

Impact of Abiotic Stress on Antioxidant Enzyme Activity and Physiological Performance of Niger (*Guizotia Abyssinica L.*)

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Annotation: Niger (*Guizotia abyssinica L.*) is a significant minor oil seed crop and is cultivated under rainfed and low-input agricultural systems in Bihar where crop productivity is increasingly becoming endangered by abiotic stresses like drought, salinity and temperature extremes. Unpredictable monsoon cycle, prolonged dry season and increasing temperature caused by climate change leads to oxidative stress in plants causing massive physiological and yield losses. The current research is a compilation of existing evidence of experiment to evaluate the impact of abiotic stress on the activity of antioxidant enzymes, biochemical measures of oxidative stress, physiological performance of niger with particular reference to agro-climatic condition of Bihar. Abiotic stresses lead to overproduction of reactive oxygen species (ROS) that leads to lipid peroxidation, chlorophyll degradation and dysfunction of photosynthetic machinery. High malondialdehyde (MDA) content, disturbed chlorophyll fluorescence (Fv/Fm) and reduced photosynthetic rates are significant parameters of oxidative stress in niger plants under stress. To neutralize these effects, niger induces an integrated antioxidant defense mechanism

that includes enzymatic antioxidants, including superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase, and non-enzymatic factors, including proline, ascorbate and glutathione. These responses vary in size and efficiency across genotypes, which highlights the significance of genetic variability in stress tolerance. The biochemical, physiological and molecular indicators can be combined to provide a complete framework of evaluation of the stress responses and the identification of resilient niger genotypes to provide Bihar. These integrated methods and improved agronomic methods and the new biotechnological methods can significantly contribute to the enhancement of stress resilience, the stabilization of oilseed production and the livelihoods of small and marginal farmers in the area.

Keywords: Niger (*Guizotia abyssinica* L.), ROS, Antioxidant enzymes, Superoxide dismutase, Catalase, Ascorbate peroxidase, MDA, Photosynthetic efficiency, Stress tolerance.

1. Introduction

1.1 Background and Importance of Niger (*Guizotia abyssinica*)

Guizotia abyssinica L. or niger or ramtil is a poorly used but economically significant oilseed crop that is grown in India, Ethiopia, and some regions of East and Central Africa (Naik and Devaraj, 2016). The crop plays an important role particularly in the rainfed and marginal agricultural systems whereby it forms part of the livelihoods of the small and resource-poor farmers. Niger seeds are also sources of about 30-35 percent edible oil, which is high in polyunsaturated fatty acids, specifically, linoleic acid, which has been well-documented to have health advantages, including a decreased cardiovascular risk (Naik & Devaraj, 2017). Besides being nutritious, niger seeds have found applications in the markets of bird feed and in the preparation of traditional foods, which makes it economically relevant.

Although it can be adapted to the low-input farming conditions, the productivity of niger is comparatively low in relation to the major oilseed crops. This is to a large extent due to the growing exposure to abiotic stresses including drought, salinity and temperature extremes as a result of climate variability. These stresses are negative impacts on plant growth, seed production and quality of oil, which underscores the importance of knowing more about the stress tolerance mechanisms in this crop.

1.2 Abiotic Stress and Crop Productivity

The abiotic stresses such as drought, salinity, extreme temperatures, and heavy metal toxicity are some of the biggest limitations to agricultural productivity in the world. These stresses interfere with physiological and metabolic activities like water relations, nutrient uptake, photosynthesis, and enzyme activity and eventually cause inhibition of growth and yield losses (Hasanuzzaman et al., 2020). The abiotic stresses have been estimated to cause over 50 percent of the world yield losses in crop plants. Niger is a crop that is mostly grown in rainfed and stress-prone conditions, and so it is especially susceptible to these conditions. Specifically, drought and salinity pressure

greatly decrease the growth of biomass, reproduction, and oil production in niger, which poses a threat to the sustainable growth and profitability of this crop (Naik & Devaraj, 2016).

1.3 Oxidative Stress and Antioxidant Defense

Another typical physiological effect of abiotic stress in plants is excessive production of reactive oxygen species (ROS), such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH). These ROS are typically generated at low concentrations in cellular metabolism when the stress conditions disrupt the redox homeostasis leading to oxidative stress (Das & Roychoudhury, 2014). Overproduction of ROS may destroy cell membranes by lipid peroxidation, inactivate proteins and enzymes, destroy chlorophyll, and damage DNA, which leads to a decrease in plant growth and productivity.

Plants have an effective antioxidant defense system to reverse the oxidative damage that includes enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) and non-enzymatic antioxidants such as ascorbate, glutathione, carotenoids, phenolic compounds, and compatible osmolytes such as proline (Hasanuzzaman et al., 2020). The concerted efforts of these elements are instrumental in ensuring the redox balance in cells and improving the ability to withstand stress.

1.4 Aim and Scope

The current research will assess the effects of significant abiotic stresses on antioxidant enzyme activity and physiological performance of *Guizotia abyssinica* L. Special attention is given to the biochemical indicators of the oxidative stress such as lipid peroxidation, antioxidant enzyme dynamics, and osmolyte accumulation and their association with physiological characteristics in the presence of stress. This study aims to offer information about the defense mechanisms of niger against stress by incorporating both biochemical and physiological responses and help in improving the resilience of niger to changing climatic conditions.

