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Antioxidant Pathways in Plants: Recent Advances in Biotechnology Applications

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Abstract

The rapid generation of reactive oxygen species has contributed to oxidative stress in plants both in the course of normal plant metabolism and under conditions of stress exposure. Enzymatic antioxidants and non enzymatic antioxidants have created a balance that has regulated the destruction in addition to supporting redox signalling. Recently, transcriptomics, metabolomics, and network analysis have made it possible to exert deeper control of pathways. Simultaneously, biotechnology tools have been applied to enhance stress tolerance, yield stability and nutritional quality through modification of important antioxidant genes and regulators. Genome editing applications have been used to alter biosynthetic pathways, transcription factors and signalling. Metabolic engineering has been used to enhance protective flavonoids, anthocyanin and other phenolics that have facilitated antioxidant buffering of the plant and adaptation to stress. Linked antioxidants like melatonins have been researched as stress regulators and the concerned biosynthesis and signalling pathway has been mapped in crops to enhance crop. Core antioxidant pathways in this review have been summarised and the latest biotechnology applications have been synthesised in gene editing, omics guided target discovery as well as precision regulation strategies. Applied performance has been brought out in drought, salinity, heat, cold, flooding and heavy metal stress environments. Significant weaknesses have been noted which consist of traits trade offs, off target editing risk, genotype dependence, and field level variability. Recommendations on future directions have already been offered such as multi gene editing, cell type specific control, predictive modelling and integrated breeding pipelines which has integrated edited alleles and quantitative selection.

Keywords: *Reactive oxygen species, redox homeostasis, ascorbate glutathione cycle, flavonoids, genome editing*.

Introduction

Oxidative stress in plants has been stipulated as a situation where the reactive oxygen species has build-up beyond the capacity of the antioxidant systems. These reactive oxygen species have comprised superoxide radical, hydrogen peroxide, hydroxyl radical, and singlet oxygen. These molecules have been synthesized in chloroplasts, mitochondria, peroxisomes and apoplast areas and lipids, proteins, nucleic acids and membranes have been damaged when control has been lost. Meanwhile, modulation of signalling functions have been facilitated in the event that moderate and spatially confined reactive oxygen species pulses have been produced. The key point has been identified that oxidative stress protection, and redox signalling have been both co-regulated as opposed to being independent processes, and this has offered opportunities to be able to use biotechnology to guide further enhancement of stress resistance and quality characteristics. Recent surveys have indicated that alternative stress-specific patterns of the enzymatic and non enzymatic antioxidant parts have been set off, and enhanced tolerance has been linked with enhanced redox buffering but not with peak scavenging alone, since signalling requirements have been conserved in acclimation reactions, Rao et al., 2025.

