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REVIEW

Physiological, biochemical, and molecular responses of rice (*Oryza sativa* L.) towards elevated ozone tolerance

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Abstract

Rice (*Oryza sativa* L.) is one of the most important staple food crops that is cultivated in South East Asia. This crop is affected by a wide range of biotic and abiotic factors, each of which is contributing to a change in its physiology, biochemistry, and genetic makeup. Ozone is produced in the troposphere as a result of reactions between abiotic factors, such as oxides of nitrogen and carbon, and UV radiation. These reactions lead to the production of a wide variety of volatile organic compounds. Rice scientists have expressed a great deal of concern regarding the impact of ozone on rice, which has necessitated the development of strategies to combat the problem. The recent advances in rice genomics have led to the discovery of molecular biology approaches such as marker-assisted selection involving quantitative trait loci linked to genes that confer tolerance to ozone stress. This trait is thought to be controlled by a large number of loci with medium effects rather than by a single locus with a large effect. The current review is an effort to provide information on the physiological, biochemical, and molecular responses of rice towards elevated ozone tolerance and also to reflect the available strategies to minimize the effect.

Keywords Rice · O_3 tolerance · Physiology and growth · Molecular breeding · Quantitative trait loci

Introduction

Food security is a severe problem in emerging nations such as India and Bangladesh (Carter et al. 2017). Ground-level ozone (O_3) is a prominent phytotoxic pollutant that harms rice plants physiologically and subsequently affects their productivity (Lakaew et al. 2021). Tropospheric O_3 concentrations have increased as a result of increased industrialization and urbanization. Although O_3 level routinely exceeds threshold limits on warm days, levels below 40 ppb have little influence on crop production (Fowler et al. 2008). Photochemical interactions between volatile organic compounds (VOCs) and nitrogen oxides (NOx) are the primary sources of tropospheric or ground-level ozone. Historically, it has been believed that, heat and sunlight are necessary for these reactions to take place, and this is why summers typically contain greater levels of ambient ozone (Peng et al. 2015). Some high-elevation places in the Western United States with significant levels of local VOC and NOx emissions have created ozone (EPA 2022). Smog or haze, caused in part by ozone, is still most common in the summer, though it can persist all year in some southern and mountain regions. The majority of ground-level ozone is caused by the interactions of man-made VOC and NOx, while some ozone from the stratosphere is carried out into the troposphere, and VOC and NOx do exist naturally. Gas stations, chemical plants, oil-based paints, auto body shops, printing presses, and

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paint stores are all major contributors to VOC pollution. Combustion at high temperatures is the primary source of NOx. However, power plants, industrial furnaces, boilers, and cars are major contributors of NOx (EPA 2022).

Tropospheric ozone is formed by the interaction of sunlight, particularly ultraviolet light, with hydrocarbons and nitrogen oxides, which are emitted by automobile tailpipes and smokestacks (UCAR 2022). In urban areas, high ozone levels usually occur during warm summer months. These points suggest that, higher ozone levels in the troposphere are largely man-made and reduction of air pollution caused by traffic alleviates the situation (UCAR 2022). During photosynthetic gas exchange, plants absorb troposphere O_3 and this is degraded after being absorbed by apoplastic reactive oxygen species (ROS). Uncontrolled necrosis is caused by toxic ROS such as superoxide, hydroxyl radicals, and hydrogen peroxide. During O_3 stress, stomata closure and the loss of crucial enzymes like Ribulose-1, 5-bisphosphate carboxylase (Rubisco) impact the entire photosynthetic process (Wilkinson et al. 2012). O_3 has a significant impact on agriculture as well as a negative effect on human health. The toxicity of O_3 is a severe issue, particularly across large parts of Asia, South America, Africa, and the Middle East (Sharma and Ravindranath 2019).

Photochemicals, intense solar radiation reactions, and high temperatures from precursor contaminants, such as carbon monoxide (CO) and volatile organic compounds produce O_3 in the troposphere layer (Fowler et al. 2008). Concentrations of Ozone less than 40 ppb commonly are not believed to impact rice crop yields. Given the availability of enough ozone precursors, this threshold is exceeded in warm and humid areas (Diaz-Torres et al. 2022). The damage due to O_3 stress is projected to be significant in crop areas by 2050 (Sharma and Ravindranath 2019). Asia is the major rice cultivating region in the world, and is a food crop of worldwide importance. Rice is sown in November–December and is harvested from March–June. The high concentrations of O_3 thus occur at later stages of reproduction like flowering and grain filling. O_3 impacts the early stage of vegetative growth of wet-season rice (Singh et al. 2022). Therefore, changing the planting time is not a suitable way of dealing with rice crop production during O_3 stress. Furthermore, O_3 lowers the activity of the photosynthetic enzyme Rubisco through the closure of stomata and reduced activity (Wilkinson et al. 2012).

