressing protein deficiency, with legumes playing a significant role. Both legumes and cereals are rich sources of folic acid, vitamin B12, dietary fiber, and iron. However, their productivity is often limited by various abiotic stresses. These crops are typically cultivated in regions with marginal soil fertility, making them especially vulnerable to adverse environmental conditions. The role of reactive oxygen species (ROS) in plant responses to abiotic stress is well established. Under stress conditions, ROS levels increase, potentially causing oxidative damage. However, plants possess both enzymatic and non-enzymatic antioxidant systems to regulate ROS levels and maintain cellular homeostasis. In this study, we aimed to assess the impact of abiotic stresses on the synthesis of antioxidant compounds during seed germination. Edible legume seeds, specifically black gram (Vigna mungo) and green gram (Vigna radiata L.), were germinated for 48 hours and then subjected to different abiotic stresses, including water stress, salt stress, and exposure to artificial light in controlled laboratory conditions. Additionally, metal stress was applied for 7 days. We focused on measuring biochemical markers such as proline, malondialdehyde (MDA), hydrogen peroxide, soluble proteins, and catalase activity to understand the oxidative response. Significant changes in total specific activity were observed, indicating stress-induced metabolic adjustments. The results highlight the activation of ROS-scavenging systems in response to increased ROS levels. This study aims to evaluate species-specific oxidative responses during various phases of germination and identify potential mechanisms for improving stress tolerance and seed performance in leguminous crops***

Keywords: oxidative stress, legumes, abiotic stresses, enzymes, reactive oxygen species

1. Introduction

Leguminous crops are essential to human nutrition and consider as one of the best agricultural crops because of their high protein content, diverse micronutrient profile, and capacity to fix nitrogen, which increases soil fertility [1]. A variety of legumes, including beans, green and black grams, pea etc. are commonly consumed by the people. The production of the crop is directly influenced by its germination. Thus, a crucial step of development of a plant is seed germination in which a dormant seed turns into a metabolically active seedling [2]. Enzymatic systems are activated, respiration begins, stored reserves are mobilized, and fast water absorption occurs during this transition [3]. Reactive oxygen species (ROS), including superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH-), are produced as a result of this increased metabolic activity [4]. If they are not effectively scavenged, these ROS can harm cellular components. ROS have a role as signalling molecules in seed germination and the early growth of seedlings, despite the fact that they are frequently perceived as detrimental [5]. Whether ROS function as lethal agents or signals depends on the plant's capacity to control ROS levels through antioxidant systems. Both enzymatic and non-enzymatic antioxidants are part of antioxidant defences in the plant which play an important role in germination. By considering the above facts, an investigation was carried out at the laboratory to find out the abiotic stress-induced changes in the oxidative state of green gram (*Vigna radiata*). and black gram (*Vigna mungo*) seeds during germination and the early stages of development.

2. Materials and Methods

2.1 Collection of Seed

For a germination experiment, two types of legume seeds such as green gram (*Vigna radiata*). and black gram (*Vigna mungo*) were used. In order to guarantee both experimental validity and seed quality, mature and in good health legume seeds were purchased from Krushi Vigyan Kendra, which is located very nearer to the university. The seeds were cleaned twice with tap water to remove any remaining dirt and debris present on it, and to prevent fungal infestation, they are then let to air dry in a shaded area and treated with low concentration of mercuric chloride. After drying, seeds are stored in moisture-proof containers at a low temperature (4°C) until the germination test. To avoid microbial effect, seeds are surface sterilized with 1% sodium hypochlorite prior to experimentation. At every stage, appropriate

documentation and handling were done to ensure the traceability and reliability of the germination of the seeds.

2.2 Method of Germination of Seed

The experiment aimed to examine the germination rate, viability, and early seedling Vigor of green gram (*Vigna radiata*). and black gram (*Vigna mungo*) seeds under controlled conditions. Selected uniform, fully developed both green gram and black gram seeds, surface sterilized the seeds using a 1% sodium hypochlorite solution. Then washed with distilled water to remove the surface sterilant. The germination experiment was carried out at the laboratory at the petri dishes. Separate petridish were used different types of seeds. Distribute 20 seeds evenly over each dish where filter paper was placed in it. The paper was moistened with distilled water, but not soggy. They were incubated for ten days at 25°C with a lid on to keep the petridish wet. Water was added as needed to maintain the area moist. Kept track of the number of seedlings that emerged every day. For a seed to be considered germination, the radicle must protrude by a minimum of 2 mm. Determined the germination percentage by applying the following formula:

Germination (%) = (Number of seeds germinated / Total seeds) × 100

2.3 Effect of Salt Stress on Seed Germination

The ability of green gram (*Vigna radiata*) and black gram (*Vigna mungo*) seeds to germinate and grow in salty conditions was evaluated by the salt stress tolerance test. This was crucial for identifying cultivars that can withstand salt in areas that were prone to salinity. The requisite amounts of NaCl were dissolved in distilled water to produce a range of concentrations, such as 0 mM, 50 mM, 100 mM, 150 mM, and 200 mM. The seeds were surface sterilized with 1% sodium hypochlorite for two to three minutes, and then washed with distilled water. Twenty seeds were placed on each petridish lined with filter paper. 10 ml of the concerned NaCl solution was added to each petridish to make it wet. Used distilled water as a control (0 mM). Petridishes were placed in indirect light at 25°C with covering. As needed to keep moisture levels stable, the same salt solution was added. Measured the lengths of the roots and shoots and recorded the number of seeds that germinated daily upto10 days of observation and germination of the seeds are calculated as follows
Germination % = (Germinated seeds / Total seeds) × 100

2.3 Measurement of Reactive Oxygen Species (ROS)

2.3.1 Hydrogen peroxide (H_2O_2): The titanium sulphate technique was used to estimate the H_2O_2 concentration. The absorbance was measured at 410 nm after homogenized seed tissue with 0.1% titanium sulphate in 20% H_2SO_4.

2.3.2. Lipid peroxidation assay: To measure the amount of malondialdehyde (MDA), an indication of lipid peroxidation, the thio barbituric acid reactive substances (TBARS) test was performed. Adjusted nonspecific turbidity at 600 nm after the mixture was incubated at 95°C and the absorbance was measured at 532 nm.

2.4 Enzymatic Antioxidant Activity

***2.4.1. Catalase activity (CAT)*:** A unit of CAT activity was defined as the enzyme's capacity to break down one micromole of $H2O_2$ per minute. Monitoring the rate at which H_2O_2 decomposes at 240 nm allowed for the measurement of CAT activity.

2.4.2. Peroxidase activity (POD): Using guaiacol as a substrate, POD activity was measured. The oxidation of guaiacol was shown to enhance absorbance at 470 nm.

2.5 Statistical Analysis

Three duplicates of each experiment were carried out. Using mean ± standard deviation, the data were presented.

3. Results and Discussion

The investigation on the germination of legume seeds, namely black gram (*Vigna mungo*) and green gram (*Vigna radiata*), revealed distinct variations in seedling vigor and germination rates among treatments. Germination started in two days under control circumstances (distilled water), and by day seven, the ultimate germination percentage was 97% (Table 1). Consistent seed germination was accompanied by healthy root and shoot development.

Table 1: Percentage of seed germination of black gram (*Vigna mungo*) and green gram (*Vignaradiata*)under controlled condition. The values are average of 3 replicates ± SEM

No of days	Percentage of seed germination	
	black gram (*Vigna mungo*)	green gram (*Vigna radiata*)
0	-	-
2	11 ± 0.02	12 ± 0.01
4	55 ± 0.07	53 ± 0.04
6	96 ± 0.06	94 ± 0.06
8	97 ± 0.04	97 ± 0.05

The salt stress effect greatly on the germination of the legume seeds. It has been observed that only 50% of the seeds were germinated at salt stress of 100 mM NaCl. At higher concentrations of NaCl, germination was still decreased and recorded below 10% at 200 mM in both the legumes (Table 2). The seedlings under salt stress had stunted development, as seen by the shorter roots and branches. Salt stress affects water absorption and the activity of germination-related enzymes, as evidenced by the reduced rate of germination and growth of seedlings in saline conditions. The delayed or failure germination of the affected seeds was likely caused by osmotic stress and ion toxicity, particularly from Na+ and Cl+ ions [6]. These findings are consistent with other studies that showed salt reduces seed vigour and germination [7].

Table 2: Effect of NaCl on seed germination of black gram (*Vigna mungo*) and green gram (*Vignaradiata*)after 7 days. The values are average of 3 replicates ± SEM

Concentration of NaCl	% of germination	
	black gram (*Vigna mungo*)	green gram (*Vigna radiata*)
0 mM (Control)	97 ± 0.01	97 ± 0.01
50 mM	71 ± 0.03	68 ± 0.04
100 mM	48 ± 0.06	50 ± 0.07
150 mM	21 ± 0.02	24 ± 0.04
200 mM	08 ± 0.01	09 ± 0.03

These results imply that the physiological processes that underlie germination are negatively affected by salt stress. Although ionic toxicity from Na^+ and Cl^+ hindered cell division and enzyme activity, the osmotic imbalance caused by high salt concentrations most likely restricted water absorption. Under salt stress, shorter roots are also a sign of inadequate nutrient absorption and early growth inhibition [8]. All things considered, the data lend credence to the notion that legumes are salt-sensitive, especially during their early development stages. This experiment highlights the need of evaluating legume cultivars for salt tolerance in order to promote sustainable agriculture in areas that are prone to salinity.