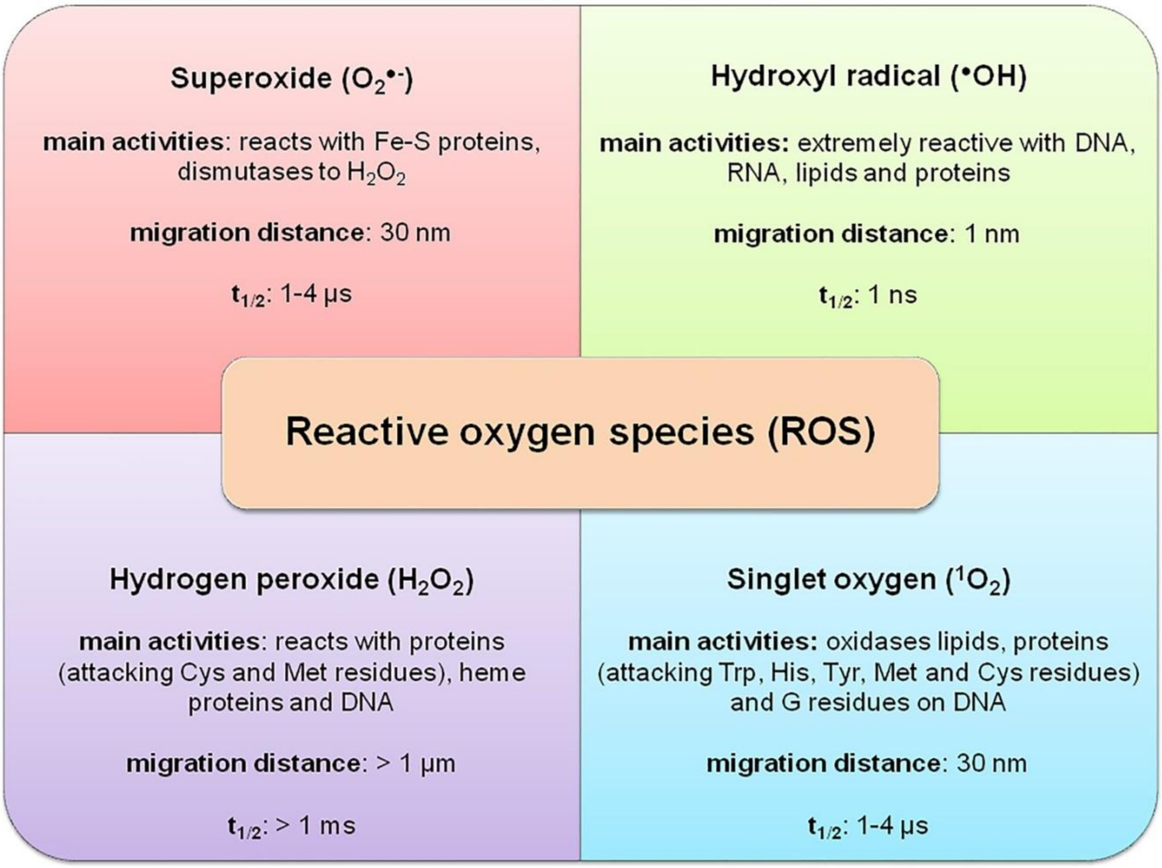

Figure 1. Major sources and control points of reactive oxygen species in plant cells

Mittler, R., 2017. ROS are good, Trends in Plant Science, 22, 1, 11 to 19. This article has given the figure of half life and migration distance of major reactive oxygen species and similar forms have been re-used in subsequent reviews.
Cores reactive oxygen species have been compared in the figure with chemical reactivity, lifetime, and movement range in cells. It has been reported that superoxide is moderately reactive and short lived, with the limited movement and quick reversion into hydrogen peroxide. It has been demonstrated that Hydrogen peroxide is less reactive but longer lived with longer movement allowing signalling functions and broader targets. Singlet oxygen has been indicated to be short lived and local acting whereas hydroxyl radical has been indicated to be highly reactive with the shortest lifetime and the least movement and hence the production site has been limited to the site of damage.
This has known information on the pathways that have been translated into practical application through biotechnology. Omics tools have been utilized to identify redox related genes, regulators and pathway bottlenecks and genome editing used to make accurate functional manipulation in crops and model plants, Cannea et al., 2025. Besides, the ascorbate glutathione cycle has been centralized in cellular redox networks that have also embraced energy status, development and stress signals, Foyer and Hanke, 2024. It is on this basis that a scrutiny attention has been vindicated on the manner in which antioxidant mechanisms have been manipulated and controlled through current developments and how beneficial crop traits have been, or might be, offered by biotechnology platforms.

Background of the Study

The plant antioxidant systems have been classified into metabolite pools and enzyme pathways. Enzyme systems have encompassed superoxide dismutase, catalase, peroxidases, glutathione peroxidases, peroxiredoxins, and enzymes which have recycled antioxidants. Non enzymatic systems have entailed ascorbate, glutathione, carotenoids, tocopherols, flavonoids, anthocyanins among other phenolics. Combinations of these have been successfully obtained through compartment specific reactions, enzyme isoforms and transporter supported distribution. Ascarbate glutathione cycle has received great attention during the past years, since ascorbate and glutathione pools have been preserved in reducing conditions to preserve a variety of cellular functions, such as photoprotection during photosynthesis, detoxification, and intracellular signalling. Post translational modifications and interactome effects have reportedly regulated component enzymes and more moonlighting functions have been postulated than the removal of reactive oxygen species, Foyer and Hanke, 2024.
Similar progress has been achieved in the knowledge of ascorbate itself. The biosynthesis of ascorbate degradation, recycling, and transport have also been referred to as development regulators with useful application to crop

productivity, quality, and storage performance, Johnson et al., 2024. This has been significant since, in addition to stress tolerance, the enhanced antioxidant capacity has been associated with post harvest characteristics, as well as nutritional value.