Every year, ozone pollution costs China, South Korea, and Japan a combined \$63 billion (£46 billion) due to the damage it causes to wheat, rice, and maize crops (Feng et al. 2022). The study by a multinational group of academics emphasizes the need of reducing ground-level ozone in East Asia to protect agricultural production. One of the most prevalent forms of air pollution is ground-level ozone. It is produced when other atmospheric pollutants, such as nitrogen oxides from fuel combustion and volatile organic compounds from paint and solvent production, combine with sunlight. Ozone's propensity to penetrate leaf pores and oxidize plant tissues has long been recognized by scientists, and this has led to concerns about the gas's impact on plant development and viability (Feng et al. 2022). For the last two decades, scientists have used ozone modelling data to make approximate estimates of worldwide crop losses due to ozone, which have raised food security worries, especially in Asia, where ozone levels are gradually rising (Feng et al. 2022).

In many industrialized nations, comparable steps have been taken for several years leading to static or even decreasing emissions of O_3 precursors (Oltmans et al. 2013). Nevertheless, several emission projections show that the precursors will further develop in the coming years (Dash et al. 2020). Therefore, developing O_3-tolerant rice varieties is essential to produce high-quality grain (Frei 2015), and for that, it is necessary to understand the biochemical, physiological, and molecular foundations of damage to rice plants caused by O_3. In this review, the signal transduction pathway under elevated atmospheric O_3 in rice and rice responses under elevated atmospheric O_3 concentrations are discussed along with methane transportation from soil to the atmosphere in paddy fields and approaches for mitigation of harmful effects of high O_3 in rice.

Signal transduction pathway

Ozone diffuses into apoplastic areas of the leaf from the environment. It quickly interacts with other reactive oxygen species (ROS), such as free oxygen (O_2), hydrogen peroxide (H_2O_2), and nitrous oxide (NO). However, if the ROS detoxifier capability is exceeded, ROS scavenging is performed enzymatic or via non-enzymatic antioxidant defence pathways, which control the regulation of ROS levels through strict compartmentalization (Fig. 1). The breakdown of O_3 into ROS takes place in the apoplastic region itself. The accumulation of ROS in this region is affected by Ascorbic acid (ASA) which in turn changes to dehydroascorbate and enters the plasma membrane. A signal transduced chain depending on heterotrimeric G-proteins is activated by apoplastic ROS.

Meanwhile, an influx of calcium into the plasma membrane takes place. The initiation of mitogen-activated protein kinases (MAPK) is dependent on calcium. Calcium induces ROS production in the chloroplast and all these ROS along with the MAPK induce transcriptional responses within the cell. Change in redox and calcium influx (Ca^{2+}) stimulate MAPK cascades that modify the expression of the genes involved in hormone production, antioxidant production, and the process of photosynthesis (Fig. 1).