1.5 Related work

Oxidative damage caused by abiotic stress is one of the key growth and productivity constraints of the oilseed crop, *Guizotia abyssinica* L. A number of studies have determined that environmental stresses like drought, salinity and extreme temperatures cause the excessive generation of reactive oxygen species (ROS), which causes oxidative stress and cellular damage in plants (Mittler, 2002; Das and Roychoudhury, 2014). In response to this, the plants use antioxidant defense systems which entail enzymatic and non-enzymatic systems. Studies of niger have established that abiotic stresses have a significant effect on the activities of antioxidant enzymes and biochemical indicators of oxidative stress. Naik and Devaraj (2016) also stated that lipid peroxidation and the activity of superoxide dismutase (SOD) and catalase (CAT) in niger plants were higher under salinity stress, which is an adaptive response to the high level of ROS. Drought stress has been identified to cause the activation of antioxidant enzymes like ascorbate peroxidase (APX) and glutathione reductase (GR) as well as proline build-up that leads to osmotic adjustment and ROS scavenging (Naik and Devaraj, 2017).

These results are supported by studies in other oilseed crops, which point to a high level of correlation between stress tolerance and the efficient antioxidant defense systems (Gill and Tuteja, 2010; Hasanuzzaman et al., 2020). Stress-induced high malondialdehyde (MDA) content has been extensively employed as a measure of lipid peroxidation and oxidative damage of membranes. Oxidative stress severity is closely linked with the physiological parameters that include low chlorophyll content and photosynthetic efficiency (Sharma and Dubey, 2005). Nevertheless, extensive research combining biochemical, physiological, and molecular reactions to various abiotic stresses in niger has not been done.

2. Sources and Effects of Abiotic Stress in Niger (Bihar Perspective)

2.1 Drought Stress

One of the most serious abiotic limitations to the niger cultivation in Bihar is drought, especially in the rainfed districts like Gaya, Nawada, Jamui, Aurangabad, as well as in some areas of Rohtas and Kaimur. The state is typified by unpredictable monsoon rainfall, intermittent dry periods and unequal distribution of water which subject crops to intermittent and terminal drought stress. Niger is moderately drought-tolerant but is severely impaired physiologically in the case of extended water deficit. Drought stress causes a reduction in cell turgor, water potential of leaves, and stomatal conductance, which results in a reduction in CO₂ assimilation and photosynthetic efficiency (Sharma and Dubey, 2005). Water scarcity also increases the production of reactive oxygen species (ROS) in both chloroplasts and mitochondria that lead to lipid peroxidation, destabilization of membranes, and decreased growth and yield in niger plants (Naik & Devaraj, 2017).

2.2 Salinity Stress

The salinity stress is becoming a local but a severe issue in a few areas of Bihar, especially in the north Bihar plains, where the soil is becoming saline due to poor drainage systems, low water table, and salty ground water being used to irrigate the land. High salt levels in niger interfere with ionic homeostasis, raising sodium (Na⁺) and lowering potassium (K⁺) uptake, which in turn impairs enzyme functions and metabolism. Salinity also causes osmotic stress, which limits the uptake of water and increases the rate of ROS production (Gill and Tuteja, 2010). Research has demonstrated that salinity stress in niger contributes to the elevation of the malondialdehyde (MDA) content, which is an indication of the increased membrane lipid peroxidation, as well as the change in antioxidant enzyme activity as part of the defense system of the plant (Naik and Devararaj, 2016).

2.3 Temperature Extremes

Climate change is becoming a growing menace to crop production in Bihar because of temperature extremes. Summer and late kharif conditions increase the rate of respiration and membrane fluidity resulting in the increased production of ROS and oxidative injury. In oil seed crops such as niger, heat stress has a detrimental impact on the pollen viability, seed set, and oil accumulation (Mittler, 2002). On the other hand, the cold stress in early winter in certain parts of Bihar interferes with the membrane integrity, enzyme kinetics, and photosynthetics. Both cold and heat stress cause a lot of pressure to the antioxidant defense systems, and inability to effectively scavenge the ROS leads to physiological deterioration.

2.4 Heavy Metals and Other Stressors

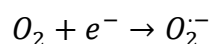
High concentrations of heavy metals including cadmium (Cd) and lead (Pb) are likely to be found in the soils in some agricultural pockets in Bihar, particularly near industrial zones, highways and receiving untreated wastewater. The metals cause oxidative stress through catalyzing free radical production and disrupting antioxidant enzymes. Exposure of heavy metals results in a decrease in root growth, damage to the ability to take up nutrients, and oxidative damage in plants (Das & Roychoudhury, 2014).

3. Mechanisms of ROS Generation and Oxidative Damage

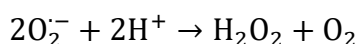
Abiotic stress situations interfere with the homeostasis of plant cells, which causes excessive production of reactive oxygen species (ROS) and consequent oxidative damage. The stress sensitivity or stress tolerance of niger (*Guizotia abyssinica L.*), like other oilseed crops, depends on the imbalance between the production of ROS and the ability to scavenging capacity during stress.