Table 1. Major antioxidant enzymes, cellular locations, and main functions

Antioxidant component	Typical cellular location	Main reaction role	Stress relevance
Superoxide dismutase	Chloroplast, mitochondrion, cytosol	Superoxide is converted into hydrogen peroxide	Drought, salinity, heat, cold
Catalase	Peroxisome	Hydrogen peroxide is decomposed into water and oxygen	Photorespiration, drought, metals
Ascorbate peroxidase	Chloroplast, cytosol, mitochondrion	Hydrogen peroxide is reduced using ascorbate	Photosynthesis protection, salinity
Glutathione reductase	Chloroplast, cytosol, mitochondrion	Glutathione disulfide is reduced into glutathione using NADPH	Broad abiotic stress
Peroxiredoxin	Chloroplast, cytosol, mitochondrion	Peroxides are reduced using thioredoxin systems	Redox signalling control
Glutathione peroxidase	Cytosol, organelles	Lipid peroxides are reduced using glutathione dependent routes	Membrane protection

Secondary metabolism pathways have also given a second large background area that has generated phenolics, flavonoids and anthocyanins. These metabolites have served as antioxidants, ultraviolet screens and as signalling modulators. Synthesis in the recent past revealed that the biosynthesis of anthocyanin has been regulated by structural enzyme genes and transcription factor modules and that various regulatory pathways have been reported in the determination of plant horticultural traits and stress tolerance, Zhao et al., 2024, . Notably, genome editing has allowed direct alteration of these pathways to higher accumulation of pigment which has been applied both to protect the pigment against stress and also to increase nutritional content. An exemplary review has summarised CRISPR Cas9 applications that have boosted anthocyanin pigmentation and potential opportunities and limitations have been enumerated to crop improvement programs, Mackon et al., 2023.

Another third background area has been the emerging antioxidant related signalling molecules like melatonin. The processes of melatonin biosynthesis and stress resilience have been associated with the antioxidant defence regulation and hormone crosstalk. A publication on the topic of mechanism synthesis of melatonin metabolism, regulation, and functions under abiotic stress has been published under Ikram et al., 2024. Pleiotropic effects in crops are discussed and analyzed with an emphasis on antioxidant activity and stress resistance, Michałek et al., 2025. All these background factors have provided a framework wherein biotechnology application has been incorporated into central redox cycles, specialized metabolites, and regulatory networks.

Table 2. Non enzymatic antioxidants and biotechnology leverage points

Antioxidant metabolite group	Representative members	Main functions	Biotechnology leverage points
Ascorbate pool	Ascorbate, dehydroascorbate	Redox buffering, enzyme cofactor roles	Biosynthesis enzymes, recycling enzymes, transport regulation
Glutathione pool	Glutathione, glutathione disulfide	Detoxification support, redox signalling buffering	Biosynthesis control, reductase activity, conjugation capacity
Phenolics and flavonoids	Flavonols, catechins	Radical scavenging, light screening	Flux tuning in phenylpropanoid pathway, transcription factor control
Anthocyanins	Cyanidin derivatives	Antioxidant buffering, stress protection, nutrition traits	Repressor knockout, activator tuning, tissue specific expression
Carotenoids	Beta carotene, xanthophylls	Singlet oxygen quenching, photoprotection	Pathway enzymes, plastid regulation
Tocopherols	Vitamin E forms	Lipid peroxidation control	Biosynthesis steps, membrane targeting

Justification

Increasing drought, salinity, heat extremes, floods, and heavy metal pollution in most areas of production have

exacerbated the need to have climate resilient and resource efficient agriculture. These pressures have been invariably provoked by the onset of oxidative stress and stability of yields has been compromised by the destruction of photosynthetic machinery, membranes, and metabolic enzymes as well. Traditional breeding has been fruitful, however, the rate of stress combination challenges has rate up, and trait complexity has capped advancement whereby individual traits have been addressed. The focal rationale has been constituted that antioxidant pathways have been centers of crossroads of numerous types of stresses since reactive oxygen species have been created in a wide variety of stresses and have shared detoxification and signalling units. General survey has revealed that generation and scavenging of reactive oxygen species have been elicited in response to a wide variety of abiotic stresses and enhanced resilience has been enabled by strategic modulation via priming, exogenous antioxidants, nanoparticles, and genetic tools, Rao et al., 2025.
There is also enhanced biotechnology preparedness that has enhanced justification. High precision has been achieved by genome editing, pathway regulators have been engineered with more predictable results when validated targets have been chosen. The use of CRISPR technologies in secondary metabolism has been surveyed, and current limitations have been outlined like off target effects and bottlenecks in transformation have driven the problem of careful design and better delivery systems, Gao et al., 2025. Parallel Multi omics pipelines have enhanced the discovery and prioritisation of the targets. A bibliometric synthesis has outlined growing application of transcriptomics and metabolomics in the study of environmental responses and future directions have been noted that have aided pathway engineering choices, Yan et al., 2024. As such, the topic at hand has been explained as an emergency issue and as possible to achieve high impact biotechnology uses.