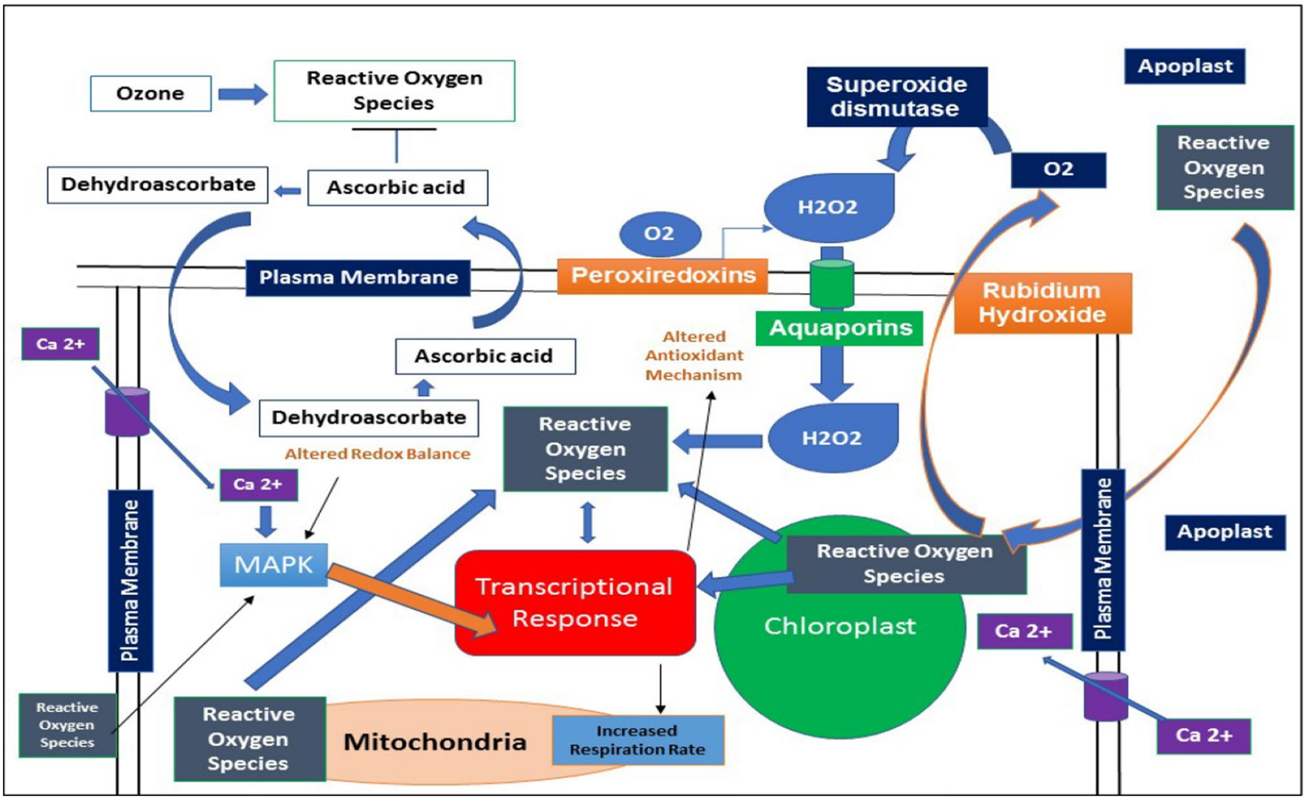

Fig. 1 Signal transduction mechanisms for the biological response of rice plants to O_3 exposure

This results in increased respirational rate in mitochondria. However, another pathway is the conversion of oxygen into hydrogen peroxide (H_2O_2) by superoxide dismutase. This H_2O_2 passes through the plasma membrane using aquaporins (AQP), and leads to the formation of ROS. H_2O_2, in addition to the oxidized ascorbate, impacts the cell redox balance through plasma membrane AQP. This leads to increased transcriptional responses. Extracellular and membrane-bound peroxidase (PRX) and plasma membrane-bound nicotinamide adenine dinucleotide phosphate oxidase (NADPHox) are transmitted into the intercellular area, affecting the activity of apoplastic proteins, including cysteine-rich receptor-like kinases (RLk) (Wrzaczek et al. 2010; Booker et al. 2012).

Rice responses under elevated ozone concentration

Effect on yield and quality

In plants, increments of surface O_3 cause apparent injury, senescence, responsive oxygen species stress enzymes, catalase, and peroxidase and diminished plant height, leaf area and number of leaves, total chlorophyll, biomass, photosynthetic rate, stomatal conductance, Rubisco activity, ascorbic corrosion, root exudates, and the soil microbial community (Xu et al. 2021). Pernicious impacts of O_3 have frequently been credited to leaf senescence, diminished light capture capacity, decrease in chlorophyll content, and reduced forage quality of C_4 plants (Singh et al. 2022). In a previous study, rice seedlings were exposed to 200 ppb O_3 and found clear damage in rice proteome with specific leaf injury (Sahoo et al. 2020). Furthermore, scientists in a previous study (Wahid et al. 1995) reported reductions of 42% and 37% in grain yield of two cultivars of rice, primarily due to a reduction in mean panicle number per plant. Yield reduction due to surface O_3 levels has been observed in cereals, pulses, vegetables, and oilseeds grown in different Asian countries. Subsequently, the effects of O_3 on final grain yield seem to change depending upon factors including O_3 fixation, crop species, and cultivars. However, elevated ozone concentration affects the rice yield drastically (Sahoo et al. 2020).