3.1 ROS Production Pathways

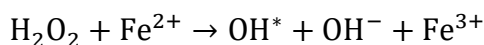
In healthy physiological states, the ROS are continually produced as inevitable by-products of aerobic metabolism, mainly in photosynthesis in chloroplasts and respiration in mitochondria. ROS act as growth, development, and stress response signaling molecules at low concentrations. In abiotic stresses like drought, salinity, extreme temperatures and exposure to heavy metals, it is observed that there is a significant increase in ROS production because of the interference with electron transport processes (Mittler, 2002). Stress conditions in chloroplasts disrupt photosynthetic electron transport chain especially photosystems I (PSI) and II (PSII) leading to an increased leakage of electrons to molecular oxygen and the generation of superoxide radicals (O_2^-). These radicals are quickly transformed to hydrogen peroxide (H_2O_2) spontaneously or by superoxide dismutase (SOD). The over-reduction of the electron transport chain is a stress response in mitochondria resulting in excessive production of ROS in the respiration process (Gill & Tuteja, 2010). The peroxisomes also help in the generation of ROS through photorespiration and fatty acid oxidation particularly during drought and heat stress. In the normal metabolic conditions, the ROS are produced as by-products of aerobic metabolism during photosynthesis and respiration. The abiotic stresses enhance leakage of electrons in electron transport chain that greatly boosts the formation of ROS. The main way of ROS generation in chloroplasts is the Mehler reaction:



Superoxide radicals are further converted into hydrogen peroxide through superoxide dismutase activity:



Hydrogen peroxide may subsequently generate highly toxic hydroxyl radicals via the Fenton reaction:



Stress conditions enhance electron leakage rates, leading to exponential ROS accumulation, particularly in chloroplasts, mitochondria, and peroxisomes.

3.2 Lipid Peroxidation and Membrane Damage

Membrane lipids especially the polyunsaturated fatty acids are one of the major targets of ROS in plant cells. ROS trigger lipid peroxidation by removing the hydrogen atoms of fatty acid chains, which leads to the production of lipid radicals and lipid hydroperoxides. This cascade damages membrane fluidity, permeability and integrity, and eventually results in cellular leakage and loss of compartmentalization (Das & Roychoudhury, 2014).

The end-product of lipid peroxidation, malondialdehyde (MDA) is a stable biochemical indicator of oxidative stress and commonly utilized. Increased levels of MDA have been observed in niger under drought and salinity stress, which is an indication of increased membrane damage and oxidative damage (Naik & Devaraj, 2016). The growth is inhibited by increased membrane permeability caused by lipid peroxidation which negatively affects ion transport, photosynthesis and metabolic efficiency. Membrane lipids that contain polyunsaturated fatty acids are very vulnerable to attack by ROS. Lipid peroxidation is a chain reaction that involves initiation, propagation and termination stages. The extent of lipid peroxidation is usually measured by the level of MDA concentration. The spectrophotometric absorbance is used to determine the concentration of MDA:

$$MDA(\mu\text{mol } g^{-1} FW) = \frac{A_{532} - A_{600}}{\epsilon \times l}$$

Where:

A_{532} = Absorbance at 532 nm

A_{600} = Correction absorbance

ϵ = Extinction coefficient ($155\text{mM}^{-1} \text{ cm}^{-1}$)

l = Path length of cuvette

Elevated MDA levels indicate increased oxidative damage and membrane permeability in stressed niger plants.

3.3 Protein Oxidation and DNA Damage

Another significant damage of ROS is on proteins. Reactive oxygen species oxidize amino acid side chains resulting in the generation of carbonyl groups, destabilisation of tertiary structure and enzyme inactivity. Further stress-induced metabolic dysfunction is caused by oxidative modification of photosynthetic, respiratory, and antioxidant defense enzymes (Hasanuzzaman et al., 2020). Nucleic acids may also be damaged by ROS leading to single and double strand breaks, base alterations, and chromosomal abnormalities. This kind of damage disrupts DNA replication, transcription and cell division and may cause mutation or programmed cell death in extreme stressful circumstances. Despite the fact that plants have DNA repair systems, chronic oxidative stress can exceed the repair ability and disrupt cell viability. ROS lead to oxidative alteration of proteins with the introduction of carbonyl groups into amino acid residues, which leads to the inactivation of enzymes. Protein carbonyl content is frequently used to measure protein oxidation using the formula:

$$\text{Protein Carbonyl (nmol mg mg}^{-1}\text{)} = \frac{\text{Absorbance}}{\epsilon \times \text{Protein concentration}}$$

The damage of DNA is also caused by oxidative stress which results in strand breaks and base oxidation. The magnitude of DNA damage is estimable by the comet assay parameters including tail length and percentage of DNA fragmentation. High concentration of ROS damages the process of DNA replication, transcription, and cell division, resulting in low plant growth and productivity (Hasanuzzaman et al., 2020).

3.4 Photosynthetic Apparatus Impairment

Oxidative stress especially affects the photosynthetic machinery. High levels of ROS destroy chlorophyll pigments and damage thylakoid membranes resulting in reduced light collection and electron transfer. Damage of PSII reaction centers due to stress decreases photochemical efficiency, which is commonly indicated by a decrease in chlorophyll fluorescence parameters like F_v/F_m (Sharma and Dubey, 2005).

Oxidative stress on the photosynthetic apparatus in niger leads to decreased CO₂ assimilation, stomatal constraints, and a reduction in biomass accumulation during stress. The long-term oxidative stress may cause an earlier senescence of the leaf and lowering of the reproductive success, which ultimately influences the seed production and the oil levels. Therefore, the defense against photosynthetic system by effective antioxidant defense is of paramount importance in stress tolerance. The photosynthetic system is very vulnerable to oxidative damage. ROS destroy chlorophyll pigments and damage thylakoid membranes, which reduces the efficiency of photosynthesis. The efficiency of photosystem II is often measured by chlorophyll fluorescence:

$$F_v/F_m = \frac{F_m - F_0}{F_m}$$

Where:

F_v = Variable fluorescence

F_m = Maximum fluorescence

F_0 = Minimum fluorescence

Healthy plants typically exhibit Fv/Fm values near 0.83 , while stress conditions significantly reduce this ratio, indicating impaired photochemical efficiency (Sharma & Dubey, 2005).

