Ascorbate-Glutathione Pathway

Subjects: Biology

Contributor: Mirza Hasanuzzaman, MHM Borhannuddin Bhuyan

The Ascorbate-Glutathione (AsA-GSH) pathway, also known as Asada–Halliwell pathway comprises of AsA, GSH, and four enzymes viz. ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase, play a vital role in detoxifying ROS. Apart from ROS detoxification, they also interact with other defense systems in plants and protect the plants from various abiotic stress-induced damages. Several plant studies revealed that the upregulation or overexpression of AsA-GSH pathway enzymes and the enhancement of the AsA and GSH levels conferred plants better tolerance to abiotic stresses by reducing the ROS.

Keywords: antioxidant defense ; free radicals ; glyoxalase system ; hydrogen peroxide ; plant abiotic stress ; reactive oxygen species ; redox biology ; stress signaling

1. Introduction

Plants have an antioxidant defense system having non-enzymatic and enzymatic antioxidants in cellular organelles, which scavenges different ROS up to a certain level. If the ROS generation is higher than the scavenging ability of the antioxidant system, then oxidative damage occurs. The antioxidant defense system comprises ascorbate (AsA), glutathione (GSH), carotenoids, tocopherols, flavonoids, etc., which are some commonly known non-enzymatic antioxidants [1]. Ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione *S*-transferase (GST), and peroxiredoxin (PRX) are well known enzymatic antioxidant components [2][3]. Among all of these, AsA, GSH, APX, MDHAR, DHAR, and GR comprise the AsA-GSH cycle.

Ascorbate is one of the most powerful substrates for scavenging H_2O_2. Ascorbate maintains the reduced state of α-tocopherol. Ascorbate is supposed to be concerned in zeaxanthin biosynthesis dissipating excess light energy in the thylakoid membranes of chloroplast and prevents oxidative stress. Ascorbate sustains reduce the state of prosthetic metal ions and maintain the activity of antioxidant enzymes [4]. Glutathione regulates various metabolic functions; it acts as an antioxidant. Glutathione peroxidase and GST utilize GSH as substrate; GPX is responsible for ROS detoxification, whereas GST is liable for xenobiotic detoxification [5]. The glyoxalase system consisting of glyoxalase I (Gly I) and glyoxalase II (Gly II) enzymes detoxifies cytotoxic and oxidative stress creator methylglyoxal (MG), where Gly I uses GSH and after finishing MG detoxification, GSH is recycled [6]. The positive role of AsA-GSH cycle components has been documented in many plants that are affected by abiotic stresses [5][6]. Participation of the GSH/glutathione disulfide (GSSG, the oxidized form of GSH) redox in maintaining a favorable cellular environment and in stress signal and adaptation were discussed in some previous findings. Glutathione participates in signal transduction, the proper pathway, of which remains unrevealed. The presence of AsA and GSH has been reported to improve osmoregulation, plant water status and nutrient status, water use efficiency, photosynthetic performance, and the overall productivity of plants. Exogenous AsA and GSH applications have been reported to enhance the antioxidant defense as well as the overall tolerance of plants against abiotic stresses. Accordingly, the enzymatic antioxidants of AsA-GSH cycle participate in scavenging ROS, whereas AsA and GSH not only directly scavenge a range of ROS but also perform many other functions to maintain a favorable state in cytosol and other cellular organelles to enhance antioxidant capacity and to reduce oxidative stress, which is induced by different abiotic stresses; AsA and GSH also improve the physiological performance of plants. Since the discovery of the AsA-GSH cycle, its most discussed topics are related to antioxidative protection.

2. Ascorbate-Glutathione Pathway—An Overview

Ascorbate-Glutathione pathway (also called as Asada–Halliwell pathway) is the major pathway of antioxidant defense, which mainly detoxify the H_2O_2 in a plant cell. Apart from AsA and GSH, its enzymes—APX, MDHAR, DHAR, and GR [4]—have significant roles. Both AsA and GSH are found in the cytosol, nucleus, chloroplast, mitochondria, and peroxisome,

where they operate the functions assisted by four enzymes and, therefore, each enzyme has several isoforms that are based on the cellular localization [7]. Both AsA and GSH are present in cellular organelles in a millimolar range, for instance, in *Arabidopsis thaliana*, AsA concentration is the highest (22.8 mM) in the peroxisome, where GSH is highest (14.9 mM) in mitochondria [8][9]. AsA and GSH both have high redox potentials and, therefore, interact with many components and pathways towards the maintenance of a generally reduced state. There are few steps, by which AsA and GSH work coordinately to detoxify H_2O_2, and at the same time, both AsA and GSH are regenerated. First, the enzyme APX converts H_2O_2 into water with the help of AsA as an electron donor, which is also converted into monodehydroascorbate (MDHA). This MDHA again regenerates AsA by the activity of MDHAR and a part of this is spontaneously converted into dehydroascorbate (DHA). Later, DHA is reduced to AsA again by using GSH, which results in its oxidation to produce GSSG. Finally, this GSSG regenerates GSH by the activity of GR using NADPH as the electron donor (**Figure 1**). Both AsA and GSH are strong antioxidants, but the maintenance of their redox state is important in conferring stress tolerance in plants, which largely depends on the activities of the four enzymes that are associated with the AsA-GSH cycle [4][10]. In the next sections, we have described all of the components of the AsA-GSH pathway.

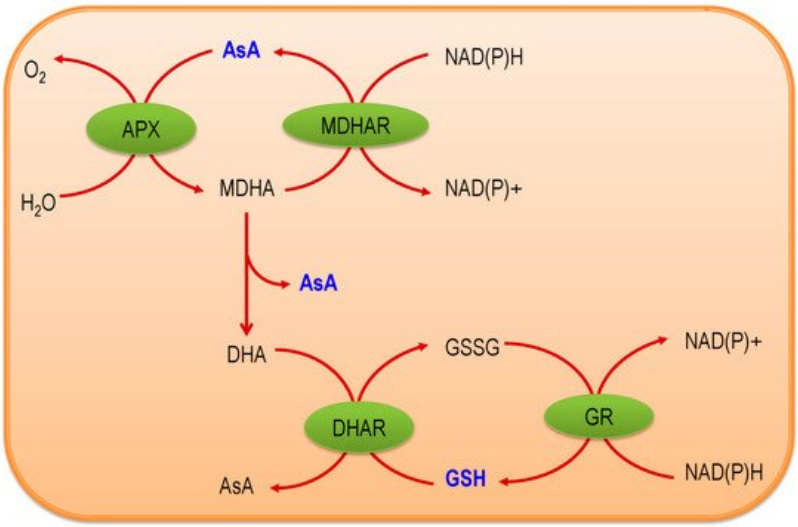

Figure 1. Ascorbate-Glutathione (AsA-GSH) (Ascorbate-Glutathione) pathway [ascorbate, AsA; ascorbate peroxidase, APX; monodehydroascorbate, MDHA; monodehydroascorbate reductase, MDHAR; dehydroascorbate, DHA; dehydroascorbate reductase, DHAR; glutathione, GSH; oxidized glutathione, GSSG; glutathione reductase, GR; Nicotinamide adenine dinucleotide phosphate (reduced form), NAD(P)H; Nicotinamide adenine dinucleotide phosphate (oxidized form), $NAD(P)^+$].

3. Role of AsA-GSH in Regulating Oxidative Stress under Abiotic Stresses

Abiotic stress-induced excess ROS causes oxidative stress in plants followed by cellular damage, even death. Hence, the plant itself defends against this higher ROS accumulation by their defense mechanism. Plant significantly activates the AsA-GSH pathway for ROS detoxification. In this section, we will discuss the involvement of AsA-GSH cycle for alleviating oxidative stress upon various abiotic stresses reviewing recently published articles (**Table 1**, **Table 2,** and **Table 3**).