Agro-geographical investigations also confirmed field crop losses due to high atmospheric levels of O_3 (Feng et al. 2015; Emberson et al. 2009). India has suffered an output loss in the 2002–2007 period of around 276 million dollars due to high O_3 levels in the atmosphere (Debaje 2014). It was also reported that, in 2005 alone, 2.1 million tonnes of rice crops were lost, equivalent to 2.1% of total production (Ghude et al. 2014). The losses in rice yield in the form of tropospheric O_3 pollutants are predicted to increase by 1% to 2% by 2030 (Van Dingen et al. 2009). Some previous findings of the effects of elevated ozone concentration on some rice quality attributes are indexed in Table 1. In Indian cultivars (BR11, BR14, BR28, and BR29), a loss of 53% in rice yields was demonstrated (Akhtar et al. 2010). Subsequently, a loss of grain yield of 43% was reported in the Chinese cultivars Jiahua 2 and Fan 3694 (Wang et al. 2012); 7–26% in the Japanese cultivar Koshihikari cultivar (Yamaguchi et al. 2014), and 16–43% in the Jiaxing cultivar (Feng et al. 2015) of rice, respectively.

Compared to a plant control, lower tillering, lower panicle spikelet number, greater sterility, and decreased individual grain mass were characterized by elevated ozone in rice cultivars (Frei 2015). In some other studies, rice plants were subjected to O_3 at 0.0 (control), 0.05, and 0.10 ppm for 8 weeks from vegetative to early heading stages to elucidate the response of growth and root functions to low concentrations of ozone (Dash et al. 2022). Subsequently, two-hybrid Indica cultivars were treated to ambient and enhanced O_3 under a free-air O_3 enrichment system to investigate the effects of O_3 on uptake and utilization of nitrogen, phosphorus, and potassium nutrients in rice for planning rational fertilization under projected O_3 elevation, which may impact nutrient dynamics in paddy systems to minimize fertilizer use and reduce possible pollution (Kou et al. 2017). Excess ozone concentration can also affect the rice quality (Table 1).

Effect on root rhizosphere microbiome

The effects of rising tropospheric ozone on plant-based ecosystems and individual plants have been studied extensively during the last few decades (Ainsworth 2008). An essential component of the holobiont is the microbes. They develop plant-microbial interactions, producing a distinct microbiome under varied environmental circumstances for each species. These microbial communities are sensitive to changes in their environment and must adapt to different ecological circumstances, such as geographical location or climate. Earlier research has found that higher ozone concentration in rice fields affects the phylogenetic diversity of bacterial and archaeal communities (Zhang et al. 2016; Ueda et al. 2016) in the rhizosphere. These analyses are confined to rhizosphere soil, and the microorganisms that immediately colonize the plant surface have received little attention.

In particular, phyllosphere microorganisms can be impacted by more significant ozone levels, since they are in direct touch with higher concentrations of ozone (Turner et al. 1974). Because of the essential roles of microorganisms and probable ecosystem impacts, further research is necessary to understand this rarely studied element of global climate change, and its potential impact on agriculture. Although the impact of O_3 on the environment has been extensively studied, there has been little research on the impact of O_3 on subsurface processes, particularly

Table 1 Effects of elevated ozone concentration on rice quality

Rice quality attributes affected by elevated ozone	References
Starch concentration reduced	Frei et al. (2012)
Increased protein concentration	Wang et al. (2012)
Reduction in starch concentration	Zheng et al. (2013)
Decrease in rice biomass	Wang and Frei (2011)
Decrease in grain minerals	Wang et al. (2014)
Decrease in grain quality	Zheng et al. (2013)
Decrease in grain quality	Frei et al. (2012)
Decrease in grain quality	Zheng et al. (2013)
Decrease in grain quality	Wang et al. (2014)
Rice grain with higher lipid concentration	Frei et al. (2012)
Smaller grain size	Frei et al. (2012)
Rice straw with more phenolics and lignin concentration	Frei et al. (2012)
Reduction in the digestibility quality of rice straw	Frei et al. (2011)
Lower grain chalkiness	Jing et al. (2016)
Lower grain protein concentration	Jing et al. (2016)
Grain filling with low foliar nitrogen and carbohydrate	Jing et al. (2016)
Irregular endosperm structure	Sawada et al. (2016)
Change in the pH, colour, cooking quality, and total solids of rice grains	Shah et al. (2015)

soil microbial populations. In a rice paddy area in China, researchers investigated the impact of O_3 on the structure and function of the soil microbial community (Chen et al. 2010). The results showed that, soil microbial biomass was significantly lowered under increased O_3 concentrations, compared to the control. Increased O_3 significantly reduced soil microbial functional parameters such as, colour development, richness, and Shannon diversity (Chen et al. 2010).