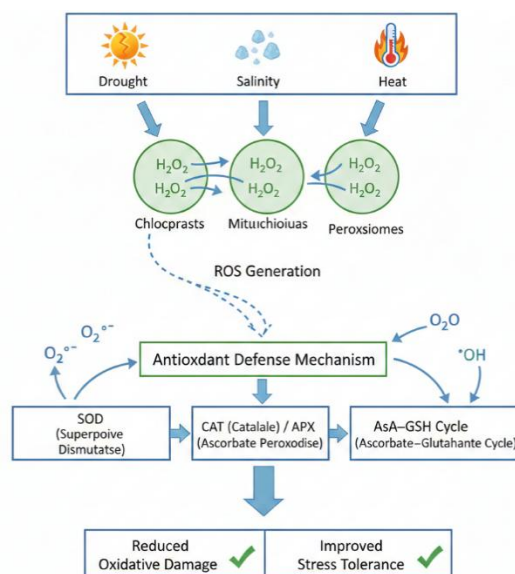


Figure 1. Abiotic Stress–Induced ROS Generation and Antioxidant Defense Mechanism in Niger

Table 1. Major ROS Types, Sources, and Biological Effects in Niger

| ROS Type | Cellular Source | Chemical Formula | Major Effects |
|--------------------|---------------------------|-------------------------------|------------------------------------------|
| Superoxide radical | Chloroplast, mitochondria | O ₂ ^{•-} | Initiates ROS cascade, enzyme inhibition |
| Hydrogen peroxide | Peroxisomes, cytoplasm | H ₂ O ₂ | Membrane damage, signaling molecule |
| Hydroxyl radical | Fenton reaction | •OH | Severe DNA, protein, and lipid damage |
| Singlet oxygen | Chloroplast PSII | ¹ O ₂ | Chlorophyll degradation, photoinhibition |

Table 2. Biochemical Indicators of Oxidative Stress in Niger

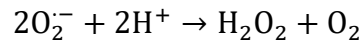
| Parameter | Indicator Role | Stress Response Pattern |
|---------------------|------------------------------------------|--------------------------------------|
| MDA content | Lipid peroxidation marker | Increases under drought and salinity |
| SOD activity | Converts superoxide radicals | Increases during early stress |
| Catalase activity | Detoxifies H ₂ O ₂ | Elevated during moderate stress |
| Proline content | Osmotic protection and ROS scavenging | Accumulates under severe stress |
| Chlorophyll content | Photosynthetic efficiency | Declines under oxidative stress |

4. Antioxidant Defense Systems

Plants have a very well-coordinated antioxidant defense system to neutralize overproduction of excess reactive oxygen species (ROS) in the abiotic stress conditions like drought, salinity, heat, and metal toxicity. These antioxidant systems are essential to the survival of cells in niger under stress, as well as, the maintenance of cellular redox homeostasis in niger grown under rainfed and marginal agro-climatic conditions in Bihar.

4.1 Superoxide Dismutase (SOD)

Superoxide dismutase (SOD) constitutes the first enzymatic barrier against oxidative stress by catalyzing the dismutation of superoxide radicals ($O_2 \cdot^-$) into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). The general reaction is expressed as:

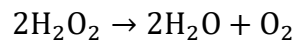


SOD comes in a variety of isoforms based on the metal cofactor and cellular localization such as Cu/Zn-SOD (cytosol and chloroplasts), Mn-SOD (mitochondria) and Fe-SOD (chloroplasts). This compartmentalization enables effective ROS detoxification in their areas of production (Gill & Tuteja, 2010).

Niger plants exhibit a great enhancement of SOD activity, especially in leaf tissues, under conditions of drought and salinity stress that are characteristic of rainfed agriculture in Bihar. High levels of SOD activity decrease the build-up of superoxide and thus, it minimizes oxidative damage to membranes and proteins. There is a positive relationship between enhanced SOD activity and stress tolerance and biomass retention in oilseed crops, such as niger (Hasanuzzaman et al., 2020).

4.2 Catalase (CAT)

Catalase (CAT) plays a crucial role in eliminating hydrogen peroxide generated by SOD and other metabolic reactions. It catalyzes the conversion of H_2O_2 into water and oxygen according to the equation:

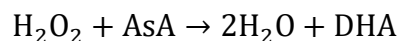


CAT is predominantly localized in peroxisomes and exhibits high turnover rates, making it particularly effective during severe oxidative stress (Apel & Hirt, 2004).

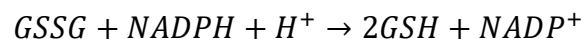
Experimental observations indicate that drought- and heat-stressed niger plants exhibit increased CAT activity, reflecting an adaptive response to excessive H_2O_2 accumulation. Under prolonged or extreme stress, CAT activity may decline due to enzyme inactivation, leading to oxidative damage (Mittler, 2017).

4.3 Ascorbate–Glutathione (AsA–GSH) Cycle

AsA-GSH cycle is a highly-regulated antioxidant mechanism that takes place in chloroplasts, cytosol, mitochondria and peroxisomes. It entails the presence of enzymes like ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR). APX reduces H_2O_2 to water using ascorbate as an electron donor:



GR regenerates reduced glutathione (GSH) from oxidized glutathione (GSSG):



Under drought and salinity stress, increased APX and GR activities have been reported in oilseed crops, suggesting enhanced H_2O_2 detoxification and redox cycling. Similar trends are expected in niger cultivated under Bihar conditions, contributing to sustained photosynthetic efficiency and cellular stability (Noctor & Foyer, 2016).