Table 1. Role of AsA-GSH in regulating oxidative stress under salinity and drought.

Plant Species	Stress Levels	Status of AsA-GSH Component(s)	ROS Regulation	References
***Triticum aestivum* L.**	**100 mM NaCl**	**GSH content increased by 15%; Stimulated APX and GR activities by 78% and 56%, respectively**	**Increased H_2O_2 content about 79%**	[11]

Plant Species	Stress Levels	Status of AsA-GSH Component(s)	ROS Regulation	References
T. aestivum L. cv. BARI Gom-21	12% PEG for 48 and 72 h	Decreased AsA content at 48 h, but after 72 h, AsA content again enhanced; Increased GSH and GSSG content where GSH/GSSG ratio decreased time-dependently; Enhanced the activities of APX, MDHAR, and GR	Enhanced the H_2O_2 content by 62% and increased O_2^- accumulation	[12]
T. aestivum L.	10% PEG	Reduced AsA/DHA and GSH/GSSG redox; Increased enzymatic antioxidants actions of AsA-GSH cycle	Increased H_2O_2 production	[13]
T. aestivum L.	35–40% field capacity (FC) water	Increased GSH/GSSG by 64% while decreased AsA/DHA by 52% respective with a duration of stress; Enhanced APX, MDHAR, DHAR and GR activities	Increased H_2O_2 along with stress duration	[14]
T. aestivum cv. Pradip	150 and 300 mM NaCl	Reduced AsA content upto 52%; Increased reduced and oxidized GSH accumulation by 55% and 18%, respectively with 32% higher GSH/GSSG ratio; Increased APX activity with 29% reduction of GR activity; Slightly increased MDHAR and DHAR activity	Enhanced H_2O_2 generation by 60%	[15]
Oryza sativa L. cv. BRRI dhan47	150 mM NaCl	Increased GSH accumulation while reduced AsA content by 49% Increased GSH content and lowered the redox status of both AsA/DHA and GSH/GSSG; Upregulated the activity of APX, MDHAR, DHAR, and GR	Increased the production of O_2^- with 82% higher H_2O_2 accumulation	[16]
O. sativa L. cv. BRRI dhan49	300 mM NaCl	Reduced AsA and GSH accumulation by 51% and 57%, respectively; Decrease GSH/GSSG redox by 87%; Showed lowered APX (27%), MDHAR (24%), DHAR and GR (25%) activities	Increased H_2O_2 content upto 69%	[17]
O. sativa L. cv. BRRI dhan54	300 mM NaCl	Improved AsA content by 51% with higher GSH content; Decreased GSH/GSSG ratio by 53%; Showed higher APX (27%) and DHAR activities while decreased both GR (23%) and MDHAR activities	Accumulated 63% higher H_2O_2 content	[17]

Plant Species	Stress Levels	Status of AsA-GSH Component(s)	ROS Regulation	References
Brassica napus **L. cv. BinaSharisha-3**	**100 and mM NaCl**	**Reduced the AsA content by 22%; Increased GSH content by 72% and GSSG content by 88%; Unaltered the GSH/GSSG ratio; Amplified APX activity by 32%, decreased DHAR activity by 17%; Slightly increased GR activity**	**Accumulated higher H_2O_2 content by 76%**	**[18]**
B. napus **L. cv. Binasharisha-3**	**200 mM NaCl**	**Reduced the AsA content (40%) along with increased GSH (43%) and GSSG (136%) contents; Decreased the GSH/GSSG ratio (40%); Amplified the APX activity (39%) and reduced the MDHAR (29%) and DHAR (35%) activities; Improved GR activity (18%)**	**Showed 90% more H_2O_2 content**	**[18]**
B. napus **L.**	**15% PEG**	**The AsA accumulation remained unaltered and reduced the AsA/DHA ratio; Enhanced GSH content by 19% and GSSG by 67% and decreased GSH/GSSG ratio; Increased APX, MDHAR, DHAR and GR activities**	**Higher accumulation of H_2O_2 by 55%**	**[19]**
B. campestris **L.**	**15% PEG**	**Decreased AsA content by 27% with a decrease of AsA/DHA ratio; Increased GSH content by 33% with higher GSSG content by 79% and lowered GSH/GSSG ratio; Decreased DHAR activity**	**Higher accumulation of H_2O_2 about 109%**	**[19]**
B. juncea **L.**	**15% PEG**	**Increased the AsA content and did not affect the AsA/DHA ratio; Increased GSH content by 48% and GSSG by 83% and decreased GSH/GSSG ratio; Increased APX, MDHAR, DHAR and GR activities**	**Accumulation of 37% higher H_2O_2**	**[19]**
B. juncea **L. cv. BARI Sharisha-11**	**10% PEG**	**Reduced AsA content (14%) while increased both GSH (32%) and GSSG (48%) contents; Enhanced APX activity (24%); Decreased MDHAR and DHAR (33%) activities along with 31% increased GR activity**	**Acute generation of H_2O_2 (41%)**	**[20]**

Plant Species	Stress Levels	Status of AsA-GSH Component(s)	ROS Regulation	References
B. juncea L. cv. BARI Sharisha-11	20% PEG	Decreased AsA content by 34% while increased the content of GSH by 25% and GSSG by 101%; Up-regulated APX activity by 33%; Decreased activity of MDHAR and DHAR (30%)	Extreme generation of H_2O_2 by 95%	[20]
B. napus L. cv. BinaSarisha-3	10% PEG	Increased AsA (21%), GSH (55%) and GSSG contents while decreased GSH/GSSG ratio Unaltered the activities of APX, and increased the activity of MDHAR, DHAR, and GR (26%)	Elevated the H_2O_2 production	[8]
B. napus L. cv. BinaSarisha-3	20% PEG	Unaltered AsA content along with higher content of GSH (46%) and GSSG and reduced GSH/GSSG ratio; Reduced the APX and MDHAR activities along with the higher activity of DHAR and GR (23%)	Showed higher H_2O_2 production	[8]
B. napus L. cv. BinaSharisha-3	10% PEG	Increased AsA, GSH (31%) and GSSG (83%) accumulation with lowered GSH/GSSG ratio; Increased APX activity while reduced MDHAR and DHAR activities, but GR activity remained unaltered	Increased H_2O_2 content by 53%	[21]
B. napus L. cv. BinaSharisha- 3	20% PEG	Slightly increased AsA content with 26% and 225% increase of GSH and GSSG content, respectively; Reduced GSH/GSSG ratio; Increased APX activity while decreased the activity of MDHAR, DHAR, and GR (30%)	Increased about 93% H_2O_2 content	[21]
B. rapa L. cv. BARI Sharisha-15	20% PEG	Slightly increased AsA content with 72% and 178% increase of GSH and GSSG content, respectively; Reduced GSH/GSSG ratio by 38%; Increased APX, MDHAR, DHAR, and GR activity	Increased about 131% H_2O_2 content	[22]
Cucumis melo L. cv. Yipintianxia No. 208	50 mM of $NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3$ (1:9:9:1 M)	Improved AsA, GSSG and DHA contents; Lowered GSH content; Reduced the ratio of AsA/DHA and GSH/GSSG; Stimulated the activity of APX by 96% and DHAR by 38% while reducing the activity of MDHAR and GR by 48% and 34%, respectively	Increased H_2O_2 accumulation	[23]