To estimate the impacts of ozone in large-scale field applications, the chemical ethylenediurea (EDU) has been suggested; however, controlled research assessing EDU's appropriateness to identify genotypic variations in ozone tolerance in rice is lacking (Macías Benítez et al. 2021). Despite the fact that, EDU application had almost no effect on plants. When ozone wasn't present, it partially mitigated the effects of ozone on foliar symptoms, lipid peroxidation, SPAD (Soil Plant Analysis Development) value, stomatal conductance, several spectral reflectance parameters, panicle number, grain yield, and spikelet sterility in the experiment (Macías Benítez et al. 2021). In contrast to tolerant genotypes, sensitive genotypes exhibited more significant EDU responses (Macías Benítez et al. 2021). Therefore, EDU somewhat mitigated the harmful impacts of ozone, particularly on sensitive rice cultivars, but had no constitutive effects on the crop overall (Macías Benítez et al. 2021). However, the biostimulants brassinosteroids (BR), amino acids (AA), nitrophenolates (NP), or biostimulant based on botanical extracts (BE) can be considered as an agronomic strategy to mitigate the adverse effects of heat stress in rice areas, where periods of high temperatures are expected during the day in Colombia (Quintero-CalderóN et al. 2021).

Physiological and biochemical responses

Rice plants are regularly subjected to two types of O_3 stress. In the first one, rice plants are exposed to 150 ppb O_3 from hours to several days in acute O_3 stress, whereas in the second one, they are exposed to 150 ppb O_3 from weeks to months in chronic O_3 stress (Sarkar and Agrawal 2010). ROS build-up in apoplasts caused by O_3 contamination results in uncontrolled necrosis in rice leaves, and the symptom is known as "leaf bronzing". It is frequently used to indicate stress (Sarkar and Agrawal 2010). Acute O_3 exposure for 6 h at 150 ppb concentration resulted in continuous ROS production for 49 h (Sarkar and Agrawal 2010).

Therefore, direct O_3 disintegration and associated metabolic pathways caused by O_3 are jointly responsible for the ROS development of rice (Ueda et al. 2013). Due to increased lipid peroxidation, O_3 stress affects the cell membrane and raises the amount of malondialdehyde (MDA) (Wang et al. 2014; Banerjee and Roychoudhury et al. 2016). Superoxide dismutase (SOD), peroxidase, and the cultivar-dependent phenolic accumulation are increased by the O_3-induced oxidative stress (Ueda et al. 2013). In comparison to the control therapy with charcoal filtered air, higher levels of O_3 raised the activity of superoxide dismutase (SOD), catalase, and guaiacol peroxidase (GPX) by 93.7%, 39.9%, and 312.4%, respectively (Zhang et al. 2008). Also, increased O_3 activity in two tropical rice cultivars: Malviya Dhan 36 and Shivani, boosted SOD, peroxidase, ascorbate peroxidase (APX), and reductase glutathione (CG) (Sarkar et al. 2015).

Furthermore, O_3 reduced plant photosynthesis efficiency (Sarkar et al. 2015). However, GDP-D-mannose-30–50-epimerase was shown to be necessary for antioxidant accumulation in rice lines with a mutation in the biosynthetic ASA gene (Frei et al. 2012), but mutants were more sensitive to O_3 since ASA and glutathione were decreased. Studies show that, in the grains of the hybrid rice cultivar Shantou 63, increasing O_3 levels have increased the overall amino acids, including total essential and non-essential amino acids, by 12–14% (Zhou et al. 2015). O_3 stress also greatly affects rice photosynthesis. As O_3-derivative ROS damages photosynthetic pigments, including chlorophyll and carotenoids, the leaves become chlorotic and their senescence is accelerated (Frei 2015). In O_3-sensitive rice-genotype leaves under stress, photosynthesis-associated proteins essential for light collection and electron transport were found to be decreased (Sawada et al. 2012). During O_3 stress, stomatal closure and biochemical damages to the photosynthesis system have further impeded rice development.

In another study, the harmful effects of O_3 on the cumulative stomatal outflow of O_3 in Koshihikari, a Japanese rice cultivar, were explored to analyse the adverse effects of O_3 on yield. A linear regression analysis revealed a correlation between relative output and cumulative O_3 influx, with a threshold of 10 nmol O_3 projected leaf surface (Yamaguchi et al. 2014). Subsequently in another study, Malviya Dhan 36 and Shivani cultivars subjected to O_3 pollution showed reduced photosynthesis rates, stomatal conductivity, and photosynthesis efficiency (Sarkar and Agrawal 2010). It has also been shown that, in the leaves of O_3 stressed rice seedlings during grain filling, net photosynthetic, stomatal, and transpiration rates were reduced (Sarkar and Agrawal 2010). Transcriptomic research has also elucidated the genes, that are engaged in the processing of information, storage, cellular processing, signalling, and metabolism in O_3-damaged rice (Cho et al. 2008). Scientists also found that, O_3 stress activates many genes involved in hormonal regulation, cell wall construction, defence, modification of the cell wall, starch mobilization, and secondary metabolite production (Cho et al. 2008).