4.4 Non-Enzymatic Antioxidants

Non-enzymatic antioxidants like ascorbic acid, glutathione, carotenoids, flavonoids and tocopherols directly bind with the ROS and stabilize cellular membranes. Osmolytes such as proline are not only able to sustain osmotic balance but also serve as ROS scavengers and molecular chaperones which help in protecting proteins and membranes in stressful conditions found in semi-arid areas of Bihar (Sharma et al., 2019).

Table 3. Antioxidant Defense Components and Their Stress Responses in Niger

| Antioxidant Component | Cellular Location | ROS Target | Response Under Stress |
|-----------------------|------------------------------------|-------------------------------|-------------------------------|
| SOD | Chloroplast, mitochondria, cytosol | O ₂ • ⁻ | Activity increases |
| CAT | Peroxisomes | H ₂ O ₂ | Strong induction |
| APX | Chloroplast, cytosol | H ₂ O ₂ | Enhanced activity |
| GR | Cytosol, chloroplast | GSSG | Increased regeneration of GSH |
| Proline | Cytosol | Multiple ROS | Accumulates under drought |

5. Biochemical Markers of Oxidative Stress in Niger

Oxidative stress has biochemical markers that are effective in determining the physiological and metabolic responses of the plants to unfavorable environmental conditions. These markers give important information on the degree of oxidative damage and stress tolerance mechanisms in niger grown in rainfed, drought-prone and marginal soil conditions in Bihar.

5.1 Malondialdehyde (MDA)

Malondialdehyde (MDA) is among the most popular biochemical indicators to assess lipid peroxidation by reactive oxygen species (ROS). It is a stable final product that is developed in the oxidative degradation of polyunsaturated fatty acids in cellular membranes. Increased levels of MDA are signs of loss of membrane integrity, permeability and dysfunction of cells. The MDA content of the niger plants exposed to drought, salinity, and temperature extremes is found to rise significantly indicating increased oxidative stress. Experiments conducted on oilseed crops cultivated in water-limited conditions indicate that there is a direct positive relationship between the severity of stress and MDA accumulation, which implies the acceleration of membrane lipid peroxidation in the presence of extended stress exposure (Apel and Hirt, 2004; Hasanuzzaman et al., 2020). High levels of MDA can therefore be employed as a sensitive measure of cellular injury induced by stress in niger in the agro-climatic environment of Bihar.

5.2 Proline Accumulation

Proline is a versatile amino acid, which accrues in plant tissues as a reaction to abiotic stress. It works as an osmoprotectant, stabilizes proteins and membranes, buffers cellular redox potential and directly scavenges ROS. The degree and length of stress are usually related to proline buildup. Drought and salinity stress factors that characterize Bihar rainfed agriculture result in large accretion of proline in leaves and roots in niger. Increased concentration of proline has been linked to better stress endurance, preservation of cell turgor and defense of photosynthetic apparatus. The concentration of proline is a biochemical indicator of stress perception and an indicator of adaptive stress responses (Sharma et al., 2019; Gill and Tuteja, 2010).

5.3 Antioxidant Enzyme Activities

The enzyme activities of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) are important biochemical indicators of the ability of the plant to neutralize ROS. In stressful situations, the amplified activities of these enzymes point to the stimulation of antioxidant defense mechanisms. An increase in the SOD activity in niger indicates effective dismutation of the superoxide radicals; high CAT and APX values indicate the higher scavenging of hydrogen peroxide. GR activity shows the restoration of decreased glutathione, which preserves the redox homeostasis in cells. Empirical evidence in oilseed crops shows that stress-tolerant genotypes have a stronger activity of antioxidant enzymes than the susceptible ones, indicating their use as predictors of oxidative stress tolerance (Noctor and Foyer, 2016; Mittler, 2017).

5.4 Chlorophyll Content and Fluorescence

Oxidative damage to photosynthetic apparatus is an indirect, but sensitive, parameter measured as chlorophyll content and chlorophyll fluorescence parameters. The degradation of chlorophyll pigments caused by ROS results in low photosynthetic efficiency and carbon assimilation.

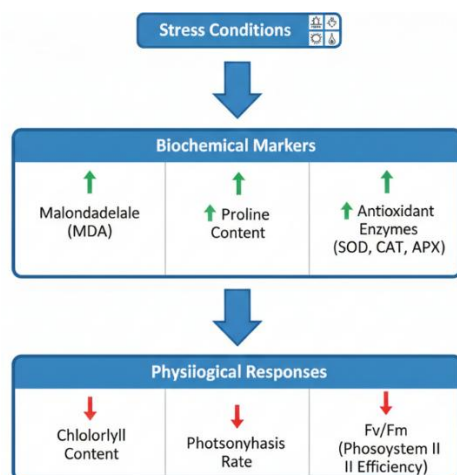


Figure 2. Biochemical and Physiological Indicators of Abiotic Stress in Niger

Photoinhibition caused by stress is normally determined by the maximum quantum efficiency of photosystem II (Fv/Fm). In the optimal conditions, the Fv/Fm values are between 0.75 and 0.85, when subjected to drought, heat, or salinity stress, Fv/Fm values of niger significantly decrease, which means that the photosystem II reaction centers are damaged. The decreased chlorophyll content and the distorted fluorescence parameters will therefore be effective indicators of the severity of oxidative stress in field grown niger crops (Maxwell and Johnson, 2000).