Plant Species	Stress Levels	Status of AsA-GSH Component(s)	ROS Regulation	References
Solanum lycopersicum L., var. Lakshmi	0.3 and 0.5 g NaCl kg^{-1} soil	Reduced AsA and AsA/DHA ratio; Lowered GSH and GSSG accumulation with decreased GSH/GSSG redox; Increased APX activity by 28%, DHAR activity by 28% and GR activity by 14%	Enhanced H_2O_2 and O_2^- accumulation	[24]
S. lycopersicum L.cv. Boludo	60 mM NaCl, 30 days	Reduced the activities of APX, DHAR, and GR; Increased MDHAR activity	Higher H_2O_2 generation	[25]
S. lycopersicum L. *var.* Pusa Ruby	150 mM NaCl	Decreased AsA and GSH content with a higher content of DHA and GSSG; Increased APX, MDHAR, DHAr and GR activities	Higher generation of H_2O_2 and O_2^-	[26]
S. lycopersicum L. var. Pusa Rohini	150 mM NaCl	Reduced AsA content by 42%; Increased both GSH and GSSG accumulation; Enhanced the activity of APX and GR by 86% and 29%, respectively with reduction of the activity of MDHAR and DHAR by 38% and 32%, respectively	Accumulated about 3 fold higher H_2O_2 content	[27]
S. lycopersicon L. cv.K-21	150 mM NaCl	Reduced AsA content by 40% with 50% higher GSH content; Lowered GSSG content by 23% while increased GSH/GSSG ratio by 112%; Increased APX (86%) and GR (92%) activity along with the lowered activity of MDHAR (32%) and DHAR (30%)	Elevated H_2O_2 content about 175%	[28]
Nitraria Tangutorum Bobr.	100,200, 300 and 400 mM NaCl	Increased AsA, DHA, GSH and GSSG accumulation decreased their redox status; Enhanced the activity of APX and GR; Unvaried the activity of DHAR and MDHAR but increased DHAR activity only at 300 mM NaCl	Increased $O_2^-$and H_2O_2 content by 38–98 and 49–102% respectively	[29]
Camellia sinensis (L.) O.Kuntze	300 mM NaCl	Enhanced the AsA and GSH content; Increased APX activity	Elevated H_2O_2 and O_2^- content	[30]
Phaseolus vulgaris L. cv. Nebraska	2.5 and 5.0 dS m^{-1} prepared from a mixture of NaCl, $CaCl_2$, and $MgSO_4$	Increased AsA, GSH, DHA and GSSG accumulations; Enhanced AsA/DHA and GSH/GSSG status; Stimulated the enzymatic activity of APX, MDHAR, DHAR and GR activities	Accumulated higher H_2O_2 content	[31]

Plant Species	Stress Levels	Status of AsA-GSH Component(s)	ROS Regulation	References
Vigna radiate L. cv. Binamoog-1	25% PEG	Reduced AsA content along with higher GSH content of 92%; Increased GSSG content by 236% and reduced GSH/GSSG ratio; Amplified the activity of APX (21%) and GR while reduced MDHAR and DHAR activities	Elevated H_2O_2 content by 114% with higher O_2^- generation	[32]
V. radiata L.	200 mM NaCl	Reduced AsA content; Increased GSSG and GSH accumulation and lowered GSH/GSSG ratio; Amplified the activity of APX, MDHAR, DHAR, and GR	Increased H_2O_2 content by 80% and O_2^- generation by 86%	[33]
V. radiata L. cv. BARI Mung-2	5% PEG	Reduced AsA content where decreased AsA/DHA ratio by 54%; Increased GSSG content; Upregulated the activity of APX and GR (42%) while downregulated the MDHAR (26%) and DHAR activities	Elevated H_2O_2 and O_2^- accumulation	[34]
Lens culinaris Medik cv. BARI Lentil-7	20% PEG	Lowered AsA content with higher total GSH content; Unaltered the APX and GR activities while the increased activity of MDHAR and DHAR (64%)	Accumulated higher H_2O_2 content	[35]
L. culinaris Medik cv. BARI Lentil-7	100 mM NaCl	Reduced AsA content by 87% while increased total GSH content by 260%; Improved the activity of APX, MDHAR, DHAR (286%) and GR (162%)	Increased H_2O_2 content by 15%	[35]
Anacardium occidentale L.	21-day water withdrawal	Enhanced total AsA and GSH content; Increased APX activity	Reduced H_2O_2 generation	[36]
Arabidopsis	12-day water withhold	Showed higher GSH and GSSG accumulation; Reduced GSH/GSSG ratio; Increased GR activity	Increased H_2O_2 accumulation rate	[37]
Cajanus cajan L.	Complete water withholding for 3, 6 and 9 days	Decreased GSH/GSSG ratio; Increased the activity of APX, DHAR, and GR	Higher H_2O_2 content	[38]
Amaranthus tricolor L.cv. VA13	30% FC	Increased AsA and GSH contents by 286% and 98%, respectively; Improved APX, MDHAR, DHAR, and GR activity by 371%, 379%, 375%, and 375%, respectively	No increment of H_2O_2 content	[39]

Plant Species	Stress Levels	Status of AsA-GSH Component(s)	ROS Regulation	References
A. tricolor L.cv. VA15	30% FC	Increased AsA and GSH contents along with higher redox status of AsA/total AsA and GSH/total GSH; Enhanced the activity of APX, MDHAR, DHAR, and GR by 37%, 45%, 40%, and 2%, respectively	Accumulated higher H_2O_2 content by 137%	[39]
C. sinensis (L.) O. Kuntze	20% PEG	Higher contents of both AsA and GSH; Enhanced the APX activity	Higher accumulation of H_2O_2 and O_2^-	[30]

Table 2. Status of AsA-GSH in regulating oxidative stress under metal/metalloid stress.

Plant Species	Stress Levels	Status of AsA-GSH Component(s)	ROS Regulation	References
Brassica napus L. cv. BinaSharisha-3	Cd (0.5 mM and 1.0 mM $CdCl_2$), 48 h	Reduced AsA content by 20% under 0.5 mM and 32% under 1.0 mM $CdCl_2$ treatment; Increased GSH content only under 0.5 mM $CdCl_2$ stress but enhanced level of GSSG by 34% under 0.5 mM and 65% under 1.0 mM $CdCl_2$ treatment; Increased function of APX by 39% and 43% under 0.5 mM and 1.0 mM $CdCl_2$ treatment but MDHAR and DHAR activity were diminished in dose dependant fashion; GR activity increased by 66% due to 0.5 mM $CdCl_2$ treatment but reduced by 24% due to 1.0 mM $CdCl_2$ treatment	Enhanced H_2O_2 content by 37% under 0.5 mM and 60% under 1.0 mM $CdCl_2$ treatment	[40]
Gossypium spp. (genotype MNH 886)	Pb [50 and 100 µM $Pb(NO_3)_2$], 6 weeks	Increased APX activity	Increased H_2O_2 content	[41]
T. aestivum L. cv. Pradip	As (0.25 and 0.5 mM $Na_2HAsO_4 7H_2O$), 72 h	Reduced AsA content by 14% under 0.25 and 34% underd 0.5 mM $Na_2HAsO_4 \cdot 7H_2O$ treatment; Increased GSH content by 46% and 34%, GSSG content by 50 and 101% under 0.25 and 0.5 mM $Na_2HAsO_4 \cdot 7H_2O$ stress; Enhanced APX function by 39% and 43% but decreased DHAR function by 33% and 30% under 0.25 and 0.5 mM $Na_2HAsO_4 \cdot 7H_2O$ treatment; Increased GR function by 31% under 0.25 mM	Increased H_2O_2 content by 41% under 0.25 and 95% under 0.5 mM $Na_2HAsO_4 \cdot 7H_2O$ treatment	[42]
B. napus L. viz. ZS 758, Zheda619, ZY 50 and Zheda 622	Cr (400 µM), 15 days	Increased GSH and GSSG content; Increased APX activity	Increased H_2O_2 content	[43]
Oryza sativa L. cv. BRRI dhan29	As (0.5 mM and 1 mM Na_2HAsO_4), 5 days	Decreased AsA content by 33 and 51% and increased DHA content by 27% and 40% under 0.5mM and 1mM Na_2HAsO_4 treatment, respectively; Decreased ratio of AsA/DHA; Enhanced GSH content by 48 and 82% under 0.5mM and 1mM Na_2HAsO_4 treatment, respectively; Enhanced GSSG content whereas lessened GSH/GSSG ratio by 25% under 0.5mM and 41% under 1mM Na_2HAsO_4 treatment; Augmented the function of APX, MDHAR, and GR, however, reduced the activity of DHAR	Increased H_2O_2 content by 65% and 89% under 0.5mM and 1mM Na_2HAsO_4 treatment, respectively	[44]
O. sativa L. cv. Disang (tolerant)	100 µM $AlCl_3$, 48 h	Increased AsA content in both roots and shoots; Enhanced the GSH content in shoots; Higher activities of APX, MDHAR, DHAR, and GR,	Elevated the generation of H_2O_2 and O_2^-	[45]