Greenhouse gas emission from rice field

Stomata allow CO_2 and water to diffuse into the tissue and are found mostly on the underside of plant leaves. High concentrations of O_3 very close to the stomata reduce photosynthesis and plant growth (McAdam et al. 2017). O_3 may initiate oxidative stress, and subsequently may harm plant cells (Vainonen and Kangasjarvi 2015). Anoxic carbon breakdown produces CH_4 (Methane), the simplest organic molecule and a member of the paraffin hydrocarbon family (Conrad 2002). Paddy soil may release CH_4 via aerenchyma in the plant, and diffusion through the soil and water layer (Fig. 2). CH_4 enters the plant via diffusion and during water absorption. Aerenchyma and intercellular gaps are thought to have a role in the gasification of dissolved CH_4 in the root cortex before it diffuses upwards to the root–shoot transition zone.

The plant aerenchyma system transports oxygen from the leaves to the roots. An area of relatively oxygen-rich rhizosphere is created as CH_4 diffuses into the root system beneath the soil surface, similar to how oxygen diffuses into the soil surface. Methanotrophic bacteria in the rhizosphere oxidize CH_4 to CO_2. Oxygen suppresses methanogenesis in the rhizosphere itself, where it occurs naturally. The rice plant's characteristics determine the amount of CH_4, that is released into the atmosphere. These characteristics affect the gas flow, as well as permeability coefficients, root concentration gradients, and the internal structure of the aerenchyma. Gas fluxes are also influenced by tillers, root mass, rooting pattern, total biomass, and metabolic activity. However, many researchers reported that more than 90 per cent of total CH_4 emitted during the cropping season is released by diffusive transport through the aerenchyma system of the rice plants, and not by diffusion or ebullition (IPCC 1996). In response to seasonal changes in soil conditions and plant growth, rice plant emissions are likely to be variable. During the rice plant development cycle, the amount of CH_4 emitted fluctuates. When the rice plant is in its early phases of development, its CH_4 emissions are modest and methanogenesis may be insignificant (Bharati et al. 2001).

Molecular breeding approach for elevated ozone tolerance

Rising tropospheric O_3 affects rice yields, grain quality, and straw quality. Breeding adapted rice cultivars is a viable way to improve global food security, since farmers cannot control atmospheric conditions. Intraspecific variation in rice O_3 adaption has been genetically dissected by utilizing bi-parental populations or genome-wide association studies (GWAS). Marker-assisted breeding and transgenic techniques targeting ozone tolerance may boost rice productivity. GWAS, which evaluate genetic combinations during the development and domestication of crops, have identified the genetic variants governing O_3 tolerance in rice