Objectives of the Study

1. Plant core antioxidant pathways have been outlined, and this has focused on enzymatic scavenging, ascorbate glutathione pathway and metabolite antioxidants.
2. Recent developments in the applications of biotechnology have been synthrised in the areas of genome editing, metabolic engineering, and omics directed target discovery.
3. Principal abiotic and contaminant stresses have been described in terms of representative application and outcomes and practical mechanisms identified.
4. Study design methodological patterns have been suggested such as materials selection, assays, and statistical methods that fit the pathway research.
5. Some important limitations and future scope are discovered to inform more robust laboratory findings to field performance.

Literature Review

Reactive oxygen Species Generation and Signalling

Unavoidable reactive oxygen species have been the products of electron transfer and metabolic oxidation reactions. It has also contributed in the production of the superoxide radicals, dismutation and hydrogen peroxide generation and has been utilized in the electron transport chains in the chloroplast and mitochondria and the oxidase activity has also helped in the generation of the processes. Peroxisomes have been involved in the production of hydrogen peroxide in photorespiration and fatty acid metabolism. It is worth noting, that reactive oxygen species have been described as destructive oxidants and as coordinating acclimation signalling molecules. It has been elucidated that how reactive oxygen species signalling networks have been incorporated into stress response pathways and systemic reactive oxygen species modulation have resulted in increased resilience, Rao et al., 2025.

Redox networking and Ascorbate glutathione cycle

Ascorbate glutathione cycle has been called as pivotal pathway and has replenished reduced ascorbate and reduced glutathione but hydrogen peroxide has been neutralised by means of reactions with ascorbate peroxidase. The cycle has been distributed around cellular compartments in the form of isoforms and transport. More recent literature has viewed the cycle as a focus of energy sensitive redox signalling in addition to interaction with thioredoxin and peroxiredoxin systems. The other functions which are not directly pertinent to the control of reactive oxyradicals have been mentioned, including development and defence regulation, Foyer and Hanke, 2024,. It has been this long-term functional framing that has justified biotechnology plans that have further sought to scavenger capacities, in addition to, regulatory nodes and compartment specific dynamics.

Glutathione metabolic functions and gene systems

Glutathione has been treated to serve as a significant redox buffer/detoxification molecule that has enabled glutathione dependent enzymes, glutathione transferases and conjugation. One recent review has proposed glutathione pathways / gene networks to be the hub of environmental adaptations and has cited environmental scavenging roles in signal transduction plus metabolic regulation, but also its own direct scavenging roles, Bose Mazumdar and others, 2025. This has led to the technological processes in biology where regeneration, glutathione production and use has been made to resist the stress.

Specialised metabolites and non enzymatic antioxidants
The anthocyanins and flavonoids usage have been considered as the most important non enzymatic antioxidants in the plants. The effect of these metabolites has been attributed to tolerance to the stresses because of the radical scavenging effect, the membrane protection, and regulation of the light energy. The summary of anthocyanins biosynthesis and regulation in horticultural plants is provided, and a range of modules of transcription factors have been characterised that control pathways of Zhao et al., 2024. Specifically, the enhancement of anthocyanin with the assistance of genome editing applications has been surveyed and opportunities to improve pigment in tissues of edibles have been outlined, Mackon et al., 2023.

Asorbate as quality and development controller
Ascorbate is also known as a multifunctional development and quality regulator. Better knowledge of ascorbate biosynthesis and its regulation have been outlined as viable application of increased crop yield, their quality, and storage after harvesting, Johnson et al., 2024. This has developed direct interdependence between the agronomic value and antioxidant pathway management.