Plant Species	Stress Levels	Status of AsA-GSH Component(s)	ROS Regulation	References
O. sativa L. cv. Joymati (sensitive)	100 μM $AlCl_3$, 48 h	Higher accumulation of AsA in both roots and shoots; Reduced the GSH content in roots while shoots content was unaltered; Increased APX, MDHAR, DHAR activities; Slightly increased GR activities	Higher accumulation of H_2O_2 and O_2^-	[45]
V. radiata L. cv. BARI Mung-2	Cd (mild: 1.0 mM $CdCl_2$, severer: 1.5 mM $CdCl_2$), 48 h	Declined AsA content by 31% due to mild and 41% due to severe stress; Enhanced DHA level and reduced AsA/DHA ratio; GSH content did not change due to mild stress but enhanced owing to stress severity; GSSG level enhanced, and GSH/GSSG ratio decreased in a dose-dependent manner; Increased function of APX but lessened MDHAR and DHAR function due to both level of stress; GR activity increased only due to severe stress	H_2O_2 level and O_2^- generation rate was augmented by 73% and 127% due to mild and 69% and 120% due to severe Cd stresses	[46]
V. radiata L. cv. BARI Mung-2	Cd (1.5 mM $CdCl_2$), 48 h	AsA content decreased by 27%, and the ratio of AsA/DHA reduced by 80% whereas DHA content increased considerably; Augmented the function of APX and GR however lessened function of MDHAR and DHAR	Increased H_2O_2 level and O_2^- generation rate	[47]
O. sativa L. cv. BRRI dhan29	Cd (0.25 mM and 0.5 mM $CdCl_2$), 3 days	AsA content and AsA/DHA ratio reduced by 37% and 57% due to 0.25 mM $CdCl_2$ and reduced by 51% and 68% due to 0.5 mM $CdCl_2$, respectively; DHA content increased significantly; GSH content enhanced due to 0.25 mM $CdCl_2$ stress, but reduced due to 0.5 mM $CdCl_2$ stress; GSSG content enhanced by 76% under 0.25 mM and 108% under 0.5 mM $CdCl_2$ stress; Reduced ratio of GSH/GSSG in dose dependant manner; Enhanced APX, MDHAR and GR activity	Enhenced H_2O_2 by 46% under 0.25 mM $CdCl_2$ and 84% under 0.5 mM $CdCl_2$ treatmen whereas O_2^- generation rate increased in dose dependant manner	[48]
O. sativa L. cv. BRRI dhan29	Cd (0.3 mM $CdCl_2$), 3 days	Lessened level of AsA and AsA/DHA ratio but enhanced DHA level; Enhanced the level of GSH and GSSG however lessened GSH/GSSG ratio; Enhanced the action of APX, MDHAR, and GR whereas declined DHAR function	Overproduced ROS (H_2O_2 and O_2^-)	[49]
O. sativa L. Zhunliangyou 608	Cd (5 μM $Cd(NO_3)_2{\cdot}4H_2O$), 6 days	Reduced AsA content; Increased GSH content; Slightly reduced the APX activity	H_2O_2 content increased by 22.73%	[50]
Abelmoschus esculentus L. Moench	Pb (100 mg L^{-1}), 21 days	Increased AsA content	Enhanced H_2O_2 content	[51]
B. juncea L. cv. BARI Sharisha-11	Cr (mild: 0.15 mM K_2CrO_4, severe: 0.3 mM K_2CrO_4), 5 days	AsA content lessened by 19% due to mild and 32% due to severe stress whereas DHA level enhanced by 83% due to mild and 133% due to severe stress as well as AsA/DHA ratio lessened by 47% due to mild and 82% due to severe stress; GSH content did not change considerably but GSSG content enhanced by 42% due to mild and 67% due to severe stress as well as GSH/GSSG ratio lessened by 26% due to mild and 41% due to severe stress; The function of APX enhanced by 21% due to mild and 28% due to severe stress; The activity of MDHAR and DHAR reduced by 25 and 32% under mild and 31 and 50%, under severe stress, respectively; Mild stress increased the activity of GR by 19% while severe stress increased by 16%	H_2O_2 level enhanced by 24% and 46% due to mild and severe stress. Similarly, O_2^- generation rate also raised in a dose-dependent manner	[52]

Plant Species	Stress Levels	Status of AsA-GSH Component(s)	ROS Regulation	References
B. campestris L. cv. BARISharisha 9, *B. napus* L. cv. BARI Sharisha-13 and *B. juncea* L. cv. BARI Sharisha-16	Cd (mild: 0.25 mM $CdCl_2$, severer: 0.5 mM $CdCl_2$), 3 days	Decreased level ofAsA, augmented level of DHA as well as decreased AsA/DHA ratio in all studied cultivars; GSH and GSSG level enhanced, but GSH/GSSG ratio lessened in all studied cultivars; APX and GR activities of all species increased significantly under both levels of Cd toxicity	Enhanced H_2O_2 level and O_2^- production rate in all tested cultivars in a concentration-dependent fashion	[53]
B. juncea L. BARI Sharisha-11	Cd (mild: 0.5 mM $CdCl_2$, severer: 1.0 mM $CdCl_2$), 3 days	Reduced AsA content with higher DHA content and thus decreased AsA/DHA ratio; Increased GSH and GSSG levels as well as declined GSH/GSSG ratio; APX activity increased where GR increased at mild stress but remained unaltered at severe stress; Decreased MDHAR and DHAR activities	Enhanced the H_2O_2 and O_2^-level	[54]
V. radiata L. cv. BARI Mung-2	Al ($AlCl_3$, 0.5 mM), 48 and 72 h	Enhanced DHA content but reduced AsA level and AsA/DHA ratio; Increased level of GSH and GSSG but the diminished ratio of GSH/GSSG; Augmented APX activity but decreased MDHAR and DHAR activity	Enhanced H_2O_2 level by 83% and O_2^- generation rate by 110%	[34]
T. aestivum L. cv. Pradip	Pb [mild: 0.5 mM $Pb(NO_3)_2$, severer: 1.0 mM $Pb(NO_3)_2$], 2 days	AsA decreased in a dose-dependent manner; Mild stress improved the GSH level, but severe stress reduced it; Increased GSSG content; Increased APX activity; Diminished activity of MDHAR and DHAR in a concentration-dependent fashion; Mild stress improved GR activity but severe stress reduced it	Mild stress increased H_2O_2 levels by 41%, but severe stress enhanced it by 95% while O_2^- generation rate also increased in a dose-dependent manner	[55]
B. juncea L. cv. BARI Sharisha-11	Cd (mild: 0.5 mM $CdCl_2$, severer: 1.0 mM $CdCl_2$), 3 days	AsA content decreased by 24% due to mild and 42% due to severe stress whereas DHA level enhanced by 79% due mild and 200% due to severe stress; Decreased AsA/DHA ratio in dose-dependent manner; GSH and GSSG content enhanced by 19% and 44%, respectively, due to mild stress, while only GSSG content enhanced due to severe stress by 72%; The ratio of GSH/GSSG declined by 17% due to mild and 43% due to severe stress; Enhanced APX by 15% due to mild and 24% due to severe stress; The activity of MDHAR and DHAR reduced by 12% and 14% due to mild stress whereas 17% and 24%, due to severe stress, respectively; The activity of GR enhanced under mild stress by 16% and lessened under severe stress by 9%	Level of H_2O_2 enhanced by 43% due to mild and 54% due to severe stress. Augmented O_2^- generation rate in a dose-dependent manner	[56]
B. juncea L. cv. varuna	Ni, (150 µM $NiCl_2.6H_2O$), 1 week	AsA content decreased by 61% whereas GSH and GSSG content increased by 75% and 151%, respectively; Enhanced function of APX by 60% and GR by 72%; DHAR and MDHAR activities were decreased by 62% and 65%, respectively	Increased H_2O_2 by 3.23-fold	[57]
Pisum sativum L. cv. Corne de Bélier	Pb (500 mg $PbCl_2$ kg^{-1}), 28 days	Increased APX and GR activity	Increased H_2O_2 content	[58]
O. sativa L. cv. BRRI dhan54	Ni (0.25 mMand 0.5 mM $NiSO_4{\cdot}7H_2O$)	Diminished content of AsA and enhanced content of DHA as well as the lessened ratio of AsA/DHA by 73% and 92% under 0.25 mM and 0.5 mM $NiSO_4{\cdot}7H_2O$ stress; GSH and GSSG level enhanced in a dose-dependent manner. However, the GSH/GSSG ratio reduced only under 0.5 mM $NiSO_4{\cdot}7H_2O$ treatment; Increased APX, MDHAR, DHAR and GR activity by 70%, 61%, 19% and 37% under 0.25 mM $NiSO_4{\cdot}7H_2O$ and 114%, 115%, 31% and 104% under 0.5 mM $NiSO_4{\cdot}7H_2O$ treatment, respectively	Increased H_2O_2 content by 28% and 35% due to 0.25 mM and 0.5 mM $NiSO_4{\cdot}7H_2O$ treatment	[59]