The effect of NaCl (salt stress) on oxidative parameters like hydrogen peroxide (H_2O_2) levels, lipid peroxidation (measured as MDA content), catalase (CAT), and peroxidase (POD) activity during legume seed germination has been well-documented (table 3). The amount of hydrogen peroxide (H_2O_2) is raised until the salt concentration reaches 150 MM, after which it was lowered. This is because salt stress causes osmotic and ionic stress, which upsets cellular equilibrium and causes reactive oxygen species (ROS) such H_2O_2 to be produced in excess [9].

Table 3: Effect of different concentration of NaCl on % of activity of biochemicals inblack gram green gram at 7 days. The values are average of 3 replicates ± SEM

Concentration of NaCl	Biochemical changes							
	Hydrogen peroxide (H_2O_2)		Lipid peroxidation assay		Catalase activity (CAT)		Peroxidase activity (POD)	
	black gram	green gram	black gram	green gram	black gram	green gram	black gram	green gram
0 mM	61 ± 0.04	59 ± 0.04	30 ± 0.04	32 ± 06	33 ± 0.04	28 ± 0.01	40 ± 0.04	45 ± 0.04
50 mM	62 ± 0.02	58 ± 0.03	29 ± 0.02	50 ± 0.04	51 ± 0.02	30 ± 0.07	53 ± 0.09	55 ± 0.02
100 mM	73 ± 0.03	74 ± 0.07	66 ± 0.03	69 ± 0.04	70 ± 0.02	74 ± 0.03	72 ± 0.01	74 ± 0.01
150 mM	81 ± 0.08	82 ± 0.04	78 ± 0.08	82 ± 0.07	84 ± 0.08	81 ± 0.04	85 ± 0.01	82 ± 0.06
200 mM	30 ± 0.01	25 ± 0.02	22 ± 0.02	26 ± 0.02	27 ± 0.02	25 ± 0.02	30 ± 0.02	33 ± 0.02

At low quantities, H_2O_2 can function as a signalling molecule, but at large concentrations, it is poisonous and causes oxidative damage. In a similar vein, lipid peroxidation (MDA content) rose as salt concentration rose, but activity dropped at 200 mM. Lipid peroxidation occurs when polyunsaturated fatty acids in membrane lipids are attacked by ROS brought on by salt stress [10]. One typical indicator of this damage is malondialdehyde (MDA). Increased MDA levels during germination are indicative of membrane degradation and loss of integrity. After initially rising, the catalase (CAT) activity subsequently fell. H_2O_2 is detoxified by catalase into oxygen and water [11]. CAT activity rose as a defence mechanism during mild salt stress. Enzyme activity, however, may decrease under extreme or protracted stress because of oxidative damage or suppressed expression. The activity of peroxidase (POD) rose as the quantity of salt rose. PODs are essential for scavenging H_2O_2 and for lignification, which strengthens cell walls under stress [12]. During germination in salinity, they are an essential part of the plant's defence strategy because their activity frequently rises to offset high ROS levels.

4. Conclusion

The oxidative reactions of black gram (*Vigna mungo*) and green gram (*Vigna radiata*) seeds during germination and the early stages of seedling growth under varied abiotic stress conditions are better. The information makes it abundantly evident that oxidative state is a dynamic, stress-sensitive metric that is intimately related to seed and seedling physiological response. Although they may be harmful, reactive oxygen species (ROS) seem to serve as both stress indicators and signalling molecules that drive important developmental processes. Germination caused a discernible rise in ROS levels. Increased lipid peroxidation and disturbed antioxidant enzyme activity were indicators of oxidative stress that was exacerbated by exposure to abiotic stressors such salinity variations. These results highlight how crucial seed genotype and physiological condition are in influencing stress tolerance. To sum up, knowing the oxidative dynamics that occur during seed germination under stressful circumstances improves our ability to choose or create green gram and black gram cultivars that are more resilient to stress. Agriculture may benefit from this information, particularly in light of climatic unpredictability and the requirement for sustainable agricultural production methods.

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