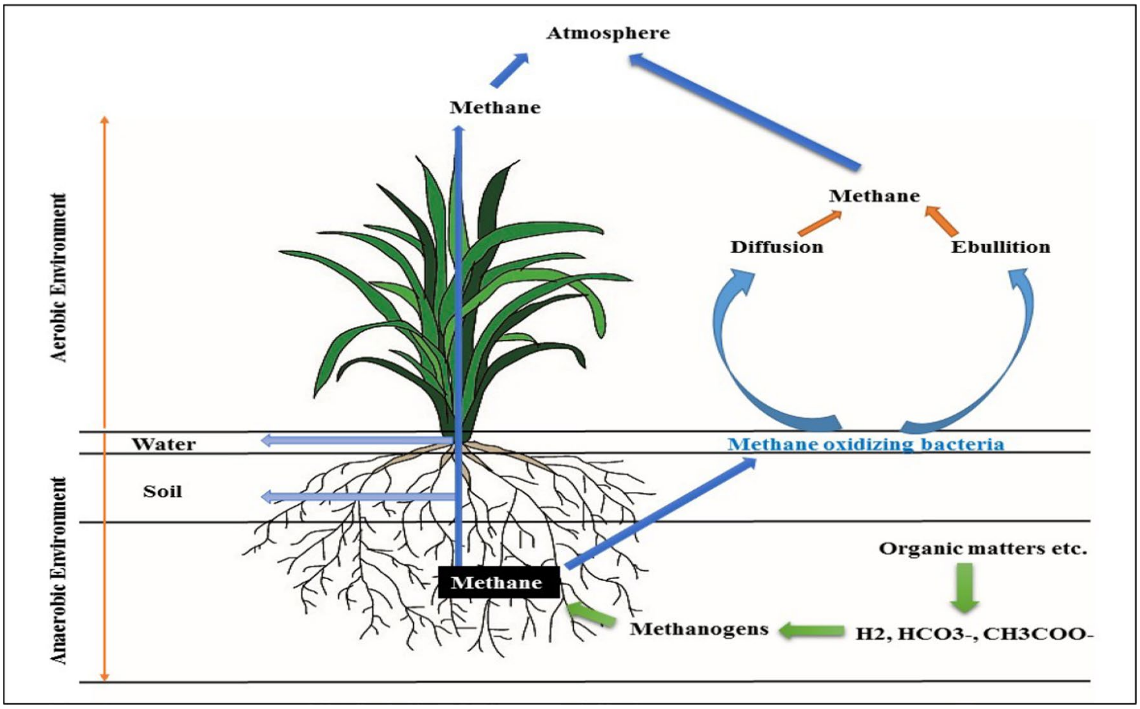

Fig. 2 Transportation of methane from paddy soil to the atmosphere

plants. Genome-wide association mapping improves genetic resolution and the ability to identify loci associated with favourable agriculture characteristics (Sahoo et al. 2020).

Rice genome sequencing, single-nucleotide polymorphism (SNP) markers, and dense marker maps for high-resolution genome-wide association mapping are all commonly employed (Morrell et al. 2012), for O_3 tolerance in rice plants. In this case, the methodology utilizes diverse populations with a morphological and phenological diversity. Hence, genotypical and phenotypic properties may not be appropriately combined (Frei 2015). In some previous studies, 328 rice accessions representing genetically diverse sub-groups of the Asian rice family and types were tested in genome-wide association mapping for O_3 tolerance, in which, 30 000 SNP markers yielded 16 significant markers throughout the genome by applying a significance threshold of $P < 0.0001$ (Ueda et al. 2013). This analysis revealed a number of novel polymorphisms in two candidate genes for the formation of visible leaf symptoms, a *RING* and an *EREBP* gene, both of which are involved in cell death and stress defence reactions (Ueda et al. 2013).

Subsequently, researchers observed a correlation between the weight of the panicle and flowering time, showing a genetic relationship of O_3 tolerance with a distinct flowering characteristic (Zhao et al. 2011). Genome-wide studies have shown that rice genotype differences are substantial, and some putative loci related to O_3 tolerance characteristics have been found (Sahoo et al. 2021). The application for O_3 tolerance of such varied genotypes and phenotypes is significant under the general title "characteristic improvements via plant breeding". Co-localization of QTLs for ozone tolerance in rice from separate bi-parental QTL mapping studies for the attribute leaf bronzing was also investigated (Kim et al. 2004), for biomass (Frei et al. 2008), grain yield (Tsukahara et al. 2013), and a genome-wide association study for the target traits leaf bronzing, plant height, biomass, number of tillers and weight of panicle (Wahid et al. 1995).

Some studies of breeding in rice using linkage mapping or GWAS for elevated ozone levels are indexed in Table 2. Breeding involves the crossing and selfing of two separate bi-parental populations to form recombinant inbred lines (RILs) (Ueda et al. 2015). Each of these lines has a genotypical divergence induced by different parental genome combinations. Before phenotyping for O_3 stress tolerance, each RIL is genotyped using molecular markers. Recombination frequency-based linkage mapping is utilized to discover the exact chromosomal tolerance characteristics (Sahoo et al. 2021). The first study, involving QTLs, was conducted with the sensitive Indian cultivars, Gihobyeo and Milyang 23, crossing the O_3-tolerant Japanese and Indica types (Kim et al. 2004). In another experiment, a set of 164 RILs from a bi-parental mapping population was generated in which three QTLs responsible for O_3-mediated foliar damage were identified on chromosomes 1, 7, and 11 (Tsukahara et al. 2013).

Subsequently, a gene named Aberrant Panicle Organisation 1 (APO1) was identified in the region of a QTL, which governs primary rachis branching (Tsukahara et al. 2013) under elevated O_3 stress. In another study, after translation and activation, the rice transcription factor, WRKY45 increases OsORAP1 expression, which is a novel protein, OZONE-RESPONSIVE APOPLASTIC PROTEIN1, and the OsORAP1 gene causes rice tissue necrosis, reducing jasmonic acid (JA) biosynthesis, preventing oxidative stress, and scheduled cell death (Ueda et al. 2013). Subsequently, a recent study provides strong correlative evidence that, OsORAP1 (Sahoo et al. 2019) is an important regulator of O_3 response and tolerance in Asian rice.