Plant Species	Stress Levels	Status of AsA-GSH Component(s)	ROS Regulation	References
Capsicum annuum L.cv. Semerkand	Cd (0.1 mM $CdCl_2$), 3 weeks	Enhanced AsA and GSH content	Increased H_2O_2 content	[60]
C. annuum L. cv. Semerkand	Pb (0.1 mM $PbCl_2$), 3 weeks	Enhanced AsA and GSH content	Increased H_2O_2 content	[60]
Zea mays L. cv. Run Nong 35 and Wan Dan 13	Cd (50 mg $3CdSO_4{\cdot}8H_2O$ kg^{-1} soil), 6 months	Decreased GSH content	Increased accumulation of H_2O_2	[61]

Table 3. Role of AsA-GSH in regulating oxidative stress under extreme temperature, flooding, and atmospheric pollutant.

Plant Species	Stress Levels	Status of AsA-GSH Component(s)	ROS Mitigation	References
Actinidia deliciosa	45 °C, 8 h	Increased content of AsA; Higher activity of APX, MDHAR, DHAR, and GR	Increased H_2O_2 content	[62]
Zea mays L. cv. Ludan No. 8	46 °C, 16 h	Decreased GSH, and GSSG content, but interestingly GSH/(GSH + GSSG) ratio increased; Reduced GR activity	-	[63]
Cinnamonum camphora	40 °C, 2 days	Reduced AsA content with higher DHA content; Increased GSH and GSSG content; Enhanced the activities of APX, MDHAr, DHAR, and GR	Higher content of H_2O_2 and O_2^-	[64]
S. lycopersicum L. cv. Ailsa Craig	40 °C, 9 h	Higher APX and GR activities by 74% and 45%, respectively	H_2O_2 content increased by 49%	[65]
S. lycopersicum L.cv. Boludo	35 °C, 30 days	Increased the APX, DHAR and GR activities; Reduced the MDHAR activity	Increased H_2O_2 content	[25]
Vicia faba L. cv. *C5*	42 °C, 48 h	Enhanced the AsA, GSH ans GSSG content significantly; The enzymatic activity of APX and GR also enhanced	Extreme accumulation of O_2^- and H_2O_2	[66]
V.radiata L. cv. BARI Mung-2	40 °C, 48 h	Decreased 64% in AsA/DHA ratio; GSSG pool increased; Higher APX (42%) and GR (50%) activities but declined activities of MDHAR (17%) and DHAR	Higher H_2O_2 content and O_2^-production rate	[34]
Z. mays cv. CML-32 and LM-11	40 °C, 72 h	Increased AsA content in both shoot and root of tolerant (CML-32) one, but unaffected in the susceptible (LM-11) one; Both APX and GR activity increased in roots of CML-32 but reduced in the shoot	Higher H_2O_2 accumulation, especially in shoots	[67]
L. esculentum Mill. cv. Puhong 968	38/28 °C day/night, 7 days	AsA+DHA and DHA increased by 220% and 99% respectively; AsA/DHA ratio decreased by 33%.; Higher GSSG (25%), but reduced GSH content (23.4%) and GSH/GSSG ratio (39%); APX, MDHAR, DHAR and GR activities declined	Enhanced O_2^- generation rate and H_2O_2 content by 129% and 33% respectively	[68]
Nicotiana tabacum cv. BY-2	35 °C, 7 days	Total GSH and AsA contents rose after 7 days heat stress; Increased MDHAR. DHAR and GR activities up to 72 h	The increasing trend of H_2O_2 generation was observed up to 72 h, and then a sharp decline occurred	[69]

Plant Species	Stress Levels	Status of AsA-GSH Component(s)	ROS Mitigation	References
Ficus concinna var. subsessilis	35 °C and 40 °C, 48 h	AsA content reduced at 40 °C but GSH content similar to control at both 35 and 40 °C; DHA content enhanced by 49% at 35 °C and by 70% at 40 °C; APX activity increased by 51% and 30% at 35 °C and 40 °C; Activities of MDHAR, DHAR, and GR increased at 35 °C, but GR activity decreased by 34% at 40 °C	At 35 °C, 103% higher H_2O_2 content and 58% higher O_2^-production rate and at 40 °C those were 3.3- and 2.2-fold respectively	[70]
T. aestivum cv. Hindi62 and PBW343	Heat stress environment, Late sown (Mid-January)	Higher activities of MDHAR and DHAR was observed in heat-tolerant (Hindi62) one whereas other enzyme activities seemed mostly to decline with time	The content of H_2O_2 was higher up to 14 DAA compared to non-stressed seedlings	[67]
G. hirsutum cv. Siza	Waterlogged pot for 3 days and 6 days	Increased content of AsA by 20% at 3 days and 30% at 6 days of waterlogging; Lower APX, MDHAR and GR activities	Enhanced O_2^- generation rate by 22 and 53% and H_2O_2 content by 10 and 39% at 3 and 6 days of waterlogging, respectively	[63]
Sesamum indicum L. cv. BARI Til-4	Waterlogged pot by 2 cm standing water on the soil surface for 2, 4, 6 and 8 days	Reduced AsA content upto 38%; Enhanced GSH and GSSG content significantly; Increased APX and MDHAR activities; Reduced DHAR activity upto 59%; GR activity decreased upto 23%	Increased H_2O_2content sharply	[71]
Z. mays cv. Huzum-265 and Huzum-55	Root portions waterlogged for 21 h	Reduced AsA content in both cultivars; Increased APX activity in both cultivars	-	[72]
Glycine max L.	Waterlogged pot for 14 days	GSH activity declined sharply in roots but shoots unaffected; Reduced GR activity in shoots but roots unaffected	-	[73]
Trifolium repens L. cv. Rivendel and *T. pratense* L. cv. Raya	2 cm standing water on the soil surface for 14 days and 21 days	Increased contents of both oxidized and reduced AsA observed in both genotypes	Higher H_2O_2 generation in both genotypes	[74]
V. radiata L. cvs. T-44 and Pusa Baisakhi; and *V. luteola*	Pot filled with water to 1–2 cm height below the soil level, 8 days	Increased activities of both APX and GR in tolerant genotypes but in susceptible one, activities reduced	Reduced contents of $O_2^-$and H_2O_2 in susceptible (Pusa Baisakhi) cultivar	[75]
O. sativa L. MR219-4, MR219-9 and FR13A	Complete submergence for 4, 8 and 12 days	APX activity declined by 88% in FR13A under 4 days of submergence but decreased about 64 and 83% under 8 and 12 days of submergence; GR activity increased in FR13A and MR219-4 cultivars by 10- and 13-fold respectively after 8 days	-	[76]
Allium fistulosum L. cv. Erhan	Waterlogging (5 cm) at substrate surface for 10 days	Lower APX and GR activities	Increased rate of O_2^- generation by 240.4% and 289.8% higher H_2O_2 content	[77]
C. cajan L. genotypes ICPL 84,023 and ICP 7035	Soil surface waterlogged (1–2 cm) for 6 days	Reduced APX and GR activities in susceptible genotype, which was higher in tolerant one	Lower accumulation of H_2O_2 and rate of O_2^- generation	[78]
S. melongena L. cv. EG117 and EG203	Flooding with a water level of 5 cm, 72 h	Increased AsA content in susceptible EG117 genotype GSH content in both genotypes; Increased APX activity but decreased GR activity	-	[79]
S. lycopersicum cv. ASVEG and L4422	Flooding with a water level of 5 cm, 72 h	Increase in both AsA and GSH contents; Non-significant changes in APX and GR activities	-	[79]