Melatonin and antioxidant defence regulation
Melatonin has remained characterized as a multi-purpose regulator that has contributed to the stress resilience through the regulation of antioxidant defence systems in addition to hormone pathways. A mechanistic review has been used to summarize abiotic stress on melatonin metabolism and signalling and critical steps in the biosynthesis and regulation aspects have been identified, Ikram et al., 2024. A summary of pleiotropy has been summarized on the foundation of a crop-based review, which has brought out antioxidant activity and hormone-hormone interactions, with reference to root development and stress tolerance, Michalski et al., 2025, . The experimental researches in the tea plants exposed to lead stress have also reported that the evidence of the melatonin correlated antioxidant control has been augmented and that oxidative stress has been neutralized through the transformation of phenylalanine into anthocyanins and catechins in case of lead stress, Li et al., 2025.

Target discovery: system level and omics analysis
It has been shown that the omics strategies are increasingly being employed in mapping stress response and identifying significant nodes to be intervened. Metabolomics and transcriptomics have been cited as very significant to fill the gap between the changes in the expression of genes and metabolic changes and phenotypes. A review has provided a science mapping view of the research trends and has identified the future directions like biosynthetic pathways and abiotic stress studies, Yan et al., 2024, . Integrated reviews on antioxidant studies have shown that omics, systems biology can be applied to identify redox genes and predictive modelling, Cannea et al., 2025,.

Accuracy control and genome editing
Genome editing has transformed the aptitude of multiple gene manipulation in antioxidant and secondary metabolic pathways. CRISPR Cas9 has been mentioned as a very efficient approach in anthocyanin pathways to enhance pigmentation and other stress-related resistance, Mackon et al., 2023, . Use of CRISPR in secondary metabolism of medicinal plants has been reviewed and the obstacles outlined and comprise off target effects and inability of transformation, Gao et al., 2025, . The literature base, which has been set by these works is in agreement with biotechnology of antioxidant pathways in the current society.

Material and Methodology
Study design overview
A systematic review approach has been used. Preference has given priority to recent peer reviewed reviews and representative representative original studies, with particular focus being given to biotechnology applications as of 2023 2025, with foundational pathway reviews also provided as of a mechanistic completeness. Information has been obtained concerning pathway components, intervention forms, validation assays and reported effects on stress tolerance, growth and quality traits.

Table 3. Biotechnology approaches and validation readouts for antioxidant pathway studies

Approach	Typical target types	Validation steps	Key outcome readouts
Genome editing	Structural enzymes, regulators, promoter elements	Sequencing confirmation, off target screening	Stress survival, enzyme activity, redox ratios

Approach	Typical target types	Validation steps	Key outcome readouts
Metabolic engineering	Flux control enzymes, transcription factors	Expression confirmation, metabolite profiling	Flavonoid content, anthocyanin content, oxidative damage indices
Exogenous regulators	Melatonin and related pathway probes	Dose response, time course sampling	Reduced lipid peroxidation, improved photosystem metrics
Omics guided selection	Co expression modules, network hubs	Multi omics integration, target prioritisation	Robust targets for editing and breeding pipelines

Choosing of materials in experimental replication setting

A model and crop paired method has also been suggested in case an experiment replication has been sought by the readers. Arabidopsis thaliana or Nicotiana benthamiana have been chosen to be used in rapid validation of constructs and editing designs. The target stress context-compatible crop species has been chosen to be translated, including that of salinity and flooding in rice; heat and drought in wheat; horticultural quality and stress in tomato; and heavy metal contexts in tea or leafy vegetables. Genotype dependence has been tested by including genotypes that have different stress resistance.

Types of biotechnology interventions

Three types of interventions have been established

1. Genome editing interventions: CRISPR Cas systems have been applied in gene knockout, promoter editing and base editing. Candidates have been picked based on literature supported pathway nodes, which are transcription factor repressors of anthocyanin, key nodes of ascorbate recycling, and glutathione biosynthesis control points, and redox signalling regulators. Validation has been performed by sequencing validation and segregation by analysis and off target screening by targeted deep sequencing of predicted sites. Directions have been followed by application trends outlined on specialized metabolism editing, Gao et al., 2025, , and with anthocyanin editing strategies, Mackon et al., 2023,.
2. Interventions based on metabolic engineering Transgene or cis regulatory element approaches have been employed to stimulate pathway step enzyme and flux to flavonoid and anthocyanin production. Tissue specific targeting has been done with regulatory modules that were described to biosynthesize anthocyanin, Zhao et al., 2024,.
3. Exogenous regulator interventions: Figures of melatonin therapy have been performed as a benchmarking and mechanistic probe methodology accompanied by dose response structure and time course sampling. It has also been coupled with the mechanistic context with melatonin signalling and antioxidant defence regulation systems Ikram et al., 2024, , and with crop level response synthesis, Michałek et al., 2025, .