Advanced genetic engineering approach for elevated ozone tolerance

Plant breeders may discuss the need for transgenic methods for O_3 tolerance, where a more extensive range of genotypic biodiversity could be found. Several screens have found putative rice QTLs, that may confer O_3 tolerance or sensitivity regulators. However, no QTL of this kind has been fine-mapped so far for O_3 stress tolerance. Transgenic technologies are the probable solution to this problem (Pramanik et al. 2021). No commercial transgenic measures

Table 2 Rice breeding using quantitative trait loci mapping for elevated ozone

Bi-parental cross or GWAS	Population type	Location of QTLs or linked markers	References
Mileage 23 × Gihobyeo	RILs	QTLs located on chr 1, 7, and 11	Kim et al. (2004)
Sasanishiki x Habataki	CSSL	Single locus associated with grain yield loss located on chr 6	Tsukahara et al. (2013)
Nipponbare x Kasalath	98 RILs	6 QTLs located on chr 3, 8, and 9	Frei et al. (2008, 2010)
Germplasm panel for GWAS	328 rice accessions	50 SNP markers associated with various agronomic characters and the QTL for grain yield clustered around 27 Mb on chr 6	Tsukahara et al. (2013)

CSSL Chromosome segment substitution lines; *RILs* Recombinant inbred lines; *QTLs* Quantitative trait locus; *GWAS* Genome-wide association study; *SNP* Single-nucleotide polymorphism, *Mb* Mega base pair, *chr* chromosome to produce O_3-tolerant rice lines have been employed to date. However, as a result of establishing a relationship between O_3 stress and rice ascorbate metabolism, a feasible alternative for improving O_3 tolerance in rice plants has been suggested. ASA-GSH (ascorbate–glutathione) enzymes, including APX (ascorbate peroxidase), and dehydroascorbate reductase, can be overexpressed to improve O_3 tolerance in rice (Frei et al. 2012).

In a previous study, it was observed that, under O_3 stress, shortage of ascorbate in rice resulted in increased grain chalkiness as the effect of L-GaLDH (L-galactose-1,4-lactone dehydrogenase) was decreased (Yu et al. 2017). ASA deficiency has been found to promote the peroxidation of lipid and ROS generation, reducing the quality of rice grain. L-GaLDH mutants displayed decreased antioxidant capacities, photosynthesis efficiencies, decreased activity of the enzyme, and transcripts related to the starch formation (Yu et al. 2017). These findings imply that, overexpression of ASA genes can improve the physiology of rice, and the quality of grain under O_3 stress. *Arabidopsis* transcription factors (TFs) like *WRKY18*, *WRKY40*, and *WRKY60* are mediators for cross-talking between abiotic and biotic stresses, and can be found as target for development of O_3 tolerance in rice. The *OSBZ8* gene, can be over-repressed in rice for enhanced O_3 tolerance (Banerjee and Roychoudhury 2016).

Furthermore, use of transgenics could be useful, if insufficient variation is available within the rice genome, for elevated ozone tolerance. However, there is a significant yawning gap between the widespread adoption of GM crops for cultivation in many countries and the global markets for food and feed, and the often-limited acceptance of these products by consumers (Lucht 2015). There has been a recent hardening of the negative political and social environment for agricultural biotechnology in Europe, a growing discussion, including calls for labelling of GM food in the USA, and a cautious development in China towards a possible authorization of GM rice (Lucht 2015). Some consumer worries about transgenic crops have been allayed by advances in breeding techniques, but it is unclear how general public opinion will change in the future (Lucht 2015). Agriculture-related discussions would be more fruitful if they shifted their emphasis from technological details to shared goals and values (Lucht 2015).

Conclusion

The geographical and temporal variability of tropospheric O_3 concentration is a major constraint for natural selection to generate O_3-resistant lines by traditional breeding. Consequently, the putative loci should be subjected to small-scale experiments under controlled O_3 conditions. It is difficult to convince farmers and breeders to grow and select O_3-tolerant plants. This is because of the high importance of breeding for widespread abiotic conditions like salinity and drought. The increasing levels of O_3 in the troposphere are adversely affecting rice cultivation. Strategies for the development of O_3-tolerant rice crops have not yet been used in the field. In terms of agricultural development, more transgenic technology initiatives may seem to be helpful. Surprisingly, an interferometric optical approach for monitoring rice development with a great temporal resolution during O_3 stress has been created. Extrapolation of this technology on a practical level can result in a stronger linkage among genotypes with improved O_3 tolerance and the physiology of rice.

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