Plant Species	Stress Levels	Status of AsA-GSH Component(s)	ROS Mitigation	References
Lolium perenne	Grown in an area with high air pollution	APX and DHAR activities decreased while MDHAR and GR activities increased	A higher concentration of H_2O_2 in pollens	[80]
Populus deltoides × *Populus nigra* cvs. Carpaccio and Robusta	O_3 treatment (120 nmol mol^{-1} for 13 h), 17 days	No impact on AsA and GSH contents; DHAR activity decreased while GR and MDHAR activity increased	-	[81]
Fragaria x *anansa*	High dose of carbon monoxide (CO) nitroxide (NO_x) and sulfur dioxide (SO_2)	The activity of both APX and GR increased upto medium dose but reduced under high dose	H_2O_2 content as well as O_2^- generation rate increased	[82]
O. sativa L. cvs. SY63 and WXJ14	Continuous O_3 exposure for up to 79 days	Both AsA and GSH contents are more likely to decrease; APX, MDHAR, DHAR, and GR activity increased up to 70 days of O_3 exposure	Both O_2^- generation rate and H_2O_2 contents increased	[83]
Prosopis juliflora	Grown in the polluted industrial region	The content of AsA and APX activity increased under polluted environment	-	[84]
Erythrina orientalis	Grown in a polluted industrial area	Increased activities of both APX and GR enzymes recorded	-	[85]
T. aestivum L. cv. BARI Gom-26	Acidic pH (4.5) of growing media	Increased AsA and GSH content; Improved redox balance of GSH/GSSG; Increased activity of APX, MDHAR, DHR, and GR	H_2O_2 contents increased by 209%	[86]

3.1. Salinity

One of the most devastating abiotic stress factors—salinity by which cultivable land is becoming barren thus reduces total crop production day by day. Oxidative stress is the most dangerous event under salt inundation is imposed by salinity-induced ionic and osmotic stress [7]. Hence, these ionic and osmotic stress both disturb the photosystem, and thus cause excess ROS, such as $^1O_2$, O_2^-, H_2O_2, and OH. Salinity-persuaded acute ROS accumulations, then bother cellular redox followed by cellular damage counting membrane dysfunction, DNA damage, collapse the enzymatic action, along with distraction of the antioxidant defense system [87][26]. At this point, the plant synthesizes cellular AsA and GSH, which act as non-enzymatic antioxidants by involving their enzymatic components to detoxify ROS up to tolerable levels (**Table 1**).

However, the enzymes of AsA-GSH pathway showed their differential responses intolerant and sensitive varieties due to saline toxicity. Among salt-tolerant (Pokkali) and sensitive (BRRI dhan29) rice cultivars. Pokkali responded by enhancing the enzymatic activities of the AsA-GSH cycle, where, lowered APX and higher DHAR activity along with unchanged MDHAR and GR activities were found from BRRI dhan29. Rahman et al. [87][16] reported about the well involvement of AsA-GSH cycle in salt-stressed *O. sativa* where ROS generation was extreme. Here, salt exposed rice enhanced the reduced and oxidized GSH content with a lesser amount of AsA by the higher APX, MDHAR, DHAR, and GR activities against overproduced ROS. *Vigna radiata* was grown under the saline condition [88] and where salt-induced oxidative stress was marked with extreme O_2^- and H_2O_2 overgeneration. Salt-stressed *V. radiata* augmented GSH and GSSG contents along with lowered AsA, whereas up-regulated the activity of all enzymatic antioxidants of AsA-GSH cycle and thus responded with elevated ROS [33]. Salt exposed *Lens culinaris* up-stimulated both MDHAR and DHAR activities, which resulted in a lesser amount of AsA and indicated the overproduced H_2O_2 detoxification [35]. Recently, Singh et al. [24] disclosed the incremental activity of enzymatic antioxidants, including APX, DHAR, and GR, with lower AsA, GSH, and GSSG contents, because of salt-induced higher ROS accumulation in *Solanum lycopersicum*. Similarly, 150 mM salt-treated *S. lycopersicum* also decreased AsA content, which might be used in H_2O_2 detoxification, while better GSH showed its role in lowering H_2O_2. Ahmad et al. [28] also observed higher APX, and GR activities, while MDHAR and DHAR activities again reduced as well as supported AsA-GSH mediated ROS regulation. Ahanger et al. [27] reported the same response of *S. lycopersicum* upon saline toxicity. Both activities of APX and GR were enhanced in salt-treated *Triticum aestivum* besides elevated H_2O_2 generation and resulted in higher GSH accumulation [11]. The activity of APX, MDHAR, DHAR, and GR enhanced in salt-stressed *S. lycopersicum* to check the excessive H_2O_2 generation, which resulted in lowered AsA and GSH contents [26].

The changes in AsA-GSH pathway were investigated in salt-stressed *Nitraria tangutorum* by applying a varied level of NaCl (100, 200, 300, and 400 mM) [29]. They noticed a gradual enhancement of AsA, DHA, GSH, and GSSG contents by keeping pace with sequential increment of salt-induced H_2O_2. Here, increased MDHAR and DHAR activities in stressed seedlings also contributed to increasing AsA, and higher DHAR and GR were responsible for better GSH and GSSG contents [26][89]. Talaat et al. corroborated these results with salt-exposed *Phaseolus vulgaris* [31]. Thus, as a part of plant antioxidant defense under salinity, AsA-GSH pathway is very efficient to regulate extra ROS for being tolerant.

3.2. Drought

Drought is another most important abiotic stress, which generates excess ROS accumulation and thus causes variation in the enzymatic activities of AsA-GSH pathway for ROS detoxification. The enzymatic responses of AsA-GSH pathways varied, depending upon plant species, plant age, drought intensity, and duration [7]. Commonly, drought up-regulated the enzymatic antioxidant activities of AsA-GSH pool [7][22]. Plant tolerance to drought stress is categorized based on stress-induced endogenous antioxidants contents along with enzymatic activities (**Table 2**). *Dendranthema grandiflorum* responded differentially according to their tolerant and sensitive varieties, where tolerant one comparatively displayed better enzyme activity of antioxidants than the sensitive ones [90]. Lou et al. [14] demonstrated how *T. aestivum* responded upon drought exposure. Hence, they noticed that the AsA-GSH cycle responded considerably with excess ROS generation by significant variation of GSH/GSSG and AsA/DHA redox along with the steady increment of H_2O_2. Their team also observed the enzymatic up-stimulation of AsA-GSH pathway to alleviate stress by scavenging excess ROS in *T. aestivum* spike. Thus, *T. aestivum* showed higher participation of AsA with higher APX activity in drought exposure for scavenging extra H_2O_2, as well as higher enzymatic activity to run the AsA-GSH pathway systematically [13].