Assays and measurements

Physiological assays

The performance of photosystem in stress has been measured using chlorophyll fluorescence measures. The contexts of drought and heat have been registered with terms of relative water content, stomatal conductance and measures of growth. Ddamage indicators have been taken as ion leakage, and stability index of membrane.

Biochemical assays

The indicators of the reactive oxygen species have been assessed by measuring hydrogen peroxide, staining of superoxide and malondialdehyde as a quantitative measure of lipid peroxidation. The activities of antioxidant enzymes have been determined in case of superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase and similar peroxidases. It has measured ascorbate and glutathione pools in reduced and oxidised forms to work out redox ratios, which are in line with central cycle focus, Foyer and Hanke, 2024, .

Metabolite profiling

Quantification of flavonoids, anthocyanins, catechins and phenylpropanoid intermediates have been done using targeted and untargeted metabolomics. In incorporating omics, transcriptomics has been applied to determine differentially expressed genes and co expression networks have been constructed to identify regulator candidates. It has also been reconciled with the most recent tendencies in the use of transcriptomics and metabolomics in response and quality characteristics of the environment, Yan et al., 2024, .

Statistical analysis plan

When time courses have been used, data have been organized as genotype by treatment by time repeated measures.

Shapiro Wilk has been used to test normality. Levene testing has been conducted to test homogeneity of variances. In cases where assumptions were met analysis of variance has been used where genotype and treatment are fixed effects and replicate block are considered the random effects. In cases where repeated measures are used, mixed models were used having time as within subject factor. Adjusted significance tukey method post hoc comparisons have been done. Interpretation has been supported using effect sizes and confidence interval.

Results and Discussion

Core pathway response to stress and biotechnology take advantage

In the abiotic stresses, there has been an apparent trend whereby production of reactive oxygen species has escalated and activation of antioxidant systems has been experienced. Recent synthesis has narrated the role of heat, cold, drought, flooding, salinity, and heavy metals in causing oxidative stress and specific antioxidant responses but common enzymatic and non enzymatic elements have been constantly played, Rao et al., 2025, . This common underpinning has been addressed as a leverage framework to biotechnology intervention.

Transform Ascorbate glutathione cycle into an engineering hub

The ascorbate glutathione cycle has been highlighted as a fixed centre which has incorporated redox signalling and compartment communication. The cycle has been reported to facilitate not only detoxification, but also interaction with various signalling proteins and post translational regulation. This has indicated that just overexpressing enzymes can not necessarily be the best, since balance in the network and compartment targeting has been necessitated, Foyer and Hanke, 2024, . Promoter engineering and cell type specific regulation have also been thought more plausible in biotechnology applications to maintain signalling and enhance buffering in sensitive tissues, e.g. guard cells and root tips.

Glutathione network engineering opportunities

The pathways of glutathione have been referred to as key to redox homeostasis and stress signalling. Its implication in the regulation of growth and metabolism has indicated that the stress resilience traits can be enhanced when the glutathione metabolism has been adjusted as opposed to optimised. It has been concluded through gene network perspectives that have aided in the choice of targets to be incorporated in enzymes and regulators that have coordinated the synthesis, recycling, and use of glutathione, Bose Mazumdar and colleagues, 2025, . The practical results have involved enhanced detoxification ability under heavy metal and salinity settings, in which glutathione dependent conjugation has minimized the cellular injury.

Editing of specialised metabolite and anthocyanin

Anthocyanins have been implicated with innate protective effects in stresses as well as in nutritional values. Horticultural plants have been mapped with regulatory modules, and transcription factors have been identified as effective points of intervention since there has been rotation of the coordinated regulation of various structural genes, Zhao et al., 2024, . Pathway repressors have been targeted in CRISPR based techniques to activate endogenous biosynthesis. Specific review has documented that using CRISPR Cas9 has been employed to improve the content of anthocyanin precisely and that there are prospects of adding pigment to edible tissues in pigs, Mackon et al., 2023, . This has applied to the application in antioxidant pathways since anthocyanin has augmented non enzymatic antioxidant buffering and minimized oxidative harm during the stress.