7. Case Studies and Experimental Evidence

Experimental research under conditions of controlled and field conditions give compelling evidence that abiotic stresses play a major role in oxidative metabolism and antioxidant defense in *Guizotia abyssinica*. Since niger in Bihar is mostly grown under rainfed and marginal soil conditions, drought, salinity and temperature stresses are of special concern.

7.1 Drought Stress Experiments

Experiments of drought stress in greenhouse and field conditions all show increased reactive oxygen species (ROS) production and oxidative injury in niger plants. A decrease in soil moisture to optimal and severe drought conditions (3040 to 10-15) causes a considerable increase in the content of malondialdehyde (MDA), which is an indicator of increased lipid peroxidation and membrane damage. At the same time, the level of proline accumulation rises significantly, which is an indicator of osmotic adaptation and perception of stress.

The activities of antioxidant enzymes are biphasic. Exposure to drought in the short term triggers higher levels of activities of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) implying the activation of the protective defense systems. The extended drought causes oxidative inactivation of enzymes and metabolic disturbances, which cause a decrease in the amount of chlorophyll and photosynthetic rate (Anjum et al., 2011; Hasanuzzaman et al., 2020).

Table 4 Measurement Framework (Indicative Data)

| Parameter | Control | Drought Stress |
|-----------------------------------|-----------|----------------|
| Soil moisture (%) | 35 ± 2 | 12 ± 1 |
| MDA (nmol g ⁻¹ FW) | 2.1 ± 0.3 | 5.8 ± 0.6 |
| Proline (μmol g ⁻¹ FW) | 0.9 ± 0.1 | 3.6 ± 0.4 |

| | | |
|-----------------------------------------------------------------------------|-------------|-------------|
| SOD (U mg ⁻¹ protein) | 85 ± 5 | 132 ± 8 |
| Chlorophyll (mg g ⁻¹ FW) | 1.85 ± 0.12 | 1.02 ± 0.09 |
| Photosynthetic rate (μmol CO ₂ m ⁻² s ⁻¹) | 18.4 ± 1.2 | 9.6 ± 0.8 |

Osmoprotectants like proline and glycine betaine have been demonstrated to prevent the drought-induced oxidative damage by stabilizing membranes, increasing the antioxidant enzyme activities, and partly restoring the photosynthetic efficiency and yield (Ashraf and Foolad, 2007).

7.2 Salinity Stress Studies

Experiments on salinity stress with NaCl levels of 50-150 mM show that niger seedlings respond to salinity levels in a dose-dependent manner. The moderate salinity (50-75 mM NaCl) leads to the stimulation of the SOD and CAT activities, which allows the successful elimination of ROS. Above 100 mM of salinity, the excessive ionic toxicity interferes with enzyme structure and activity leading to lower antioxidant activity and increased oxidative damage. The contents of MDA and proline gradually rise with the salinity, whereas the chlorophyll content and membrane stability index (MSI) decreases significantly. The presence of combined salinity and drought stress conditions is a negative factor that contributes to oxidative damage, and this synergistic effect is detrimental to plant performance (Munns and Tester, 2008).

Moderate salinity → ↑ SOD, CAT → enhanced ROS scavenging

High salinity → ↓ enzyme activity → increased lipid peroxidation

Salinity + drought → severe reduction in photosynthesis and growth

7.3 Temperature Stress Responses

Another important abiotic limitation to the niger culture in Bihar is temperature extremes. The presence of heat stress at 35-40 °C boosts the generation of the ROS in leaf tissues greatly by increasing the rate of respiration and destabilizing the thylakoid membranes. The first stage is the increase in activity of antioxidant enzymes in response to the oxidative stress but with long exposure, the enzyme activity decreases and the chlorophyll is damaged. Cold stress (410 °C) is less prevalent, but also results in the accumulation of ROS because it inhibits the photosynthetic electron transport. Interestingly, cold stress induces different regulation of antioxidant enzymes, and APX, GR tend to be more activated than CAT, which indicates stress-specific antioxidant responses (Mittler, 2017). These case studies affirm that the relationship between the antioxidant defense capacity and the ROS production is the determinant of stress tolerance in niger. These biochemical and physiological responses can only be understood to come up with stress-resistant niger varieties that can be used in the agro-climatic conditions of Bihar.

8. Molecular and Genetic Regulation of Stress Responses

The molecular and genetic control of antioxidant defense mechanisms is important in designing abiotic stress-tolerant varieties of niger (*Guizotia abyssinica*) particularly in the agro-ecological environment of Bihar that is climate-sensitive. Although the biochemical responses are a marker of instant adaptation to stress, the molecular regulation defines the efficiency, duration and specificity of the responses.

8.1 Gene Expression of Antioxidant Enzymes

Transcriptional reprogramming in plants in response to abiotic stresses including drought, salinity, and temperature extremes results in increased expression of antioxidant enzyme genes that include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR). Increased transcription of these genes leads to increased enzyme production, which increases the ROS detoxification ability.

Stress-responsive cis-regulatory elements that are commonly present in promoter regions of antioxidant genes include ABA- responsive elements (ABRE), dehydration- responsive elements

(DRE), and antioxidant response elements (ARE). The motifs help in quick transcriptional activation in response to stress-induced signaling molecules (Mittler et al., 2011). Even though there is limited molecular research in the niger, it has been demonstrated that molecular studies in related oilseeds indicate that the same regulatory processes may be in effect in *Guizotia abyssinica L.* and help in explaining genotype-specific variation in stress tolerance.