Drought-stressed *A. thaliana* enhanced GSH and GSSG content along with the higher GR activity [37]. Hence, *Arabidopsis* showed the GSH dependent H_2O_2 detoxification to attain tolerance. Higher total AsA was accumulated in *Cajanus cajan* upon complete water restriction conditions for up to nine days to defend against excess H_2O_2 toxicity [38]. Hence, drought enhanced the enzymatic activity of APX, DHAR, and GR for decreasing GSH/GSSG, as well as controlling ROS levels.

Similarly, the tolerant genotype VA13 of *Amaranthus tricolor* showed comparatively better tolerance under drought stress than the sensitive one (VA15) by expressing differential responses of the enzymatic and non-enzymatic ROS detoxification pathways [39]. Hence, VA13 expressed a remarkable increment in AsA-GSH redox by accelerating the enzymatic antioxidative actions by which increased non-enzymatic antioxidants (AsA and GSH) accumulation, which are vital for ROS detoxification.

Vigna radiata responded differently regarding different drought intensities [32] to control diverse levels of ROS. Moderate drought imposed by 10% polyethylene glycol (PEG) induced comparatively lowered ROS than severe drought (by 20% PEG). Therefore, severe drought-stressed *Brassica* showed a larger use of AsA-GSH pathways against higher H_2O_2 generation than moderate stress. Here, higher stress caused a higher increase of APX activity along with the lowest MDHAR and DHAR activity, while GR activity reduced differently than lower stress exposure to rapeseeds seedlings. Additionally, Hasanuzzaman et al. [21] also observed AsA and GSH both antioxidants contents reduced under severe drought condition, but increased under moderate stress. Bhuiyan et al. [22] found increased AsA content in *B. rapa* under drought (20% PEG). They also observed increased APX activity in drought-stressed seedlings, which assisted in efficiently scavenging the H_2O_2. Another two enzymes related to AsA regeneration MDHAR and DHAR also upregulated, as a result the AsA level was increased and strongly maintained its redox balance during oxidative stress situation. Nahar et al. [32] narrated the function of AsA as ROS detoxifier under drought stress where AsA content reduced in *V. radiata* with the increasing of ROS generation. Here, drought-induced higher APX activity enhanced the oxidation of AsA by scavenging H_2O_2, and improved GR activity increased the supply of GSH for involving ROS detoxification. *Anacardium occidentale* also showed the active participation of AsA-GSH cycle by integrative responses of both non-enzymatic and enzymatic antioxidants for drought-induced excess ROS regulation, where the higher accumulation of AsA and GSH, along with APX activity, coordinately reduced the overproduced H_2O_2 [36]. Thus, the AsA-GSH pathways involve in ROS detoxification as well as ROS homeostasis by eliminating excess ROS for keeping them up to the requirement of functioning cell signals.

3.3. Toxic Metals/Metalloids

Due to the fast industrialization of the modern world and unrestrained anthropogenic activities, toxic metals/metalloids stresses have become a gargantuan problem for the plant growth and development [91]. Plants experience toxic metals/metalloids stress try to survive to some extent by using their well-established antioxidant defense system. But, the

activity and performance of defense system differ with stress concentration, stress duration, plant type, and age of the plant.

The enzymes of AsA-GSH pathway confirmed their differential responses to different toxic metals/metalloids stress (**Table 2**). Mahmud et al. [52] confirmed that due to Cr stress, the few components of AsA-GSH pathway increased their amount or activity in *B. juncea* L. cv. BARI Sharisha-11. They found five days duration of 0.15 mM and 0.3 mM K_2CrO_4 treatment decreased the content of AsA, but did not change the GSH content. Moreover, activities of APX and GR were enhanced; however, the activities of MDHAR and DHAR were diminished. The higher APX and GR activity might play a function in scavenging excess ROS. A similar upregulation of APX and GR was also recorded in *B. napus* L. cv. Binasharisha-3 due to Cd treatment [92]. From two separate experiments, they also found Cd stress (0.5 mM and 1.0 mM $CdCl_2$) for 48 h decreased the AsA content, but increased GSH content only under 0.5 mM $CdCl_2$ treatment. Exposure of *Gossypium* to 50 and 100 µM $Pb(NO_3)_2$ for six weeks increased the H_2O_2 content and APX activity [41]. The addition of 150 µM $NiCl_2{\cdot}6H_2O$ in growing media of *B. juncea* L. for one week increased the H_2O_2 content. Moreover, Ni stress decreased the AsA level but augmented the content of GSH and GSSG. Nickel also diminished the function of DHAR and MDHAR, however enhanced APX and GR activity [57]. Similar differential responses of AsA-GSH pathway components were also observed under As [44] and Al [34] toxicity. It can be stated that overproduced ROS plays the signaling role to some extent and inaugurate the higher activity of AsA-GSH enzymes under metals/metalloids toxicity. The upregulation of enzymes plays a significant role in maintaining the redox balance of AsA-GSH pathway under stress condition.

3.4. Extreme Temperature

Along with the rise in average global temperature, HT stress has been turned into a topic to be concerned about among environmentalists and researchers worldwide. In general, a 5 °C temperature rise above the optimum temperature of growth is considered to be extreme temperature stress or HT stress or heat shock to any plant species [93][62]. Heat stress causes denaturation of protein and membrane lipids, enzyme inactivation, inhibited protein synthesis, and loss of membrane integrity [94], which results from the disruption of cellular homeostasis through the ROS formed in a mass amount under heat stress [62][95]. Focusing on the role of AsA-GSH pathway to scavenge these ROS, different crop species under different levels of extreme or HT stress have been studied (**Table 3**).

Khanna-Chopra and Chauhan [96] selected a warmer season to induce HT stress to two different cultivars of wheat (*T. aestivum*), which are Hindi62 (heat-tolerant) and PBW343 (heat-sensitive). They sowed the wheat seeds in mid-January and considered it as heat stress environment, while the control plants were sown in mid-November and considered as the non-stress environment. Data were collected at seven days interval up to 35 days after anthesis (DAA), and the results showed a sharp increase in H_2O_2 content up to 14 days, but then declined. Whereas, MDHAR and DHAR enzymes' activity only increased in Hindi62, but APX and GR activities showed a fluctuating pattern of alteration in both cultivars [96]. Another cereal *Z. mays* when experimented similarly with two different cultivars; LM-11 (heat-sensitive) and CML-32 (heat-tolerant), exposed to 40 °C for 72 h, resulted in higher APX and GR activities in CML-32 roots, while a reduction occurred in the shoot. In LM-11, none of the enzyme activity or AsA content was affected [67]. Higher levels of O_2^- production rate and H_2O_2 content were observed in *Ficus concinna* seedlings under 48 h of HT (35 °C and 40 °C) stress condition, where AsA and GSH contents were unaffected at 35 °C, while declining AsA at 40 °C temperature [70]. The activity of APX, MDHAR, DHAR, and GR enzymes increased at 35 °C, but then again reduced at 40 °C to the level of control plants [70]. Under similar heat stress condition (40 °C, 48 h), *V. radiata* seedlings resulted in decreased GSH content and MDHAR-DHAR activities, but higher APX-GR activities [34]. Kiwi fruit (*Actinidia deliciosa*) seedlings, when exposed to 45 °C in an incubator for 8 h, resulted in higher AsA content and enhanced activity of all the AsA-GSH cycle enzymes [62]. Tomato seedlings were studied in two different aspects: short-term heat shock (40 °C, 9 h) [65] and long-term heat stress (38/28 °C day/night, seven days) [68]. In both experiments, the enhancement of O_2^- generation rate and H_2O_2 content were recorded, but enzyme (APX and GR) activity was only increased at short-term stress condition [65], while the long-term heat exposure reduced all four enzymes activities and GSH content [68]. Similar enzymatic activity was observed in *Nicotiana tabacum* seedlings after seven days of heat (35 °C) stress [69]. From the above discussion, it can be stated that heat stress prevailing for a longer duration is less likely to have the capability to modulate AsA-GSH pathway as compared to short-term heat stress.