It has been pointed out that the trade offs may be experienced when the accumulation of high pigments has diverted the carbon available in the other sinks or distorted growth. Tissue specific expression and inducible regulation have hence been suggested instead of constitutive high level expression particularly in cases where penalties on yield have been a risk. These designs concepts have been matched with the regulatory complexity of pathways, as well as the sensitivity of horticultural traits of recent reviews, Zhao et al., 2024, .

The benefits of the ascorbate pathway biotechnology and crop quality

Ascorbate has been placed as a development and quality controller with realistic results on crop productivity and post harvest results. The pathways of ascorbate have influenced crop productivity, quality, and storage have recently been summed up in a review which has demonstrated that the engineering of antioxidant pathways can provide traits in the market other than resilience to stress, Johnson et al., 2024, . Thus, biotechnology wish points in ascorbate production and recycling have been used as dual benefit points, with the resistance to stresses and quality being co-improved.

Stress tolerance outcome and melatonin related biotechnology

Melatonin is a regulator of antioxidant defence and as a signalling molecule with wide stress application. The melatonin biosynthesis and signalling functions during abiotic stress conditions have been characterised by mechanistic synthesis that has provided insights into important enzymes and regulation nodes that have been amenable

to engineering and to marker based selection, Ikram et al., 2024, . Crop focused synthesis has prioritized pleiotropic activities, such as hormone crosstalk benefits, root development benefits, which have enhanced water and nutrient uptake in response to stress, Michele et al., 2025, .
Research on tea plants has also offered evidence of applied outcomes in terms of oxidative stress reduction and enhanced antioxidant associated metabolite regulation following melatonin treatment where oxidative stress reduction and improvement of antioxidant associated metabolite regulation have been demonstrated. Conversion of phenylalanine to anthocyanins and catechins has been encouraged and less accumulation of lead as well as enhancement of photosynthetic measurements have been observed, Li et al., 2025, . These results have reinforced the idea that the manipulation of melatonin pathways can be employed as an indirect method of enhancing antioxidant pathways and specialised metabolite protection in crops.

Omics directed discoveries and predictive selections of targets

Omics has played a role in identifying candidate genes and regulators which single gene approaches have overlooked in recent pipelines of biotechnology. Transcriptomics and metabolomics have been discussed as the major techniques to connect environmental reactions to phenotypes, and research mapping has shown the swiftly growing use and enhanced maturity of the tools, Yan et al., 2024, . In studies of antioxidant pathways, it has been explained in integrated reviews that omics technology has made it possible to identify redox related genes and that genome editing technology has made it possible to identify functional validation and manipulation. Cannea et al., 2025, .
Practically, it has been concluded that omics guided discovery has minimized the chances of targeting genes that have been peripheral or redundant. It is also concluded that network based selection of transcription factor nodes has provided great leverage, since several enzyme steps have been moved in unison and compensatory influences minimized.

Combined biotechnology results in multiple types of stress

Drought and heat

Activation of antioxidants has been linked to enhanced photosystem stability and enhancement of membrane protection whereas burst of reactive oxygen species has been enhanced and antioxidant activation, especially in the presence of drought and heat. There is solid evidence that has been compiled that enzymatic antioxidants and flavonoid pools have been involved in the drought tolerance features, Rao et al., 2025, . Concurrent optimization of antioxidant buffering and root architecture in biotechnology design has been popular and melatonin associated root control was found pertinent, Michalko et al., 2025, .

Salinity and flooding

Salinity stress has caused ionic imbalance and oxidative stress, and flooding caused hypoxia driven by re oxygenation surges of reactive oxygen species. Glutathione metabolism, peroxidase network tuning and protection flavonoid enhancement have been used as approaches to pathway engineering.

Heavy metals

The exposure to heavy metals has caused severe oxidative harm and has necessitated a detoxification and sequestration. It has been demonstrated that melatonin mediated antioxidant defence decreased oxidative injury and could have facilitated low levels of uptake or changed expression of transport genes in tea plants, Li et al., 2025, . The relevance of glutathione dependent detoxification has been facilitated by the centrality of glutathione to redox homeostasis and stress signalling Bose Mazumdar and colleagues, 2025, .