8.2 Signal Transduction Pathways

Reactive oxygen species are not only by-products which are cytotoxic but also secondary messengers in stress signaling pathways. The regulated accumulation of ROS triggers mitogen-activated protein kinase (MAPK) cascades that transmit cytoplasmic stress signals to the nucleus. These cascades control the action of transcription factors including NAC, WRKY, MYB and bZIP which subsequently regulate the expression of antioxidant and stress-responsive genes (Sharma et al., 2012).

The plant hormones are the key to the combination of stress signals and antioxidant reactions. Abscisic acid (ABA), which is one of the stress hormones, increases during the drought and salinity stress and activates the expression of antioxidant genes by modulating ABA-dependent signaling pathways. The regulation via ABA improves the expression of SOD, CAT, and APX genes, which facilitates the elimination of ROS and cellular protection (Fujita et al., 2011). ABA-dependent antioxidant regulation is especially important to the survival and productivity of nigers in rainfed habitats of Bihar where drought stress is often combined with high temperature.

8.3 Genetic Variation in Niger

The genetic variability in the niger genotypes is also very important in the determination of the stress tolerance capacity. Various landraces and cultivars have significant differences in activities of antioxidant enzymes, ROS scavenging activities, and stress-induced patterns of gene expression. Genotypes that have naturally increased basal antioxidant activity or greater upregulation of genes due to stress are better membrane stable, photosynthetic and yield better under stress. The expression quantitative trait loci (eQTLs) and stress-responsive gene markers are molecular markers associated with antioxidant characteristics, which can be utilized in marker-assisted selection (MAS) programs. Even though the genomic resources of niger remain immature, the progress in comparative genomics and transcriptomics of related species of the Asteraceae family offer useful resources to find candidate genes related to stress tolerance (Varshney et al., 2018). The use of molecular screening based on biochemical and physiological characteristics would greatly improve breeding efficiency.

9. Agronomic and Biotechnological Strategies to Enhance Stress Tolerance

A combination of the traditional agronomy, breeding, and the use of modern biotechnology is necessary to improve abiotic stress tolerance in niger (*Guizotia abyssinica L.*). Considering the fact that rainfed agriculture is the main activity in Bihar, unpredictable rainfall, and low quality soils, these strategies are very important in stabilizing production and enhancing the livelihoods of the farmers.

9.1 Conventional Breeding

One of the simplest ways of improving stress resistance of niger is through traditional breeding. Very useful in enhancing resilience can be the use of genotypes with naturally high antioxidant enzyme activity (SOD, CAT, APX, GR), lower malondialdehyde (MDA) levels, stable chlorophyll levels, and higher membrane stability under stress. Physiological and biochemical phenotyping under controlled stress conditions can be used to identify tolerant genotypes adapted to drought, salinity and extreme temperatures by the breeders. The use of biochemical signatures such as proline accumulation, chlorophyll fluorescence (Fv/Fm), and antioxidant enzyme phenotypes in breeding programs can be used to improve the selection process. The breeding against multi-stress conditions is particularly relevant in Bihar where prevalence of

multi-stress conditions is high. The breeding cycle is protracted and genomic resources in niger are few, which makes the implementation of the modern tools additional to the traditional ones (Ceccarelli et al., 2010).

9.2 Use of Biostimulants and Priming

Biostimulants and seed priming methods are also cost effective and easy methods of improving stress tolerance that is optimal in small scale systems of Bihar. Priming of seed with antioxidants such as ascorbic acid, salicylic acid and hydrogen peroxide has been found to cause so-called stress memory resulting in acceleration of the rate and intensity of antioxidant defense response to subsequent stress. Primed niger seeds have improved germination, anti-oxidant enzyme activities, low lipid peroxidation and early seedling vigor during drought and salinity stress conditions. The ability to fight oxidants also rises with zinc and selenium foliar application of micronutrients as cofactors to the antioxidant enzymes and stabilizing cellular redox homeostasis (Hasanuzzaman et al., 2018). Interventions based on low input are especially applicable in those cases when farming is resource constrained.

9.3 Genetic Engineering and CRISPR

The genetic engineering methods offer potent instruments of direct improvement of the antioxidant defence mechanisms. Excessive expression of genes that encode important antioxidant enzymes like SOD, APX, and GR in numerous crops has been found to lower the accumulation of ROS, preserve photosynthetic activity, and enhance drought, salinity, heat tolerance, etc.

Recent advances in the CRISPR/Cas genome editing provide a degree of accuracy that has never been experienced in the editing of stress-regulatory genes and regulatory factors. Stress responses may be optimized without the insertion of foreign DNA through editing promoter regions to increase expression that may be induced by stress or by targeting negative regulators of antioxidant pathways. Transgenic and genome-edited lines of Niger are still not well-investigated, though the experience with other oilseeds crops shows that there is a serious possibility of using them when the genomic resources and regulatory frameworks are more developed (Chen et al., 2019).

9.4 Microbial Interactions

Plant-microorganism interactions should also be positive to enhance abiotic stress tolerance. Plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi promote water and nutrient uptake, regulate concentrations of phytohormones, and trigger antioxidant defense. The activity of PGRs induces antioxidant enzymes to reduce the levels of ROS and improve membrane stability under drought and salinity stress. The mycorrhizal associations enhance the phosphorus uptake and utilization of soil moisture which indirectly reduces oxidative stress. The application of microbial inoculants to niger culture practice can thus provide a sustainable and ecologically safe solution to the reduction of stresses in Bihar agro-ecosystems that are delicate (Vurukonda et al., 2016).