3.5. Flooding

Changes in global climate result in the frequent or unexpected occurrence of heavy rainfall in different regions of the globe, which causes a sudden flood and disrupts the normal ecosystem [6]. Such changes in the ecosystem may cause the extinction of plants species and imbalance in the natural environment [6]. Flooding-induced production of ROS and subsequent cellular damage has been authenticated in many studies so far [76][73][63]. Following are the discussion regarding crop species facing flooding stresses and modulation of their AsA-GSH pathway by flooding stress (**Table 3**).

Pigeon pea (*C. cajan*) seedlings that are exposed to waterlogged condition for six days revealed that tolerant cultivar could increase APX and GR activities, but a susceptible one cannot [78]. They also observed that, unlike other cases, waterlogging caused a lower accumulation of H_2O_2 and O_2^- [78]. In another experiment with *V. radiata*, Sairam et al. [75] showed that waterlogging similarly reduced the H_2O_2 and O_2^-production rate in susceptible cultivar, while the tolerant ones remained unaffected. However, both APX and GR enzymes' activity increased in tolerant genotypes, while the susceptible one got reduced [75]. The enhanced production rate of $O_2^-$and H_2O_2 content under flooding stress has been reported in cotton [63], Welsh onion [77], and clover [74] plants. Cotton (*G. hirsutum* cv. Siza) plants after three and six days of flood exposure raised the AsA content but reduced the activity of APX, MDHAR, and GR [63]. A similar reduction in APX and GR enzymes activities was also recorded in Welsh onion (*Allium fistulosum* L.) after 10 days of waterlogging stress [77]. When *Z. mays* seedlings were waterlogged for 21 h at their root portions, they resulted in reduced AsA content and increased APX activity [72]. On the other hand, under long duration (14 days) flooding stress, *Glycine max* L. plants showed a reduction of GSH activity in roots and GR activity in the shoot, but the GSH in shoot and GR in root were not affected [73]. In case of complete submergence of *O. sativa* L. plants for two, four, or eight days, elevated levels GR enzyme activity was recorded, while APX enzyme activity increased only in tolerant cultivar [76]. Accordingly, the discussion reveals that the impact of flooding stress on AsA-GSH pathway varies depending upon the plant species and duration.

3.6. Atmospheric Pollutants

Atmospheric pollutants are the substances that are assembled in the air to a level or magnitude that is dangerous for living beings. Plants that are grown under different levels of atmospheric pollution have shown their oxidative stress responses and AsA-GSH pathway regulation in different manners (**Table 3**).

Erythrina orientalis plants were grown in three different locations of Philippines: La Mesa (a non-polluted area); and, Makati and Quezon (highly air-polluted cities). The results revealed that plants grown in the non-polluted area had lower activities of APX and GR as compared to the ones grown in highly polluted areas [85]. A similar increase in APX activity along with higher AsA content was recorded in *Prosopis juliflora* plants grown under polluted industrial region [84]. In a recent experiment, Lucas et al. [80] studied *Lolium perenne* plants that were grown under two different areas of Spain, Madrid, and Ciudad Real, where Madrid was considered to be more polluted than Ciudad Real. The findings indicated that the pollens of *L. perenne* accumulated a higher concentration of H_2O_2 and in shoots APX and DHAR activity declined, but the activity of MDHAR and GR increased in the shoot of *L. perenne* plants that were grown in Madrid [80]. When rice seedlings were exposed to continuous O_3 treatment, the results showed a remarkable increase in both O_2^- generation rate and H_2O_2 content. In addition, contents of AsA and GSH reduced, while APX, MDHAR, DHAR, and GR activity increased up to 70 days of O_3 exposure in SY63 cultivar and up to 79 days of O_3 exposure in WXJ14 cultivar [83]. Ascorbate and GSH contents were not affected by O_3 exposure in the *Populus* seedlings, but DHAR activity was lower, while the activity of GR and MDHAR was higher after 17 days of O_3 treatment [81]. Young strawberry (*Fragaria* x *anansa*) seedlings were exposed to three different levels of CO, NO_x, and SO_2, which are as follows: CO @ 133, 267, and 533 ppm, NO_x and SO_2 @ 25, 50, and 199 ppm corresponding to low, medium, and high dose, respectively. As a result of exposure to these atmospheric pollutants, H_2O_2 content as well as O_2^- generation rate increased. However, at low and medium doses of their exposure APX and GR activity increased, while at a high dose that decreased [82]. All sorts of atmospheric pollutants have a remarkable effect on AsA-GSH pathway, but further studies are required to demonstrate that those pollutants completely induced the modification of the AsA-GSH pathway.

3.7. Other Stress

Conklin et al. confirmed the positive role of AsA in protecting plants from ultraviolet (UV) radiation [72], where they found that Vit-C deficient mutant of *A. thaliana* was suffered by stress-induced damages than that of wild type. AsA-deficient mutants also showed sensitivity to O_3 stress due to a lower biosynthesis of AsA [97]. Gao and Zhang [98] reported that vitc1 mutants of *A. thaliana* showed physiological disorders and greater oxidative damages than the wild type, which was due to lower activities of antioxidant enzymes. Mutant plants also showed lower GSH/GSSG and higher DHA/(AsA+DHA) ratio than the wild type. Singh et al. [99] observed a decrease in AsA-GSH cycle enzymes in UV-exposed plants, which in turn affected the plants with oxidative stress. Similar to higher plants, marine macroalga *Ulva fasciata* also showed a positive correlation between enhanced the functions of AsA-GSH cycle and better tolerance of plants to UV radiation [100]. In their study, the scavenging of H_2O_2 was regulated by AsA-GSH cycle components, especially APX and GR. Noshi et al. [101] reported that AsA-GSH redox pool provided better protection of *Arabidopsis* from high-light mediated oxidative stress, which was mainly attained due to the higher activities of DHAR. However, both AsA and GSH were found to be responsible for conferring high light (HL) stress [101]. Later, Zheng et al. [102] that susceptibility of *Arabidopsis* mutant was to HL stress was related to the deficiency of AsA and GSH. When AsA deficient *A. thaliana* mutant (vtc2-1) was exposed to HL, they generated a high level of H_2O_2 (an oxidative stress marker) than the wild type, which was highly and

negatively correlated with the total AsA content. The lack of AsA also resulted in lower chlorophyll (chl) content, chl fluorescence parameters, and PSII photochemistry [102]. Recently, Choudhury et al. [103] studied the metabolomics of *A. thaliana* grown under HL and found that the increased biosynthesis of GSH supports the photochemistry that supports *Arabidopsis* better survival under HL stress.

The pivotal role of the AsA-GSH cycle was observed in low pH stress also. Bhuyan et al. [86] tested five spring wheat cultivars at different levels of low pH stress. Their observation exhibited that low-pH stress resulted in elevated $O_2^-$and H_2O_2 generation. A decrease in AsA content with increased DHA content was observed, although the APX activity decreased. Increased MDHAR activity was observed, but the ratio of AsA/DHA was not increased. Decreased GSH content and increased GSSG content were found where DHAR and GR activity decreased, resulting in a drop in the GSH/GSSG ratio.

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