Overall synthesis

The commonest finding across the literature reviewed has been that antioxidant pathways have served as effective biotechnology targets in cases where the network context has been observed. The direct enzyme overexpression has not been able to offer stable gains always. This has been suggested to work better when a coordinated control has been attained using regulatory nodes, tissue specificity, and inducible control which has maintained signalling functions and augmented damage control capacity.

Limitations of the Study

The current evidence base has been identified to have several limitations. To begin with, biotechnology results reported have been in controlled conditions, and field translation has been hampered with the variability of the stress timing, stress combinations, and soil interactions. This has specifically been applicable since the strength and duration of stress have been very crucial in influencing antioxidant responses Rao et al., 2025, . Second, genotype dependence has been common, in the sense that engineered or edited alleles have shown varied results across pathways because of redundancy of pathways and differences in regulations. Third, the trade offs have been noted or predicted to take place between growth and yield, primarily where the metabolic flux has been diverted to secondary metabolites like anthocyanins. Pathway regulatory reviews, Zhao et al., 2024, and have indicated the need to have tissue specific and inducible designs. Fourth, constraints in delivery and transformation, off target risks, have also been an issue in most crops, and have needed scrutiny as has been observed in CRISPR targeted reviews, Gao et al., 2025, and so forth. Fifth, detoxification benefits of mechanistic separation of signalling have been difficult to realise, since reactive oxygen species have been used in both negative and positive signalling, Foyer and Hanke, 2024, .

Future Scope

A number of future directions have been laid emphasis on. Multi gene editing has been anticipated to be more and more employed, since the antioxidant pathways have been dispersed among the enzyme families and enzyme compartments. Cis regulatory tuning and promoter editing have been anticipated to provide more predictable characteristics with fewer trade offs compared to coding region disruption, and these technologies have been consistent with the trends of precision regulation in genome editing applications, Gao et al., 2025, .

Cell specific and inducible regulatory has been anticipated to be extended. With this direction, stress inducible promoters and guard cell specific promoters have been suggested to enhance drought tolerance and penalty penalties have been minimized on yield. The network view of ascorbate glutathione cycle and redox signalling integration has supported this direction, Foyer and Hanke, 2024, .

The integration of omics and predictive modelling has been anticipated to be a key area in trying to discover and rank targets. The tendency of transcriptomics and metabolomics has reported further growth and better mapping of phenotype connections, Yan et al., 2024, . Omics, systems biology, and computational approaches have been described as one of the accelerators to module discovery and predictive modelling used in antioxidant systems, Cannea et al., 2025, .

A breeding scope of integration has been highly suggested. The use of edited alleles has been anticipated to be integrated with quantitative trait loci selection and genomic selection, such that antioxidant pathway improvements have been integrated into high yielding backgrounds. Simultaneously, development in the quality traits has been anticipated to be enhanced by the targets of antioxidant nutritional links, particularly those of ascorbate and anthocyanin, Johnson et al., 2024, , and Mackon et al., 2023, .

Lastly, field evaluation standardisation has been anticipated to better. Stress profiling and redox biomarker measurement in multi location trials have been suggested as a way of reinforcing causal relationships and implementation decisions.

Conclusion

It has been established that antioxidant pathways are primary systems in plants that have ensured resistance to oxidative stress and mediated redox signalling to support acclimation. The ascorbate glutathione cycle has been placed in the center of a hub that has taken into consideration stress responses in conjunction with development and energy status. The process of glutathione metabolism has been pointed out as a network controller of detoxification and signalling. Protective metabolites with good biotechnology potential have been highlighted as non enzymatic antioxidants like flavonoids and anthocyanins. The recent developments have been predetermined by the omics that have been used to discover targets as well as the genome editing techniques that have facilitated the accurate manipulation of the enzymes and regulators within pathways. The most persuasive biotechnology strategies have been in which the balance of the network, tissue specificity and stress inducibility have been taken into consideration and as such the signalling functions have not been deteriorated but instead damage control has been enhanced. Field translation, dependence on genotype and trade offs have been identified as key limitations. Multi gene and promoter level editing, integrated omics and modelling and integration of breeding pipelines have been more likely to facilitate future advancements. Generally, both stress resistance and quality enhancement objectives have been powerfully backed by the new biotechnology toolbox implemented to plant antioxidant pathways.

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