10. Integration of Biochemical and Physiological Indicators for Stress Assessment

The problem of abiotic stress tolerance in niger (*Guizotia abyssinica* L.) can be only clearly comprehended within an integrated assessment framework, which brings together biochemical, physiological and molecular indicators. The stress intensity, plant resilience, and adaptive capacity cannot be measured properly in the agro-climatic conditions of Bihar that are variable using individual parameters alone, but rather a holistic approach should be used.

The biochemical evidence of the oxidative stress and cellular damage is responsive to malondialdehyde (MDA), proline levels, and antioxidant enzyme (SOD, CAT, APX, GR) activity. Membrane lipid peroxidation is signified by high levels of MDA and osmotic adjustment and stress signaling by high levels of proline. Increased antioxidant enzyme activities

are indicative of activation of ROS-scavenging activities. The entire performance of the plant in the field may not be adequately explained by biochemical reactions (Mittler, 2017; Hasanuzzaman et al., 2020). Physiological traits provide functional information of how biochemical differences are translated into plant functionality. The parameters that reveal the integrity of photosynthetic machinery, water relations and membrane systems are the rate of photosynthesis, stomatal conductance, chlorophyll content, chlorophyll fluorescence (Fv/Fm) and membrane stability index (MSI). The reduction in the rate of photosynthesis and Fv/Fm values is often associated with the augmentation in the oxidative damage and the diminution in the antioxidant activity as seen in Bihar where drought and salinity stress is widespread. The gap between the physiological measures of the stress responses at the cellular level and the yield-based outcomes is filled with the help of the physiological measures (Flexas et al., 2014).

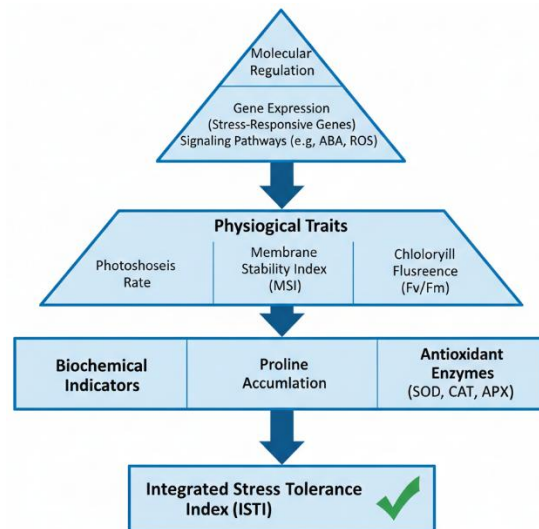


Figure 3. Integrated Stress Assessment Framework for Niger under Bihar Conditions

Molecular information may also enhance in the measurement of stress by revealing the regulation procedure of the biochemical and physiological responses observed. The patterns of gene expression of antioxidant enzyme encoding genes, stress responsive transcriptional factors and signaling factors gives early indications of stress perception and adaptive capacity. The data on gene expression can be analyzed with the data on the biochemical and physiological features, which will enable to determine the tolerant genotypes, with powerful and synchronized responses to stress (Varshney et al., 2018). The multi-level indicators can be used to produce composite stress indices that can be quantitatively measured to determine stress tolerance. These indices can be used by individuals to screen genotypes of niger on a large scale and it can be used to assist breeding programs to enhance the resilience to drought, salinity and extreme temperatures. Integrated stress indices would be a viable and scientifically applicable crop enhancement and sustainable oil production tool of the Bihar region where crops are likely to experience multiple abiotic stresses at the same time.

11. Conclusions

Abiotic stress has been one of the major limiting factors to sustainable production and growth of niger (*Guizotia abyssinica* L.) especially in rainfed, resource-limited and climate-varying environments, which are characteristic of Bihar. As noted in this research paper, environmental stressors such as drought, salinity, extreme temperatures among others, cause overproduction of reactive oxygen species (ROS) that cause oxidative stress to cellular membrane, proteins, nucleic acids and photosynthetic apparatus. Such damage would be highly indicated by high levels of biochemical indicators like malondialdehyde (MDA) and chlorophyll content and chlorophyll fluorescence. The synthesis continues on the fact that the niger plants in Bihar depend on the antioxidant defense mechanism as a means of reducing the oxidative stress. Increased activity of antioxidant enzymes including superoxide dismutase, catalase, ascorbate peroxidase and

glutathione reductase and accumulation of osmoprotectants like proline are important in the redox maintenance, membrane stability and other fundamental physiological activities in the case of stress. These defenses are incapable of coping with the long or extended stress that is increasingly becoming common because of climate variability in Bihar that causes loss of yields. One of the potential and effective models of stress tolerance measurement of niger in agro-climatic Bihar environment is an integrated system of biochemical indicators, physiological characteristics, and molecular indicators. This type of coordination will warn about the discovery and production of stress-resistant genotypes, which is specific to local habitat. This strategy together with the enhanced agronomic practices, application of biostimulants, positive microbial inoculants and novel biotechnological tools has a enormous potential in the improvement of niger productivity. It will be done by guaranteeing the increased stress tolerance of niger so that there is sustainable yield of oilseeds and better livelihoods of the small and marginal farmers in Bihar.

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